Gastric cancer carcinogenesis and tumor progression

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BACKGROUND: *Gastric cancer (GC) remains one of the leading causes of cancer-related deaths worldwide, even though a decline has been observed in its incidence and the mortality rate in recent decades. Gastric carcinogenesis is a complex phenomena involving multiple epigenetic and genetic factors; several genetic, environmental and infectious agents interact causing a cumulative effect in the early steps of gastric carcinogenesis.*
MATERIALS AND METHODS: *The most commonly used classifications of GC are the World Health Organization (WHO) and the Laurén classifications which describes two main histological types, diffuse and intestinal, which have different clinicopathological characteristics. Diffuse cancer occurs more commonly in young patients, can be multifocal, is not often accompanied by intestinal metaplasia and can be hereditary, in which E-cadherin alteration plays a pivotal role.*
Intestinal type is more frequently observed in older patients and follows multifocal atrophic gastritis. This is usually accompanied by intestinal metaplasia and leads to cancer via dysplasia, and thus intestinal metaplasia is considered a dependable morphological marker for GC risk. Intestinal adenocarcinoma predominates in the high-risk areas whereas the diffuse adenocarcinoma is more common in low-risk areas.
DISCUSSION: *Classically, genetic instability and Helicobater pylori (H. Pylori) infection are often identified in intestinal GC. The great majority of GCs are sporadic and result from the cumulative effects of different environmental risk factors; smoking, alcohol consumption and dietary habits have been addressed as significant. H. Pylori infection and proinflammatory cytokine gene polymorphisms represent other features of this compound process that leads to the development of GC. Other molecular pathway well described in GC is microsatellite instability (MSI) that related with specific clinic-pathological features.*
CONCLUSION: *In this review we focused on the role of H. Pylori infection, MSI and alterations of CDH1 (E-Cadherin) gene.*

KEY WORDS: E-cadherin, Gastric tumorigenesis, Genomic instability, Helicobacter Pilory

Introduction

Gastric cancer (GC) remains one of the leading causes of cancer-related deaths worldwide, even though a decline has been observed in its incidence and the mortality rate in recent decades. Gastric carcinogenesis is a complex phenomena involving multiple epigenetic and genetic factors; several genetic, environmental and infectious agents interact causing a cumulative effect in the early steps of gastric carcinogenesis.

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The great majority of GCs are sporadic and result from the cumulative effects of different environmental risk factors; smoking, alcohol consumption and dietary habits have been addressed as significant. *H. Pylori* infection and proinflammatory cytokine gene polymorphisms represent other features of this compound process that leads to the development of GC. Other molecular pathway well described in GC is microsatellite instability (MSI) that related with specific clinic-pathological features.

In this review we focused on the role of *H. Pylori* infection, MSI and alterations of *CDH1* (E-Cadherin) gene.

Helicobacter Pylori infection

Several prospective studies reported a strong association between chronic *H. Pylori* infection and GC; as such, the World Health Organization's International Agency for Research on Cancer recognized H. Pylori as a Group 1 carcinogen for humans. Chronic gastric inflammation and the interaction between *H. Pylori* and gastric epithelial cells have been suggested as potential mechanisms in gastric carcinogenesis 1. However, only a few individuals infected by *H. Pylori* eventually develop GC, probably this is due to environmental factors, host-inflammatory genetic susceptibility and variation of the bacterial strains.

H. Pylori is a Gram-negative bacterium that colonizes the human gastric epithelium, the severity of *H. Pylori*–related disease is correlated with the presence of *cag* pathogenicity island (PAI) associated with production of the *cagA* antigen. The *cagA* gene, the strain-specific *H. Pylori* gene, is located in the right half of the PAI, and encodes the protein *cagA*, which is secreted via a type IV secretion system and translocated into gastric epithelial cells affecting host cell physiology [2,3]. Infection with *cag* PAI–positive *H. Pylori* strains has been recognized as a marker for strains that confer increased risk for peptic ulcer disease, gastric mucosal atrophy and GC. The *cagA* gene is one of several genes of a PAI called the *cag* PAI and the presence of *cagA* is considered to be a marker of the presence of the *cag* PAI. The *vacA* gene is present in all *H. Pylori* strains and encodes the vacuolating cytotoxin vacA, which induces epithelial cell injury. *H. Pylori* colonizes the atrophic stomach poorly, and intestinal metaplasia hardly at all, suggesting that the bacteria may create the environment for intestinal-type gastric carcinogenesis (atrophy and hypochlorhydria) rather than causing the cancer directly. The risk of developing GC for infected persons is estimated to be between two and three times higher compared to none infected ones [4]. More recently, the role of individual susceptibility has been stressed and proinflammatory cytokine gene polymorphism has been demonstrated to interact in the compound process of gastric carcinogenesis in several studies [5]. In particular, some Authors investigated the relationship between interleukin-1 gene polymorphism and GC risk. The conclusions supported the theory in which host genetic factors affecting the inflammatory and immune response to *H. Pylori* infection may determine a higher risk of GC development, in particular in high risk area [6]. In addition, a variety of studies have indicated that salted, smoked, pickled and preserved foods (rich in salt, nitrite, and preformed N-nitroso compounds) as well as meat intake are associated with an increased risk of non-cardia gastric cancer [7]. In GC, the absence of *H. Pylori* infection is related with specific clinicopathologic factors impacting GC long term survival. Negative *H. Pylori* status is associated with cardia tumor location, advanced pT classification, noncurative surgery, and a lower 5-year survival rate after R0 resection. Marrelli et al. demonstrate that *H. Pylori* status is a significant prognostic factor in which negative *H. Pylori* status appears to be an indicator of poor prognosis in patients with GC [8].

Microsatellite instability and oncogenic mutations

MSI is defined as the presence of replication errors resulting in insertions or deletions of bases within nucleotide repeats, known as microsatellite regions.

MSI and chromosome instability are two major genomic instability pathways involved in GC pathogenesis; in particular, MSI is the hallmark of hereditary non-polyposis colorectal cancer (HNPCC) tumors and it also occurs in about 15% of sporadic carcinomas of the HNPCC spectrum. In stomach cancer, MSI occurs in 15-30% of cases and it tends to occur preferentially in intestinal tumors of the antrum of elderly patients with good prognosis [9,10]. Other pathologic features of MSI gastric tumors are less invasion of the serosa, and involvement of fewer lymph node with lower rate of lymphovascular invasion, relating with less tumor aggression and lower disease stage. Among GC with MSI phenotype, recently, Corso et al. described a subset of tumors with all unstable markers showing a good long term survival. This data delineate a novel MSI tumor with less biological aggression and favorable prognosis [10].

Germline defects in mismatch repair genes (MMR), such as *hMSH2*, *hMLH1* and *MSH6*, have been described in HNPCC. Tumors with MMR defects show an increased rate of instability that can lead to the inactivation of

specific target genes [11] which play a role in MSI tumorigenesis. Very few cases of sporadic GC show mutations of MMR genes [12], whilst MSI phenotype is observed in 15-30% of the cases.

MSI gastric carcinomas harbour an increased rate of mutations in repeat regions, namely in non-coding sequences and in target genes, as well as in oncogenes, namely in genes belonging to the mitogen-activated protein kinase (MAPK) cascade and phosphatidylinositol 3-kinase (PI3K) pathway. Within the MAPK pathway *KRAS* gene is commonly mutated in all types of cancer but in GC the frequency of mutations in this gene is low and it varies between 3 to 28% depending on the series under analyses. Further whenever present *KRAS* mutations cluster in MSI GC [13, 14]. In MSI GC, as in other types of cancer, the majority of *KRAS* somatic mutations have been detected in hotspots regions of the gene, namely in codons 12 and 13. Activation of *RAS* gene potentiates the MAPK by the engagement of the cytosolic protein *RAF*. *BRAF* mutations occur in about 40% of MSI colorectal cancinomas, in a mutually exclusive manner with *KRAS* mutations, but in GC activating mutations of this gene were rarely found in most series [15-19]. Within the *PI3K* pathway, *PIK3CA* gene mutations are found in different tumour types and represent an important molecular event in carcinogenesis [20]. Velho et al. previously reported that *PIK3CA* gene mutations occur in MSI gastric carcinoma with a high/ moderate mutation rate [21].

E-cadherin (CDH1) gene deregulation

CDH1 gene maps to chromosome 16q22.1 and consists of 16 exons occupying about 100 kb of genomic DNA and encodes for a 120 kDa protein called E-cadherin (E-cad). E-cad is a member of transmembrane glycoproteins family expressed on epithelial tissue and responsible for calcium-dependent cell-to-cell adhesion. E-cad has been demonstrated to be critical for establishing and maintaining polarized and differentiated epithelia through intercellular adhesion complexes. Human E-cad is considered an invasion suppressor and its deregulation is often found in advanced cases of sporadic GC. Underexpression of E-cad is also correlated with infiltrative and metastatic ability of the tumor [22]; this is due to the disruption of cadherin-catenins complex, and the consequently loss of cell adhesion and concomitant increase in cell motility. E-cad gene is regarded as a classical tumor suppressor gene that occurs early in GC carcinogenesis; it is hence expected to suffer inactivation of both alleles to be suppressed. As reported, patients with autosomal dominant inherited tumors have a mutated allele in the germline while the other (wild-type) allele is inactivated only in tumor tissue. The impairment of gene function on the wild-type allele is known as the 'second inactivating hit' and it is due to one of the following mechanisms: somatic mutation, loss of heterozygosity (LOH) and promoter hypermethylation.

Somatic mutations of *CDH1* are frequently described in sporadic diffuse GC (DGC). It has been demonstrated in about 50% of DGC but not in intestinal histotype [23]. Berx and colleagues reported that the most frequently observed changes in DGC are splice site variants that cause skipping in exon 8 or 9 and account for in-frame deletions. Conversely, missense and truncating mutations are seldom observed [24]. Machado and colleagues searched for *CDH1* gene somatic mutation in 23 GC patients. Among these, somatic mutations were identified in 9 (39.1%) patients affected by DGC. The authors concluded that E-cad mutation stands frequently for the second inactivating hit in DGC but not in intestinal GC [25].

In sporadic GC (diffuse as well as intestinal), LOH is reported with a wide range of frequency (3-60%). Liu and colleagues reported a high frequency (38%) of LOH of *CDH1* gene for intestinal and diffuse GCs. The authors concluded that LOH is one of the major mechanism of E-cad inactivation [26]. Differently, Machado and colleagues found LOH to be responsible of *CDH1* gene impairment in only one out of 9 (11%) sporadic DGC patients [25].

CDH1 gene promoter hypermethylation is the most frequent event underlying second genetic hit in GC. DNA is methylated (addition of $-CH_3$ groups) at cytosine located 5' to guanosine in the CpG island; this mechanism has an important regulatory effect on gene expression especially when the promoter region of the gene that controls transcription process is involved. Aberrant promoter methylation and the associated loss of gene expression commonly occur in several types of human cancer. Tamura and colleagues indicated that *CDH1* promoter hypermethylation may play a major role in causing the inactivation of E-cad gene in GC, and especially in DGC. At immunohistochemical analysis, hypermethylation of the promoter region was associated with a reduced E-cad tumor tissue expression [27]. Considering the experience reported by Machado and colleagues, *CDH1* promoter hypermethylation was reported in 56.2% and 28.6% of DGC and intestinal GC, respectively [25]. Liu and colleagues reported promoter hypermethylation in 76% of DGC and in 50% of intestinal GC [26]. Graziano and colleagues investigated the relationship between the epigenetic change of *CDH1* and the outcome of 73 patients with surgically resected DGC. This experience showed *CDH1* promoter hypermethylation in 40 cases (54%); a significant association was found between the tumors with hypermethylation and the distribution of disease-free and relapsed patients [28]. Grady and colleagues demonstrated that hypermethylation of *CDH1* promoter region is the most important mechanism underlying the second genetic hit in HDGC. In this study, the authors postulated that promoter methylation had a reversible nature with a possible attrac-

tive target for the development of new anti-cancer therapies [29].

Conclusions

The exact mechanisms underlying gastric carcinogenesis are not yet fully understood, but evidence points to an association with pathways involved in developmental processes.
Several factors (infectious, nutritional and genetic) have been demonstrated to interact in the multifactorial process of gastric carcinogenesis. A strict correlation between *H. Pylori* and gastric cancer has been reported and *H. Pylori* is currently classified as type I (definite) carcinogen by the International Agency for Research on Cancer. The risk of developing GC for infected persons is estimated to be between two and three times higher compared to none infected ones. More recently, the role of individual susceptibility has been stressed and proinflammatory cytokine gene polymorphism has been demonstrated to interact in the compound process of gastric carcinogenesis in several studies between interleukin-1 gene polymorphism and GC risk. In addition, a variety of studies have indicated that salted, smoked, pickled and preserved foods (rich in salt, nitrite, and preformed N-nitroso compounds) as well as meat intake are associated with an increased risk of non-cardia gastric cancer.
MSI is the hallmark of HNPCC tumors and it also occurs in about 15% of sporadic carcinomas of the HNPCC spectrum. Probably, environmental factors may play a role in generating MMR deficiency leading to MSI phenotype. Some studies showed an association between MSI, positive family history of GC and high consumption of red meat and nitrates, suggesting that environmental factors, such as nutritional habits may play a key role in inducing genomic instability and an increased risk for GC. General environmental exposures, such as diet, were considered as potential generators of increased microsatellite alterations in tumors with an increased tolerance to DNA damage associated with reduced MMR activity.
The etiology of GC includes also two well-known autosomal dominant hereditary syndromes: the (HNPCC) caused by mutations or epigenetic silencing in DNA mismatch repair genes, mainly mutations of MLH1 and MSH2, and the Hereditary Diffuse Gastric Cancer due to E-cadherin gene (*CDH1*) mutation.
A role for E-cadherin in tumor development is well established, with many human carcinomas exhibiting reduced E-cadherin expression relative to their normal cellular counterparts. In sporadic diffuse gastric cancers and lobular breast cancers, E-cadherin inactivation is associated with somatic point mutations of the E-cadherin gene, as well as loss of heterozygosity, promoter hypermethylation or overexpression of transcriptional repressors. In most carcinomas loss of E-cadherin is usually a late event associated with invasion and metastasis; nevertheless, the study of early hereditary diffuse gastric cancer lesions in germline E-cadherin (*CDH1*) mutation carriers suggests that E-cadherin loss could be an early or initiating event in tumorigenesis.

Riassunto

Il cancro gastrico (GC) rimane una delle cause principali di morte per cancro in tutto il mondo, anche se negli ultimi decenni è stato osservato un calo nella sua incidenza e nel tasso di mortalità.
La carcinogenesi gastrica è un fenomeno complesso che coinvolge molteplici fattori epigenetici e genetici; diversi agenti genetici, ambientali e infettive interagiscono provocando un effetto cumulativo nelle fasi iniziali della carcinogenesi gastrica.
Le classificazioni più comunemente utilizzate di CG sono quelle dell'Organizzazione Mondiale della Sanità (OMS) e la classificazione di Laurén che descrive i due principali tipi istologici, diffuso ed intestinale, che hanno diverse caratteristiche clinico-patologiche. Il cancro diffuso si verifica più comunemente nei pazienti giovani, può essere multifocale, spesso non è accompagnato da metaplasia intestinale e può essere ereditario; in questa forma l'alterazione della E-caderina svolge un ruolo fondamentale.
Il tipo intestinale è più frequentemente osservato nei pazienti anziani ed è associato ad una gastrite atrofica multifocale. Questa forma è di solito accompagnata da metaplasia intestinale e porta al cancro attraverso displasia, pertanto la metaplasia intestinale è considerata un marker morfologico affidabile per il rischio di GC. La forma intestinale di adenocarcinoma gastrico predomina nelle zone ad alto rischio, la forma diffusa invece è più comune nelle zone a basso rischio. Classicamente, l'instabilità genetica e l' infezione da Helicobater pylori (*H. Pylori)* sono spesso identificate nella forma intestinale di GC. La grande maggioranza di GC sono sporadici e risultato dagli effetti cumulativi dei diversi fattori di rischio ambientale; il consumo di fumo, l'alcool e le abitudini alimentari sono stati considerati significativi.
L'infezione da H. Pylori ed i poliforfismi dei geni delle citochine pro-infiammatorie fanno parte di questo complesso meccanismo che conduce allo sviluppo del GC.
Un altro percorso molecolare ben descritto nel GC è l'instabilità dei microsatelliti (MSI), che si collega con specifiche caratteristiche clinico-patologiche.
In questa review ci siamo concentrati sul ruolo delle infezioni da H. Pylori, MSI e alterazioni del gene CDH1 (E-caderina).

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