World Journal of Gastrointestinal Oncology

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.4251/wjgo.v8.i1.40

World J Gastrointest Oncol 2016 January 15; 8(1): 40-54
ISSN 1948-5204 (online)

REVIEW

Helicobacter pylori infection and gastric carcinoma: Not all the strains and patients are alike

Natale Figura, Luigi Marano, Elena Moretti, Antonio Ponzetto

Natale Figura, Department of Medical, Surgical and Neurological Sciences, University of Siena and Policlinico S. Maria alle Scotte, 53100 Siena, Italy

Luigi Marano, General, Minimally Invasive and Robotic Surgery, Department of Surgery, Hospital San Matteo degli Infermi, 06049 Spoleto, Perugia, Italy

Elena Moretti, Department of Molecular and Developmental Medicine, University of Siena, 53100 Siena, Italy

Antonio Ponzetto, Department of Medical Sciences, University of Torino, 10126 Torino, Italy

Author contributions: All the authors contributed equally in the design of the review, acquisition and interpretation of data and drafting the manuscript, which was approved by them all.

Conflict-of-interest statement: The authors have no conflict of interest or financial ties to disclose.

Correspondence to: Natale Figura, Professor, Department of Medical, Surgical and Neurological Sciences, University of Siena and Policlinico S. Maria alle Scotte, Viale Bracci, 53100 Siena, Italy. natale.figura@unisi.it
Telephone: +39-5-77585463
Fax: +39-5-77233446

Received: June 14, 2015
Peer-review started: June 17, 2015
First decision: August 4, 2015
Revised: October 6, 2015
Accepted: November 3, 2015
Article in press: November 4, 2015
Published online: January 15, 2016

Abstract

Gastric carcinoma (GC) develops in only 1%-3% of *Helicobacter pylori* (*H. pylori*) infected people. The role in GC formation of the bacterial genotypes, gene polymorphisms and host's factors may therefore be important. The risk of GC is enhanced when individuals are infected by strains expressing the oncoprotein CagA, in particular if CagA has a high number of repeats containing the EPIYA sequence in its C'-terminal variable region or particular amino acid sequences flank the EPIYA motifs. *H. pylori* infection triggers an inflammatory response characterised by an increased secretion of some chemokines by immunocytes and colonised gastric epithelial cells; these molecules are especially constituted by proteins composing the interleukin-1beta (IL-1β) group and tumour necrosis factor-alpha (TNF-α). Polymorphisms in the promoter regions of genes encoding these molecules, could account for high concentrations of IL-1β and TNF-α in the gastric mucosa, which may cause hypochlorhydria and eventually GC. Inconsistent results have been attained with other haplotypes of inflammatory and anti-inflammatory cytokines. Genomic mechanisms of GC development are mainly based on chromosomal or microsatellite instability (MSI) and deregulation of signalling transduction pathways. *H. pylori* infection may induce DNA instability and breaks of double-strand DNA in gastric mucocytes. Different *H. pylori* strains seem to differently increase the risk of cancer development run by the host. Certain *H. pylori* genotypes (such as the *cagA* positive) induce high degrees of chronic inflammation and determine an increase of mutagenesis rate, oxidative-stress, mismatch repair mechanisms, down-regulation of base excision and genetic instability, as well as generation of reactive oxygen species that modulate apoptosis; these phenomena may end to trigger or concur to GC development.

Key words: *Helicobacter pylori* infection; CagA; *CagA* gene polymorphism; Haplotype; Human gene mutation;

Gene methylation; Gastric carcinoma; Inflammatory cytokine

Core tip: CagA and the *cagA* types may play different roles in the intestinal and diffuse histotypes of gastric carcinoma (GC); The current criteria of *Helicobacter pylori* (*H. pylori*) strain classification based on their carcinogenic potential gave rise to confusion and should be unified. The possible role of inflammatory cytokine haplotypes in GC development should be reassessed taking into account some host's factors, the most important being different ethnic origin. Infection by the *cagA* positive *H. pylori* genotype may determine an increased inflammatory response and a consequent enhancement of mutagenesis rate, oxidative-stress, reactive oxygen species generation, dysfunction of DNA repair mechanisms, genetic instability and resultant high risk of GC development.

Figura N, Marano L, Moretti E, Ponzetto A. *Helicobacter pylori* infection and gastric carcinoma: Not all the strains and patients are alike. *World J Gastrointest Oncol* 2016; 8(1): 40-54 Available from: URL: http://www.wjgnet.com/1948-5204/full/v8/i1/40.htm DOI: http://dx.doi.org/10.4251/wjgo.v8.i1.40

INTRODUCTION

Gastric carcinoma (GC) is the second most frequent cause of death from cancer worldwide and the most common example of a neoplasia developing on a ground of a chronically inflamed mucosa. GC has also another record: It is the only known malignant tumour that can develop as a consequence of a chronic bacterial infection[1]. In 1994, the International Agency for Research on Cancer classified the organism responsible for the infection, *Helicobacter pylori* (*H. pylori*) - a Gram negative, microaerophilic and spiral-shaped species that finds its *habitat* in human stomachs - as a definite carcinogen to humans (Group 1): The connection of *H. pylori* with gastric cancer was considered similar to that existing between the cigarette smoke and lung cancer[2].

The bacterium H. pylori: Not all the strains are alike

It soon became clear, however, that such comparison was reductive and too simplistic, especially because the ability of these bacteria to trigger a neoplasm is not limited to the inflammatory and immune response to the infection that they cause, but it also resides in a series of bacterial factors capable of prompting and modulating the carcinogenic process[3].

As is the case for all diseases, also GC develops from the concomitance of three factors: The etiological agent, the host and the environment. Of course, many other factors may occur; for example, the cancer histological variant, the degree of differentiation of the neoplasia *etc.*[4]. Regarding the etiological agent, *H. pylori*, there are many indications that not all strains are equivalent in their carcinogenic potential and that those expressing an immunodominant peptide determinant called *CagA* (cytotoxin associated gene A), are endowed with an increased inflammatory and carcinogenic potential[5-9]. A first point has therefore been established: Strain genomic diversity corresponds to different ability to promote cancer. The possibility that a bacterial factor (*CagA*) could trigger or concur to the development of GC is one of the most important scientific achievements following the isolation of *H. pylori*.

The importance of being called CagA positive

It is worthwhile mentioning the steps that paved the way to the discovery of CagA. At the end of the 80ies, Leunk *et al*[10] first proposed that *H. pylori* should not be considered a clonal pathogen, as a relevant proportion of isolates produce a vacuolating toxin, which could account in part for the gastric mucosa damage observed in infected individuals. Afterward, our group suggested that infection by cytotoxic strains increased the risk of developing peptic ulceration[11] and that virtually all cytotoxic isolates also secreted a 120 kDa highly immunogenic protein, later called CagA[12]. In 1992, Crabtree *et al*[13] demonstrated, through *ex vivo* experiments, that such a protein was produced either by the bacteria isolated in culture and also by the organisms colonizing the gastric epithelium: Gastric antral explants of patients with GC and other pathologies were cultured *in vitro* for a few days; the bacteria that colonized the mucosa kept on secreting this peptide, which could lastly be detected in the culture medium by using immunological methods[13]. In 1993, the same team established, for the first time, the existence of a relationship between infection by strains expressing the 120 kDa protein and GC development[14]. Their observations were important also because these researchers found anti-120 kDa protein mucosal IgA antibodies even in the absence of systemic IgG to this protein and, in some patients, also in cases with urease negative biopsies (false negatives). In the same year (1993), the gene encoding for the 120 kDa protein was cloned, sequenced and called *cagA* due to the strict association of protein expression with cytotoxin production[5]. As a result of these findings, the number of studies dealing with the characterisation of CagA and its potential carcinogenicity increased exponentially and results lead to the common conclusion that such peptide is a major factor in gastric carcinogenesis.

CagA is the product of the homonymous gene placed at the end of the so-called pathogenicity island (PAI) *cag*, a fragment of DNA encompassing an approximately 40 kb cluster of genes involved in virulence. In the field of bacteriology there are numerous examples of PAIs harboured by diverse bacterial species or their virulent variants, whether they are human (*Bordetella*

pertussis, *Escherichia coli, Salmonella enterica, etc.*) or plant pathogens (*Agrobacterium tumefaciens*). In some species, PAI genes cooperate to translate effectors (mainly proteins) endowed with carcinogenic potential inside colonised cells. In *H. pylori*, such determinant is CagA. Similarly, *A. tumefaciens* exploits the Type Ⅳ secretion system *vir* to translate a single-stranded form of T-region (T-strand) coated by the ssDNA-binding protein VirE2 (T-Complex) into the host's vegetal cell nuclei. Once inside the nucleus, the T-strand can be converted in a double-stranded form (T-DNA), whose expression causes an uncontrolled host cell proliferation and tumour development[15].

Epidemiological and genomic studies suggest that the development of GC is a possible consequence of infection by strains expressing CagA[1,8,9,14,16]. In effects, using Mongolian gerbils infected experimentally, it was shown that only CagA positive (CagA+) *H. pylori* strains were able to induce stomach tumours[17]. In addition, a study of our group revealed that, while virtually all patients with intestinal histotype of GC had serum antibodies to CagA, the prevalence of anti-CagA antibodies in patients with the diffuse GC variety was similar to that observed in infected controls without neoplasia[16]. These data were confirmed by the results of an epidemiological study: The overall GC risk in infected people lacking anti-CagA antibodies (CagA-) was increased, but in non-significant way; in any case, CagA- *H. pylori* infection was associated with the growth of the diffuse variety of GC (with an OR of 9.0)[8].

It therefore seems that infections by CagA+ strains expose people to an increased risk of GC respect to infections by CagA- *H. pylori* strains, which can only be associated with the diffuse histotype. In effect, things work slightly differently. In a recent study, we examined for the presence of *cagA* up to 25 distinct, well separated colonies per patient with GC; even though only individuals with diffuse histotype GC harboured *cagA* negative (*cagA*-) organisms, in all cases patients were also infected by at least one *cagA* positive (*cagA*+) strain[18]. These observations do not corroborate the supposed propensity of strains lacking *cag* PAI to concur to diffuse GC development and suggest that, for a better comprehension of the role played by *cagA* in the histological variety of GC, many colonies per patient should be examined genomically.

CagA phosphorylation by mucocytes: Like shooting oneself in the foot

H. pylori organisms expressing CagA differ in their carcinogenic potential. Let us have a look at the mechanisms that may influence the ability of such a protein to trigger and/or concur to GC formation. CagA, following colonisation, is translated into the gastric epithelial cells through a conjugative apparatus encoded by the *cag* PAI genes upstream *cagA*; then, a portion of intracellular CagA is phosphorylated by numerous kinases, members of the host cell Src family (such as Yes, Lyn, Fyn and c-Src,) at the EPIYA C'-terminal site (Glu-Pro-Ile-Tyr-Ala) motif of tyrosine[19]. Phosphorylated CagA physically interacts with the oncogenic tyrosine phosphatase SHP-2 (Src homology phosphatase 2), modifying cellular functions and altering mammalian signal transduction machineries. SHP-2, in fact, is implicated in the regulation of cell adhesion, spreading and migration. In this manner, phosphorylated CagA causes deregulation of SHP-2 and induces abnormal proliferation, as well as movement of cells of the gastric epithelial layer, activates mitogenic signalling and disturbs host-signalling routes[20]. All these events may also predispose cells to accumulate multiple genetic and epigenetic alterations involved in gastric tumorigenesis[21].

Unphosphorylated CagA, on the other hand, interacts with the tumour suppressor protein of p53 (ASPP2), which also exert an apoptosis-stimulating activity[22]. In normal conditions, following genotoxic and oncogenic stimuli, ASPP2 associates with tumour suppressor p53, activates it and induces apoptosis. After interaction with CagA, cytosolic p53 is recruited by ASPP2 and subsequently is degraded by an enzyme complex that control cell-cycle and apoptosis, the proteasome. As a consequence, the apoptotic response of host cells is inhibited. In other words, unphosphorylated CagA takes control of ASPP2 and subverts the tumour suppressor pathway of apoptosis-stimulating protein p53 with consequent promotion of cell survival and cell transformation[20-22]. The resultant abnormal proliferation of gastric epithelial cells may contribute to GC development.

Individual CagA proteins have different biological activity and tumorigenic potential: When size matters

H. pylori strains secreting CagA protein endowed with increased biological activity can be considered more virulent and even more closely associated with gastric cancer. At the end of 90's, the group of Graham discovered the ability of distinct CagA proteins to perturb cellular functions might vary in different isolates[23]. The CagA C'-terminal region contains one or more repeats of the same amino acids in sequence (Glu-Pro-Ile-Tyr-Ala, or EPIYA)[5]; the teleonomic significance of such phenomenon reflects the bacterial strategy to generate antigenic diversity, which may protect the organisms from the immune response. The number of EPIYA motifs correlates with the size of the *cagA* variable region. Yamaoka *et al*[23] basing of the amplicon sizes obtained with primers encompassing the entire *cagA* variable region, classified *H. pylori* isolates in *cagA* structural types A, B, C and D (amplicons characterising types B and D have the same size and can be differentiated by sequencing). Strains with the *cagA* structural type C have the highest number of EPIYA phosphorylation motifs and were isolated significantly more often from patients with GC[23], confirming a previous observation that individuals with GC are infected by strains expressing CagA proteins with higher mass[24]. The incr-

eased carcinogenic potential of the *cagA* structural type C was also confirmed by another study of the same group, which showed that patients infected by *H. pylori* with this *cagA* genotype run a higher risk of developing gastric mucosa atrophy, a precancerous condition[25].

Now, it should be highlighted that the primers used in these studies to amplify the *cagA* variable region were designed on oriental (Japanese) strains, which may differ from western strains in the nucleotide sequence encoding the C'-terminal variable region[26]. Probably for this reason not all surveys on the same subject have confirmed the Yamaoka *et al*[23,25]'s findings. In an initial study of our group, in which we used the Yamaoka's primers to amplify the *cagA* variable region of Italian strains[27], we sometime obtained amplicons shorter than the PCR product that characterises the *cagA* structural type A; strains producing such amplicons, which presented a deletion of about 100 bp respect to *cagA* type A, were named type A(I), with (I) standing for Italy, because, as far as we know, similar *cagA* structural variety had not previously been described. In a more recent investigation, we observed similar proportions of the various *cagA* structural types in Italian *H. pylori* organisms isolated from GC cases and from controls (patients without neoplasia, with chronic gastritis only)[28]. In addition, in the control subjects, we frequently detected strains with the *cagA* structural type A(I). The increased prevalence of such a *cagA* type in individuals without GC prompted us to hypothesise that the reduced dimensions of the encoded CagA may decrease the ability of these bacteria to trigger a neoplastic process. As a matter of fact, the capability of CagA of binding SHP-2, and therefore of disturbing the various cellular functions, is regulated by the amounts of tyrosine phosphorylation site sequences, *i.e.*, the CagA size[20]; therefore, the shorter the protein, the less numerous are the EPIYA repeats that undergo phosphorylation and the lower is the carcinogenic potential of strains.

The employment of primers proposed by Yamaoka *et al*[23] to amplify the *cagA* variable region of western strains has sometimes led to results similar to those of the Japanese researchers: South African researchers, for instance, have confirmed that patients with GC have an increased prevalence of strains with type C CagA[29]. Investigations dealing with this important subject, however, are not numerous; in addition, sometimes they attained different conclusions and have even contributed to create confusion in this subject. Malaysian authors, for instance, using the primers designed by Yamaoka *et al*[23], determined the presence and distribution of *cagA* variants among different ethnic groups and various gastroduodenal diseases[30]. They obtained three types of amplicons and named the *cagA* subtypes with capital letters, A, B and C (like did Yamaoka *et al*[23]), but, just to complicate this topic further, they used a different criterion (respect to that of the previous study) and called subtype C the strains with the smallest amplicon size and subtype B those with the greatest one. Specific *cagA* subtype A strains (those yielding amplicons of intermediate size) were predominantly isolated from Chinese compared to Malays and Indians patients. Since Chinese patients have the highest risk of GC disease respect to the other ethnic groups, these investigators concluded that *cagA* subtyping could be used as a clinical biomarker for severe outcome of infection. Such statement, however, was based on indirect observations because they did not examine strains from GC cases[30].

In 2002, Higashi *et al*[20] observed that the ability of CagA secreted by different strains to disturb host-cell functions can be influenced by the strength of SHP-2 binding activity, which was increased in *H. pylori* strains obtained from patients living in East Asian areas (Japan, Chorea and China) respect to those isolated from patients of Western countries (Europe, America, and Australia). In addition to the number of EPIYA repeats, it was also found that polymorphism in the nucleotide sequence flanking the regions that encode EPIYA could affect the potential of different *H. pylori* strains to promote gastric carcinogenesis[31]. According to the geographic regions in which strains are isolated, it is possible to characterise the *cagA* variable region on the basis of the number and the type of sequences. The Western *H. pylori* CagA has two segments of 32 and 40 amino acids flanking EPIYA (EPIYA-A and EPIYA-B types) and one to three 34 amino acid EPIYA-C segments (A-B-C type CagA)[21]. Eastern CagA presents EPIYA-A and EPIYA-B segments, but none of the EPIYA-C fragment; it has instead one copy alone of a segment called EPIYA-D, which represents the main tyrosine phosphorylation site[20,21]. In western strains, the major site of tyrosine phosphorylation of CagA is EPIYA-C; the tyrosine residues that characterize EPIYA-A and EPIYA-B segments are phosphorylated only very weakly. Such a difference in phosphorylation degrees resides in the diverse consensus high-affinity binding sequence for the SH2 domains of SHP-2. Unlike what happens for EPIYA-D type, the western EPIYA-C type of CagA differs by a single amino acid from the consensus SHP-2 binding sequence[21].

In conclusion, the carcinogenic potential of *H. pylori* varies according to the number and the *cagA* structural types of the EPIYA flanking regions. East Asian CagA has an increased virulence and a strong ability to trigger GC, while, among western isolates, more carcinogenic are the helicobacters with two or three CagA EPIYA-C sites. This was also demonstrated by a study in which it was observed that 83.3% of GC strains possessed multiple EPIYA-C sites, *vs* only 5.2% of strains isolated from patients with chronic gastritis only (controls)[32].

Often, *cagA*+ strains are also isolated from patients with duodenal ulcer (NF personal observation). This finding may create confusion, because is common knowledge that patients with duodenal ulcer are like protected from GC development; however, the results of a recent study[33] showed that the increased virulence

of strains with CagA EPIYA-C type augmented the risk of gastric cancer and not peptic ulceration. Some studies diverge from these conclusions: Findings of an investigation carried out in Colombia suggest that polymorphic CagA proteins, based on sequences flanking the EPIYA motifs, are not clearly associated with the outcome of the infection[34]. The absence of association between the CagA polymorphisms and pathogenesis of gastroduodenal diseases could be due to geographic factors and/or the host's genetic features and environmental determinants.

In conclusion, the results of this kind of investigations are potentially useful, but the confusion existing in this field ought to be rectified and the different researchers should use the same criteria for classification. The various groups, however, have reached a common conclusion: Not all the strains are alike in their carcinogenic potential.

NOT ALL PATIENTS ARE ALIKE: THE ROLE OF THE HOST'S INFLAMMATORY CYTOKINE HAPLOTYPES IN GC DEVELOPMENT

Background

The hypothesis that human genetic polymorphisms may affect predisposition to GC has recently been explored. GC develops in only 1%-3% of *H. pylori* infected individuals, which suggests that the host background matters in this neoplasia. Several pro-inflammatory cytokines are produced by the immune system against *H. pylori*; among them, IL-1β is of paramount importance; a second one is tumor necrosis factor-alpha (TNF-α); both of them are closely related to epithelial injury and gastric hypochlorhydria[35,36]. At low concentrations, TNF-α enhances the protective inflammatory response; at high concentrations, it can injure the gastric mucosa and cause severe pathology[37]. IL-1β increases the surface molecule expression on endothelial cells, causing leukocytes to adhere; IL-1β also induces the production of macrophage chemokines leading to neutrophil activation. Recent investigations have revealed that there is a genetic regulation of the host cytokine response to inflammatory stimuli. Genomic variants of *IL-1β* and *TNF-α* were shown to correlate with the clinical outcomes of tumors, including GC[38,39]. The *IL-1β* gene cluster is polymorphic, with some alleles present at relatively high frequencies. Particular *IL-1β* haplotypes enhance the risk of GC because they induce an over expression of its product in the stomach, causing chronic hypochlorhydria, which in turn may produce gastric atrophy and, eventually and in the presence of other risk factors, GC[40]. In addition, patients with a particular haplotype of the gene that encodes IL-RA (receptor antagonist) have an elevated risk of developing GC. IL-RA is an anti-inflammatory cytokine, which is a competitor for IL-1β receptors, thus regulating the possible harmful effects of IL-1β receptors.

The role of the host in GC development could be important because the inflammatory response to infections varies from patient to patient due to the gene polymorphism of inflammatory and anti-inflammatory cytokines. Many studies have been performed in the last 15 years, with the scope of identifying a genetic marker that can determine whether or not people carrying the infection might be at risk of developing GC in *H. pylori* infected patients[41-59]. Such a marker is still lacking, and we shall try to explain some of the reasons underlying this problem.

The association between chronic inflammation and cancer has been known since Virchow, in 1864, wrote that cancer would arise from sites of inflammation: "Chronic irritation which is manifested by a chronic inflammation is a key promoter of cancer" (Quoted by Balkwill and Mantovani[60]). Individual cytokines were specifically examined, in particular the proinflammatory ones. The most well known cytokine is IL-1, along with its receptor (IL-1R) and the antagonist of this receptor (IL-1RA); all of them share the chromosomal location of the *IL-1* gene family (namely 2q13-14). IL-1β has thus been established as an important regulator of carcinogenesis, characteristic of interactions between the host and environment[54].

IL-1β haplotypes

The *IL-1β* gene displays considerable polymorphism[54]; the presence of C to T transition was frequently found either in the promoter region at positions -511 (CT; dbSNP: rs16944), at position -31 (TC; dbSNP: rs1143627) or in the coding region at position +3954 (CT; dbSNP: rs1143634) base pairs from the origin of transcription. The two single nucleotide polymorphisms (SNPs) within promoter region are in linkage disequilibrium. The *IL-1β* -31 TC substitution disrupts a TATA-box motif; this leads to several transcription factors having altered binding affinities, resulting in modified IL-1β transcription. The *IL-1β* +3954 CT substitution is a synonymous SNP. It was demonstrated *in vitro* that the C to T transition at positions -511 and +3954 correlated with elevated IL-1β levels as a result of lipopolysaccharide (LPS)-stimulated IL-1β protein secretion[54].

The paradigm of all subsequent studies regarding GC with respect to different haplotypes in cytokines was the focus of the paper by El-Omar *et al*[38], who noted for the first time that the presence of two polymorphisms (rs16944 and rs1143627) in the promoter region of the *IL-1β* gene, identified an increased risk of hypochlorhydria, as a result of *H. pylori* infection and GC[38]. These polymorphisms determine an increased secretion of IL-1β[61], a conclusion confirmed and generalized by later reports[62]. The discrepancy of results reached in different papers was clarified only after several years, once taking into account the origin of the population and the type of GC. Overall, more than 90 publications dealt with this issue, over half of them from Asia, and a single one

from North America, a clear indication of the perceived relevance of this neoplasia in the different populations.

Since the single studies are extremely inconsistent and, if taken alone, contribute little to the general overview, we opted for reporting from selected meta-analyses in this paper. Meta-analyses accrued from time to time in a number of studies originating from different countries, which is one of the main causes of contradictory results, as well as from different histological variety of stomach malignancies, another cause of strong difference in results[41-59].

The overall findings from the large amount of efforts can be summarized as follows:

(1) *IL-1β receptor antagonist* (*IL-1β RA*) polymorphism: The most credible and consistent association of peculiar genetic variation with GC was found for *IL-1β RA* haplotypes. Four alleles, numbered 1 to 4, are widely present in the general populations. People carrying the homozygous allele 2/2 (*IL-1β RN2*) were found to be at higher risk of developing cancer among non-Asian populations. Moreover, the analysis of GC patients altogether, without stratifying according to histological type, anatomic site or country of origin, showed that patients carrying homozygous allele 2, or *IL-1 RN*2 had an increased risk of developing cancer, which was statistically significant. The risk was found both in cardia and non-cardia types of neoplasia. A possible explanation for the risk stems from the high IL-1β levels circulating among *IL-RA* allele 2/2 carriers[63].

(2) *IL-1β* -31 CT polymorphism: A second plausible association was the decreased risk of GC in Asians carrying the haplotypes in the *IL-1β* -31 CC promoter region. A decreased risk of GC among *IL-1β* -31C carriers was confirmed, but solely for Asian patients.

(3) *IL-1β* -511 CT polymorphism in populations of different ethnic origin: Sub-analysis of various populations revealed a statistically significant association of stomach cancer with the *IL-1β* polymorphism at promoter region -511 CT in case-control studies based on populations (OR = 1.20, 95%CI: 1.00-1.43)[54]. The association is more consistent if only Caucasian populations are analyzed. Nevertheless, if taken together, the studies failed to show the association when stratified by ethnicities; *IL-1β* -511 CT polymorphism according to tumor site: A significant association of *IL-1β* -511 CT promoter region polymorphism was observed for stomach cancers when the tumor site (cardia *vs* non-cardia) was taken into account, as well as for histology subtypes (intestinal or diffuse/mixed). The association was present both in the case of non-cardia GC (OR = 1.57, 95%CI: 1.06-2.31) as well as intestinal GC (respectively OR = 1.57, 95%CI: 1.06-2.31 and OR = 1.24, 95%CI: 1.04-1.49)[54]; *IL-1β* +3954 CT polymorphism: Recently, Xu *et al*[54] performed a meta-analysis that was confirmed by that one by Xue *et al*[49]: There is a lack of association between *IL-1β* +3954 CT and GC risk.

(4) *IL-10* haplotypes: IL-10 - regarded as the major anti-inflammatory cytokine - will bind in form of homodimer its complex receptor, comprising four IL-10 receptor molecules, namely 2 IL-10 R1 and 2 IL-10 R2. The binding induces STAT3 signaling *via* the phosphorylation of the cytoplasmic tails of IL-10 receptor 1. IL-10 can inhibit the synthesis of pro-inflammatory cytokines; moreover it can block the function of nuclear factor-kappa B (NF-κB), and has other regulatory properties, *e.g*., JAK-STAT signaling[64]. The *IL-10* gene is known to possess several SNPs, some in the distal region upstream of the coding gene (-1082 A/G, -819 T/C) and a proximal one (the -592 A/C). Again, the complex signaling and polymorphism of *IL-10* can explain the contradictory results of the investigations.

(5) *IL-10* -1082 AG polymorphism: A clear and curious dichotomy is evident, that is, when the studies were stratified according to Asian and non-Asian populations the observations reached opposite results. The Asian populations had greater risk of GC among *IL-10* -1082 G carriers; conversely, there was a decreased risk among the non-Asian populations. Meta-analysis specific for *IL-10* confirmed for Asian population the increased risk for intestinal type of gastric neoplasia in *IL-10* -1082 GG or GA haplotypes[53]; *IL-10* -592 AC polymorphism: The -592 AC polymorphism failed to show any association, as the odd ratios for GC were 0.93 and 0.94 for homozygous and heterozygous population[55,65]; *IL-10* -819 TC polymorphism: Little data is available for this polymorphism, confirming a protective effect in Asian populations. Nevertheless, it was not found to be associated with the reduced susceptibility to GC in individuals infected with *H. pylori* compared to uninfected controls. The *IL-10* -819 TT genotype was found to be inversely correlated with the risk of the diffuse subtype, but not the intestinal subtype GC[51].

(6) *IL-8* - 251 polymorphisms: Continuous expression of human IL-8 in transgenic mice (whereby IL-8 is under the control of its own regulatory elements) increased tumorigenesis. Therefore, IL-8 may play an important role in gastrointestinal cancers. Elevated IL-8 levels could be linked to a poor prognosis of neoplasia, henceforth its levels may be indicative of more aggressive GCs.

Early data seemed to provide a possible association in GC as well[66]. A recent meta-analysis showed that the *IL-8* -251 AA genotype in the Han population correlates with augmented risk of developing GC and AA genotype carriers appear to be more likely to develop GC in Asian populations. In addition, the *IL-8* -251 AA genotype tended to be related to intestinal GC, but not with *H. pylori* infectious status[52]. There was no link between *IL-8* polymorphisms and *H pylori*-related gastric malignancies in non-Asian populations in all the meta-analyses examined[48,67].

(7) *TNF-α* polymorphism: Experimental studies have implicated TNF-α in processes that are involved in cancer progression, including promotion of metastatic behaviour and cancer associated cachexia[68,69]. The lack of *TNF-α*

in mice makes them resistant to carcinogenesis[70]. Clearly, such observation highlighted the link between genetic haplotypes for *TNF-α* and GC.

TNF-α -308 AG polymorphism: It was surprising to find a lack of association of this polymorphism with increased risk of GC, with only one exception: Non-Asian patients with distal cancer and homozygous for -308 AA alleles; the association, moreover, appeared to exist for cancer of diffuse type only. However, this association was not confirmed when only good quality studies were taken into account, according to Persson *et al*[48]. Opposite conclusions were obtained by Zhu *et al*[59]; they recently analyzed all studies and concluded that, in the Caucasian populations, *TNF-α* rs1800629 (-308 AG) polymorphism indeed posed increased risk of GC. They used several genetic comparison models, *i.e.*, A *vs* G, AA *vs* GG and AA *vs* GG/GA that gave OR respectively of 1.32, 1.76 and 1.62, all highly significant (A *vs* G: OR = 1.32, 95%CI: 1.12-1.56, $P = 0.001$; AA *vs* GG: OR = 1.76, 95%CI: 1.37-2.26, $P < 0.001$; AA *vs* GG/GA: OR = 1.62, 95%CI: 1.27-2.07, $P < 0.001$)[59].

TNF-α -238 polymorphism did not correlate with an increased cancer risk[48,58].

TNF-α 857 CT polymorphism: Reports on this topic are quite controversial. Cen *et al*[57] recently published his analysis of nine studies (all the reported ones); overall, they confirm that the *TNF-α* 857 CT polymorphism posed an elevated risk of GC solely among Asians; all four genetic models considered T *vs* C, TT *vs* CC, CT *vs* CC and TT *vs* CT gave consistent data, respectively with OR of 1.19, 1.44, 1.19 and 1.21 (their statistical significance being $P = 0.002$, $P = 0.032$, $P = 0.008$, $P = 0.003$ respectively).

TNF-β 252 AG polymorphism: A weak association with stomach malignancy was present in Asian populations, according to Xu *et al*[71]. Analysis by ethnicity revealed that the *TNF-β* 252 AG polymorphism correlated with a minor risk of GC (G *vs* A: OR = 1.10, 95%CI: 1.02-1.19, $P = 0.015$) exclusively in Asians, not in Caucasians.

Dutch patients were analyzed for their polymorphism of IL-1β; they were found to carry lower risk of GC when heterozygous for either the IL-1B -511 and for the IL1β -31 TATA-box (genotype T/C)[72]. The EBV status of the patients did not affect this correlation and there could therefore be an early shared molecular mechanism in the progression of EBV-positive and negative GCs[72].

IL-6 polymorphism was not studied in relation to GC.

IL-6 knockout mice develop cancer less frequently[73]. It is therefore plausible that high IL-6 levels will promote tumorigenesis. Today, IL-6 is considered to be a relevant tumor-promoting factor also in humans. Indeed, it was correlated with glioma, lymphoma and melanoma at first, then with solid cancers such as breast and colorectal neoplasia (also ovarian and pancreatic), prostate, renal and colorectal cancers.

IL-6 is a critical factor during chronic inflammation, since it is required for the induction of effector Th17 cells and inhibits the differentiation of regulatory T cells.

Stomach cells, however, lack IL-6 receptors; hence it cannot dimerize with the second receptor (gp 130) and the "classic signaling" is restricted to cells bearing both mIL-6R and gp130 on their surface. The latter is widely expressed, whilst mIL-6R expression is limited to some leukocytes, hepatocytes and cancer cells. It is therefore quite understandable that, as recently reported, a large meta-analysis on 105000 people established the lack of association of cancer risk with *IL-6* polymorphism in Caucasians[74], despite the association which holds true for Africans.

Other cytokines

Gene polymorphism concerning cytokine different from those we have dealt with was recently considered in relation to the risk of developing GC. The studies are not sufficiently large so far; however it is worth reporting that a haplotypes of *IL-17*, *IL-17F* rs763780 TC, was significantly associated with GC development in Asian population[75].

IL-11 was taken into consideration in a single study[76]. A reduced risk for developing cancer at the gastric site was found for a polymorphism in the *IL-4* -590 CT gene in Caucasian but not in Asian populations. *H pylori* status was not taken into consideration in these studies[77].

Different results in different studies: The origin of the problem

IL-1β, TNF-α and the remaining dozens of cytokines are not the final executor of immune signaling or the resulting consequences in cancer promotion and spread. IL-1, TNF-α, together with bacterial antigens, LPS and several other signaling molecules bind their respective receptors on the cell membrane; a cascade of signals ensues upon receptor activation, which depends on the levels of Mg-ATP availability (in turn on Mg^{2+} concentration in cells). Numerous different proteins are involved and regulate the signaling pathway, which finally results in the activation of a large family of DNA-binding proteins, the NF-κB family[78], which is a complex that regulates DNA transcription. NF-κB dimers are formed upon activation, stimulating the transcription of genes that encode cytokines, growth factors, chemokines, and anti-apoptotic factors[79]. However, some NF-κB dimers act by repressing, whilst others activate specific genes.

Cytokine polymorphism and Epstein-Barr virus-associated GC

Worldwide, it was noted that Epstein-Barr virus (EBV) is present in a relevant proportion of malignant tumors of the stomach, with an incidence that is inversely proportional to that of GC. In the USA 16% to 18% of all stomach tumors were found EBV-associated (EBVaGC), in Southern China only 4.3%[80]; a survey of 101 published papers reported that EBVaGC was evident in 7.08% of intestinal type GC, while diffuse type GC had

an incidence of 9.82%[80]. Western and Central Asian countries had significantly more EBV positive cases than South-Eastern countries; in Europe, the frequency of EBV infection ranged from 1.7% in the United Kingdom to 40% in Poland[80].

An *in vitro* model of EBVaGC was used to demonstrate that gastric cells, following EBV infection, have a high IL-1β expression, compared to EBV-negative gastric tumour cells. EBV-positive clones rapidly proliferated and were shown to be anchorage-independent in colony-forming assays[81].

Since EBV infection is highly prevalent in all populations, whilst EBVaGC is quite rare, there were attempts to identify people who run an increased risk of developing GC. Polymorphisms of proinflammatory, as well as anti-inflammatory cytokines were studied, in particular in the promoter regions of *IL-10* and *TNF-α*. For the latter, the allele -308 A (linked to high levels of TNF-α) had significantly higher frequency among EBVaGC individuals (23.3%) when compared to control subjects (12.0%, $P < 0.05$). The opposite was found in the case of the anti-inflammatory *IL-10*: The high-producer allele (-1082 G) was found to be less frequent in EBVaGC patients in comparison to controls (6.3% *vs* 3.0%, $P < 0.05$)[39,72].

The extreme complexities of all these interactions can explain the great variability in data when investigating the possible correlation between cytokine haplotypes and GC.

Gleanings on the usefulness of characterising H. pylori infected individuals for inflammatory haplotype

In addition to the complexity of this subject, the expectations created by the assertion that the host's factors could contribute to the development of GC are disappointing, at least as far as the host's inflammatory response to *H. pylori* infection is concerned. Once we get into details, we realize that in fact, the only determinant that really matters is the infection. The examination of the scientific literature on the cytokine subject has led to contradictory results: For each cytokine, the observations made by studying Caucasian people cannot be applied tout course to Asians; in certain cases, we get opposite results. In the different surveys, one can find association of determined haplotypes of inflammatory cytokines with an increased risk of GC, the opposite, or nil. Even the results of meta-analyses do not agree one another, according to whether studies are carried out by Chines or researchers from other nations. What does it mean? Is it because cytokines are not the final effectors, as they principally work on the long and winding road paved by the broad NF-κB family, which leads to GC (which means that the final response to inflammatory stimuli is far from hitting its target)? And what about the observations that people suffering from diseases far more inflammatory than chronic gastritis, such as rheumatoid arthritis, are likely protected from developing GC[82].

These observations may suggest that, if host factors are important in GC development, they probably have to be sought outside of the genes encoding the inflammatory cytokines.

NOT ALL PATIENTS ARE ALIKE: MOLECULAR BIOLOGY

The recent advances of molecular biological techniques allowed researchers to reach important insights into the oncogenesis mechanisms in gastric cancer. Besides the well-known pathogenic factor, *H. pylori*, several oncogenes and tumour suppressor genes, including cell cycle regulation genes involved in the growth and signal transduction pathways, have been identified[83-85]. In particular, alterations of genes involved in signalling pathways deregulation, patterns of aberrant DNA methylation, and chromosomal imbalances have been evidenced[86,87].

CHROMOSOMAL INSTABILITY

Chromosomal instability (CIN) represents one of the main type of genomic instability observed in several neoplasms and it has been observed in a large cohort of patients with gastric cancer[88]. In particular, it is commonly detected in gastric malignant tumours and has been shown in up to 84% of gastrointestinal cancers[89].

CIN is characterized by chromosomal anomalies, including gain or loss of the complete chromosome (aneuploidy) and segments of chromosomes (loss of heterozygosity, amplifications and translocations)[90]. These abnormalities can impact on the oncogenes expression, tumour suppressor genes and other genes, as well as those involved in digestion, DNA repair, growth regulation, and control of cell cycle checkpoint[91-93]. The genetic mechanisms leading to CIN are not entirely known; *H. pylori* infection, smoking habit and some chemical substances such as nitrates and nitrites probably have an effect on inducing CIN; anyway their influence is actually uncertain[94]. On the other side, defects of chromosome segregation (CS), imperfect DNA damage response (DDR), anomalies in cell cycle regulators and telomere dysfunction have been identified as factors leading to numerical and structural chromosome alterations[95,96]. These carcinogens may alter chromosomes and the cytoskeleton promoting malignant modification[97].

CS alterations

CS represents an important cellular process inducing the gastric epithelial cells division. Alterations of CS regulating mechanisms can cause DNA alterations or mitotic failures, leading to unfixable mutations as well as chromosomal number alterations[98]. In particular, the three recently proposed ways producing CIN are: Altered expression, polymorphisms and/or mutations of mitotic genes implicated in CS and the carcinogen activity upon

susceptible genetic background of individuals[99,100]. Many authors showed an aberrant expression of mitotic genes in CS. Moreover, the altered expression of BUB1 protein (involved in controlling the spindle assembly checkpoint), was significantly increased in patients with diffuse type gastric adenocarcinoma, but not related to DNA ploidy[89]. Furthermore, in another study, BubR1 and AURKB (proteins involved in the mitotic spindle assembly) expression resulted in association with a low risk of GC progression[101-103]. Aurora kinase A (AURKA/STK15), a cell-cycle-regulated kinase with important role in microtubule formation and stabilization during CS, is often overexpressed in adenocarcinomas of the stomach, showing a suggestive new oncogenic pathway in GC[104].

Defective DDR

The mucosa of the stomach is continually subject to several environmental and intracellular mutagens, like ROS, *H. pylori* infection, nitrates, natrium, nitrites, and other water and food contaminants, able to induce DNA damage through different mechanisms[105,106]. Failure of the most important mechanisms of repair [nucleotide excision repair, base excision repair, mismatch repair (MMR) and recombination and/or DDR] may conduce to CIN and genetic aberrations, favouring carcinogenic process[107,108]. Several studies revealed differential mRNA expression of genes implicated in DNA repair process: *ATM* and *HMGB1* (implicated in base excision repair), *RAD23B* (involved in nucleotide excision repair), *UBE2V2*, *MUS81* [involved in resolving Holliday junctions (a branched DNA structure that contains four double-stranded arms joined together, considered the central intermediate in homologous recombination)], *REV3L* (involved in replication post-DNA damage), *TP53, hHR23A* and *DDB1* (implicated in nucleotide excision repair), and *XRCC1* (implicated in single-strand breaks repair) and *MUTYH* (implicated in base excision repair)[109-113].

H. pylori

H. pylori has been shown to be able to induce DDR and double-strand breaks in gastric cancer with a mechanism of adhesion of bacteria that takes place between Lewis epitopes of the host and BabA adhesin[114]. Anyway, gastric mucosa cells can repair the DNA lesions induced by short-term infections. On the other side, prolonged infections induce saturation of repair mechanisms with a consequent ineffective DNA repair and malignant process begin. Moreover, continued infections lead to chronic inflammation, with resulting increase of mutagenesis rate, oxidative-stress, down-regulation of MMR mechanisms, instability of genes and modulation of apoptosis by means of ROS formation[115-119]. Gastric inflammation represents an important host response able to induce *H. pylori*-related carcinogenesis[120]. In fact, in infected patients with *IL-1β*, *TNF-α*, *IL-10* and *IL-8* polymorphisms, has been observed an increased risk of distal gastric cancer progression[120,121]. Furthermore, different *H. pylori* strains seem to differently increase cancer risk by means of host genotypes[122] as these bacteria are able to communicate with their hosts. The equilibrium is determined both by host and bacterial features and may explain the reason why some *H. pylori* strains augment the carcinogenesis risk. For example, CagA positive strains promote severe gastritis and increase the pro-inflammatory cytokines' level. This may lead to an environment favourable to the growth of other bacteria that can support inflammation and continually induce oxidative stress, increasing the risk for GC[1].

MICROSATELLITE INSTABILITY

Microsatellite instability (MSI) represents a genomic instability commonly detected in almost half of patients with GC. It is often observed in the Lynch syndrome (hereditary non-polyposis colorectal cancer) and in several sporadic cancers[123]. MSI phenotype is characterized by a high replication mistake rate leading to insertions and/or deletions of nucleotides within microsatellite repeats in neoplastic areas[123]. The MMR proteins are able to detect and repair these alterations, causing the dysfunction in *MMR* genes (*MLH1* and *MSH2*) a MSI phenotype's establishment, with a consequently power off of cancer suppressor genes' and loss of heterozygosity[124,125]. To this address, genes that are frequently modified induce cell cycle regulation and apoptosis (*TGFβ RII, RIZ, IGFIIR, TCF4, BAX, FAS, CASPASE5, BCL10* and *APAF1*) or are involved in the maintenance of genomic integrity (*MSH6, MED1, MSH3, BLM, RAD50, ATR*, and *MRE11*)[126].

DEREGULATION OF SIGNALLING TRASDUCTION PATHWAYS

The effects of genomic destabilization consist of aneuploidy and gain or loss of the chromosome tracts involved in mRNA transcription. Genomic alterations can modify the normal cellular biology with a consequent neoplastic switch[127]. The clearly explored pathways that probably are involved in gastric pathogenesis are Wnt/betacatenin, extracellular signal-regulated MAPK, Hedgehog, Notch, NF-κB, TGF-β/BMP pathways, COX2/PGE_2, and tyrosine kinase signalling[128-143].

Finally, several studies evidenced that pathway deregulation involved in systemic inflammatory response, such as IL-11/STAT1/gp130/STAT3, can induce a carcinogenic transformation too[144,145].

CONCLUSION

GC is a multifactorial disease. The main determinant, *H. pylori* infection, can be considered a *sine qua non* for GC development; however, despite almost all individuals who get GC are currently, or have been infected, it

is neither a necessary nor a sufficient condition. The intricacy of this topic resides in the proportion of infected people that will never get GC: 97% to 99%, according to the ethnic groups and geographic areas. Other remarks are unraveling the tangle: Almost all *H. pylori* strains from Japan and East Asia, the areas with the highest incidence of GC, are *cagA*+ and can be considered highly carcinogenic; in addition, the infection by certain *cagA* genotypes in western countries increases by far the risk of GC.

At this point, one may wonder why these strains keep on infecting people. Following along with the evolution, only the characteristics that provide a selective advantage continue to be transmitted; this is a basic rule in eukaryotic and prokaryotic worlds. What is the benefit of being infected by carcinogenic strains? Why do they not disappear? People infected by strains that multiply the risk of GC by many times over, cannot be considered advantaged. Possible answers could reside in the following observations: (1) 97% to 99% of people never acquire GC; (2) the development of the sequence gastritis-metaplasia-dysplasia-cancer takes 40 years, or more, after the infection; which means that all women, as well as men, are fertile before the age at which GC occurs (many men also in old age, but they are less important, statistically speaking); hence, fertility is not affected by cancer development; and (3) women, who develop GC far less frequently than men, are the necessary genetic traits holders. Could these answers satisfy the laws of evolution (or distract them)? And how can the occurrence of GC in younger and younger ages be explained?

Apart from the complexity of this subject, the prospect created by the assertion that the host's factors, such as the way the host reacts to infectious stimuli, may be important in the development of GC is discouraging. Despite cytokines involved in the inflammatory response to infection, there are more than 30, just over half a dozen that have been examined in the relationship of *H. pylori* infection with GC and only haplotypes of *IL-1* and *TNF-α* genes were found to possibly increase the GC risk, but only if the ethnicity of patients is not considered. Pursuing this line of inquiry makes us run the risk of sounding racist.

The hypothesis that the dissection of oncogenes and tumour suppressing genes could provide us with an answer to the question whether host factors are important in GC development has only been partly proved. However, the conclusions have led us to a starting point, that is, they ended to indirectly confirm the pathogenic role of strains expressing CagA. The local and systemic levels of substances endowed with an increased mutagenic potential, ROS, generated by immunocytes, and the consequent DNA damage are far higher when the infecting organisms harbour the *cag* PAI.

In conclusion, as regards the development of GC, not all the *H. pylori* strains and patients are alike and not all share the same responsibility, but the only determinant that really matters is the infection.

REFERENCES

1 **Peek RM**, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. *Nat Rev Cancer* 2002; **2**: 28-37 [PMID: 11902583 DOI: 10.1038/nrc703]

2 **International Agency for Research on Cancer**. IARC monographs on the evaluation of the carcinogenic risks to humans. Schistosomes, liver flukes and Helicobacter pylori. Lyon: International Agency for Research on Cancer, 1994; **61**: 177

3 **Odenbreit S**, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Science* 2000; **287**: 1497-1500 [PMID: 10688800 DOI: 10.1126/science.287.5457.1497]

4 **Wang X**, Wei M, Sun Z. An association study of histological types of gastric carcinoma with Helicobacter pylori infection. *Cell Biochem Biophys* 2014; **70**: 1283-1287 [PMID: 24898806 DOI: 10.1007/s12013-014-0052-z]

5 **Covacci A**, Censini S, Bugnoli M, Petracca R, Burroni D, Macchia G, Massone A, Papini E, Xiang Z, Figura N, Rappuoli R. Molecular characterization of the 128-kDa immunodominant antigen of Helicobacter pylori associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993; **90**: 5791-5795 [PMID: 8516329 DOI: 10.1073/pnas.90.12.5791]

6 **Censini S**, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA* 1996; **93**: 14648-14653 [PMID: 8962108 DOI: 10.1073/pnas.93.25.14648]

7 **Blaser MJ**, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, Stemmermann GN, Nomura A. Infection with Helicobacter pylori strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995; **55**: 2111-2115 [PMID: 7743510]

8 **Parsonnet J**, Friedman GD, Orentreich N, Vogelman H. Risk for gastric cancer in people with CagA positive or CagA negative Helicobacter pylori infection. *Gut* 1997; **40**: 297-301 [PMID: 9135515 DOI: 10.1136/gut.40.3.297]

9 **Enroth H**, Kraaz W, Engstrand L, Nyrén O, Rohan T. Helicobacter pylori strain types and risk of gastric cancer: a case-control study. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 981-985 [PMID: 11008919]

10 **Leunk RD**, Johnson PT, David BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. *J Med Microbiol* 1988; **26**: 93-99 [PMID: 3385767 DOI: 10.1099/00222615-26-2-93]

11 **Figura N**, Guglielmetti P, Rossolini A, Barberi A, Cusi G, Musmanno RA, Russi M, Quaranta S. Cytotoxin production by Campylobacter pylori strains isolated from patients with peptic ulcers and from patients with chronic gastritis only. *J Clin Microbiol* 1989; **27**: 225-226 [PMID: 2913034]

12 **Figura N**, Bugnoli M, Cusi MG, Pucci AM, Lusini P, Quaranta S, Barberi A, Rossolini A, Di Tommaso A, De Magistris T, Rappuoli R, Marri L, Musmanno RA, Russi M, Guarna M, Losi M. Pathogenic mechanisms of Helicobacter pylori: production of cytotoxin. In: Malfertheiner P, Ditschuneit H. Helicobacter pylori, Gastritis and Peptic Ulcer. Berlin Heidelberg Springer Verlag, 1990: 86-95 [DOI: 10.1007/978-3-642-75315-2_13]

13 **Crabtree JE**, Figura N, Taylor JD, Bugnoli M, Armellini D, Tompkins DS. Expression of 120 kilodalton protein and cytotoxicity in Helicobacter pylori. *J Clin Pathol* 1992; **45**: 733-734 [PMID: 1401190 DOI: 10.1136/jcp.45.8.733]

14 **Crabtree JE**, Wyatt JI, Sobala GM, Miller G, Tompkins DS, Primrose JN, Morgan AG. Systemic and mucosal humoral responses to Helicobacter pylori in gastric cancer. *Gut* 1993; **34**: 1339-1343 [PMID: 8244098 DOI: 10.1136/gut.34.10.1339]

15 **Souza RC**, del Rosario Quispe Saji G, Costa MO, Netto DS, Lima NC, Klein CC, Vasconcelos AT, Nicolás MF. AtlasT4SS: a curated

database for type IV secretion systems. *BMC Microbiol* 2012; **12**: 172 [PMID: 22876890 DOI: 10.1186/1471-2180-12-172]
16 **Figura N**, Vindigni C, Pinto E, Gennari C, Presenti L, Tosi P, Roviello F. Gastric cancer, H. pylori (HP) infection, serum antibodies to CagA. *Gut* 1996; **39** suppl 2; A17
17 **Ogura K**, Maeda S, Nakao M, Watanabe T, Tada M, Kyutoku T, Yoshida H, Shiratori Y, Omata M. Virulence factors of Helicobacter pylori responsible for gastric diseases in Mongolian gerbil. *J Exp Med* 2000; **192**: 1601-1610 [PMID: 11104802 DOI: 10.1084/jem.192.11.1601]
18 **Figura N**, Valassina M, Moretti E, Vindigni C, Collodel G, Iacoponi F, Giordano N, Roviello F, Marrelli D. Histological variety of gastric carcinoma and Helicobacter pylori cagA and vacA polymorphism. *Eur J Gastroenterol Hepatol* 2015; **27**: 1017-1021 [PMID: 26067222 DOI: 10.1097/MEG.0000000000000414]
19 **Higashi H**, Tsutsumi R, Fujita A, Yamazaki S, Asaka M, Azuma T, Hatakeyama M. Biological activity of the Helicobacter pylori virulence factor CagA is determined by variation in the tyrosine phosphorylation sites. *Proc Natl Acad Sci USA* 2002; **99**: 14428-14433 [PMID: 12391297 DOI: 10.1073/pnas.222375399]
20 **Higashi H**, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. *Science* 2002; **295**: 683-686 [PMID: 11743164 DOI: 10.1126/science.1067147]
21 **Hatakeyama M**, Higashi H. Helicobacter pylori CagA: a new paradigm for bacterial carcinogenesis. *Cancer Sci* 2005; **96**: 835-843 [PMID: 16367902 DOI: 10.1111/j.1349-7006.2005.00130.x]
22 **Buti L**, Spooner E, Van der Veen AG, Rappuoli R, Covacci A, Ploegh HL. Helicobacter pylori cytotoxin-associated gene A (CagA) subverts the apoptosis-stimulating protein of p53 (ASPP2) tumor suppressor pathway of the host. *Proc Natl Acad Sci USA* 2011; **108**: 9238-9243 [PMID: 21562218 DOI: 10.1073/pnas.1106200108]
23 **Yamaoka Y**, Kodama T, Kashima K, Graham DY, Sepulveda AR. Variants of the 3' region of the cagA gene in Helicobacter pylori isolates from patients with different H. pylori-associated diseases. *J Clin Microbiol* 1998; **36**: 2258-2263 [PMID: 9666002]
24 **Sepulveda AR**, Dore MP, Gutierrez O, Miehlke S, Go MF, Kim JG, Figura N, Graham DY. Helicobacter pylori CagA proteins of higher size are associated with gastric carcinoma. *Gut* 1997; **41** suppl 1: A112
25 **Yamaoka Y**, El-Zimaity HM, Gutierrez O, Figura N, Kim JG, Kodama T, Kashima K, Graham DY. Relationship between the cagA 3' repeat region of Helicobacter pylori, gastric histology, and susceptibility to low pH. *Gastroenterology* 1999; **117**: 342-349 [PMID: 10419915 DOI: 10.1053/gast.1999.0029900342]
26 **Miura M**, Ohnishi N, Tanaka S, Yanagiya K, Hatakeyama M. Differential oncogenic potential of geographically distinct Helicobacter pylori CagA isoforms in mice. *Int J Cancer* 2009; **125**: 2497-2504 [PMID: 19588494 DOI: 10.1002/ijc.24740]
27 **Figura N**, Valassina M, Roviello F, Pinto F, Lenzi C, Giannace R, Marrelli D, Valentini M, Valensin PE. Helicobacter pylori cagA and vacA types and gastric carcinoma. *Dig Liver Dis* 2000; **32** Suppl 3: S182-S183 [PMID: 11245289 DOI: 10.1016/S1590-8658(00)80272-8]
28 **Figura N**, Moretti E, Roviello F, Papini F, Marrelli D. cagA structural types of Helicobacter pylori strains isolated from patients with gastric carcinoma and chronic gastritis only. *Intern Emerg Med* 2012; **7** Suppl 2: S103-S105 [PMID: 22311514 DOI: 10.1007/s11739-012-0759-z]
29 **Kidd M**, Lastovica AJ, Atherton JC, Louw JA. Heterogeneity in the Helicobacter pylori vacA and cagA genes: association with gastroduodenal disease in South Africa? *Gut* 1999; **45**: 499-502 [PMID: 10486355 DOI: 10.1136/gut.45.4.499]
30 **Ramelah M**, Aminuddin A, Alfizah H, Isa MR, Jasmi AY, Tan HJ, Rahman AJ, Rizal AM, Mazlam MZ. cagA gene variants in Malaysian Helicobacter pylori strains isolated from patients of different ethnic groups. *FEMS Immunol Med Microbiol* 2005; **44**: 239-242 [PMID: 15866222 DOI: 10.1016/j.femsim.2005.02.001]
31 **Hatakeyama M**. Oncogenic mechanisms of the Helicobacter pylori CagA protein. *Nat Rev Cancer* 2004; **4**: 688-694 [PMID: 15343275 DOI: 10.1038/nrc1433]
32 **Argent RH**, Kidd M, Owen RJ, Thomas RJ, Limb MC, Atherton JC. Determinants and consequences of different levels of CagA phosphorylation for clinical isolates of Helicobacter pylori. *Gastroenterology* 2004; **127**: 514-523 [PMID: 15300584 DOI: 10.1053/j.gastro.2004.06.006]
33 **Batista SA**, Rocha GA, Rocha AM, Saraiva IE, Cabral MM, Oliveira RC, Queiroz DM. Higher number of Helicobacter pylori CagA EPIYA C phosphorylation sites increases the risk of gastric cancer, but not duodenal ulcer. *BMC Microbiol* 2011; **11**: 61 [PMID: 21435255 DOI: 10.1186/1471-2180-11-61]
34 **Acosta N**, Quiroga A, Delgado P, Bravo MM, Jaramillo C. Helicobacter pylori CagA protein polymorphisms and their lack of association with pathogenesis. *World J Gastroenterol* 2010; **16**: 3936-3943 [PMID: 20712055 DOI: 10.3748/wjg.v16.i31.3936]
35 **Crabtree JE**. Gastric mucosal inflammatory responses to Helicobacter pylori. *Aliment Pharmacol Ther* 1996; **10** Suppl 1: 29-37 [PMID: 8730257 DOI: 10.1046/j.1365-2036.1996.22164003.x]
36 **Beales IL**, Calam J. Interleukin 1 beta and tumour necrosis factor alpha inhibit acid secretion in cultured rabbit parietal cells by multiple pathways. *Gut* 1998; **42**: 227-234 [PMID: 9536948]
37 **Strieter RM**, Kunkel SL, Bone RC. Role of tumor necrosis factor-alpha in disease states and inflammation. *Crit Care Med* 1993; **21**: S447-S463 [PMID: 8403983]
38 **El-Omar EM**, Carrington M, Chow WH, McColl KE, Bream JH, Young HA, Herrera J, Lissowska J, Yuan CC, Rothman N, Lanyon G, Martin M, Fraumeni JF, Rabkin CS. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. *Nature* 2000; **404**: 398-402 [PMID: 10746728 DOI: 10.1038/35006081]
39 **Wu MS**, Huang SP, Chang YT, Shun CT, Chang MC, Lin MT, Wang HP, Lin JT. Tumor necrosis factor-alpha and interleukin-10 promoter polymorphisms in Epstein-Barr virus-associated gastric carcinoma. *J Infect Dis* 2002; **185**: 106-109 [PMID: 11756988 DOI: 10.1086/324771]
40 **Machado JC**, Figueiredo C, Canedo P, Pharoah P, Carvalho R, Nabais S, Castro Alves C, Campos ML, Van Doorn LJ, Caldas C, Seruca R, Carneiro F, Sobrinho-Simões M. A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. *Gastroenterology* 2003; **125**: 364-371 [PMID: 12891537 DOI: 10.1016/S0016-5085(03)00899-0]
41 **Hamajima N**, Naito M, Kondo T, Goto Y. Genetic factors involved in the development of Helicobacter pylori-related gastric cancer. *Cancer Sci* 2006; **97**: 1129-1138 [PMID: 16879717 DOI: 10.1111/j.1349-7006.2006.00290.x]
42 **Kamangar F**, Cheng C, Abnet CC, Rabkin CS. Interleukin-1B polymorphisms and gastric cancer risk--a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1920-1928 [PMID: 17035400 DOI: 10.1158/1055-9965.EPI-06-0267]
43 **Camargo MC**, Mera R, Correa P, Peek RM, Fontham ET, Goodman KJ, Piazuelo MB, Sicinschi L, Zabaleta J, Schneider BG. Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1674-1687 [PMID: 16985030 DOI: 10.1158/1055-9965.EPI-06-0189]
44 **Wang P**, Xia HH, Zhang JY, Dai LP, Xu XQ, Wang KJ. Association of interleukin-1 gene polymorphisms with gastric cancer: a meta-analysis. *Int J Cancer* 2007; **120**: 552-562 [PMID: 17096351 DOI: 10.1002/ijc.22353]
45 **Gorouhi F**, Islami F, Bahrami H, Kamangar F. Tumour-necrosis factor-A polymorphisms and gastric cancer risk: a meta-analysis. *Br J Cancer* 2008; **98**: 1443-1451 [PMID: 18319718 DOI: 10.1038/sj.bjc.6604277]
46 **Vincenzi B**, Patti G, Galluzzo S, Pantano F, Venditti O, Santini D, Ruzzo A, Schiavon G, Caraglia M, Marra M, Graziano F, Tonini G. Interleukin 1beta-511T gene (IL1beta) polymorphism is correlated with gastric cancer in the Caucasian population: results from a

meta-analysis. *Oncol Rep* 2008; **20**: 1213-1220 [PMID: 18949424 DOI: 10.3892/or_00000132]
47 **Peleteiro B**, Lunet N, Carrilho C, Durães C, Machado JC, La Vecchia C, Barros H. Association between cytokine gene polymorphisms and gastric precancerous lesions: systematic review and meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 762-776 [PMID: 20200422 DOI: 10.1158/1055-9965.EPI-09-0917]
48 **Persson C**, Canedo P, Machado JC, El-Omar EM, Forman D. Polymorphisms in inflammatory response genes and their association with gastric cancer: A HuGE systematic review and meta-analyses. *Am J Epidemiol* 2011; **173**: 259-270 [PMID: 21178102 DOI: 10.1093/aje/kwq370]
49 **Xue H**, Lin B, Ni P, Xu H, Huang G. Interleukin-1B and interleukin-1 RN polymorphisms and gastric carcinoma risk: a meta-analysis. *J Gastroenterol Hepatol* 2010; **25**: 1604-1617 [PMID: 20880168 DOI: 10.1111/j.1440-1746.2010.06428.x]
50 **He B**, Zhang Y, Pan Y, Xu Y, Gu L, Chen L, Wang S. Interleukin 1 beta (IL1B) promoter polymorphism and cancer risk: evidence from 47 published studies. *Mutagenesis* 2011; **26**: 637-642 [PMID: 21653279 DOI: 10.1093/mutage/ger025]
51 **Xue H**, Lin B, An J, Zhu Y, Huang G. Interleukin-10-819 promoter polymorphism in association with gastric cancer risk. *BMC Cancer* 2012; **12**: 102 [PMID: 22436502 DOI: 10.1186/1471-2407-12-102]
52 **Xue H**, Liu J, Lin B, Wang Z, Sun J, Huang G. A meta-analysis of interleukin-8 -251 promoter polymorphism associated with gastric cancer risk. *PLoS One* 2012; **7**: e28083 [PMID: 22279522 DOI: 10.1371/journal.pone.0028083]
53 **Ni P**, Xu H, Xue H, Lin B, Lu Y. A meta-analysis of interleukin-10-1082 promoter polymorphism associated with gastric cancer risk. *DNA Cell Biol* 2012; **31**: 582-591 [PMID: 22335769 DOI: 10.1089/dna.2011.1440]
54 **Xu J**, Yin Z, Cao S, Gao W, Liu L, Yin Y, Liu P, Shu Y. Systematic review and meta-analysis on the association between IL-1B polymorphisms and cancer risk. *PLoS One* 2013; **8**: e63654 [PMID: 23704929 DOI: 10.1371/journal.pone.0063654]
55 **Pan XF**, Yang SJ, Loh M, Xie Y, Wen YY, Tian Z, Huang H, Lan H, Chen F, Soong R, Yang CX. Interleukin-10 gene promoter polymorphisms and risk of gastric cancer in a Chinese population: single nucleotide and haplotype analyses. *Asian Pac J Cancer Prev* 2013; **14**: 2577-2582 [PMID: 23725178]
56 **Rokkas T**. Answer to Professor Kountouras's letter. *Eur J Gastroenterol Hepatol* 2014; **26**: 123-124 [PMID: 24280805 DOI: 10.1097/MEG.0b013e3283657e0f]
57 **Cen G**, Wu W. Association between tumor necrosis factor-alpha 857C/T polymorphism and gastric cancer: a meta-analysis. *Tumour Biol* 2013; **34**: 3383-3388 [PMID: 23821300 DOI: 10.1007/s13277-013-0910-0]
58 **Yu JY**, Li L, Ma H, Liu K, Cheng X, Li YL, Song XL. Tumor necrosis factor-α 238 G/A polymorphism and gastric cancer risk: a meta-analysis. *Tumour Biol* 2013; **34**: 3859-3863 [PMID: 23900678 DOI: 10.1007/s13277-013-0972-z]
59 **Zhu F**, Zhao H, Tian X, Meng X. Association between tumor necrosis factor-α rs1800629 polymorphism and risk of gastric cancer: a meta-analysis. *Tumour Biol* 2014; **35**: 1799-1803 [PMID: 24142527 DOI: 10.1007/s13277-013-1240-y]
60 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545 [PMID: 11229684 DOI: 10.1016/S0140-6736(00)04046-0]
61 **Pociot F**, Mølvig J, Wogensen L, Worsaae H, Nerup J. A TaqI polymorphism in the human interleukin-1 beta (IL-1 beta) gene correlates with IL-1 beta secretion in vitro. *Eur J Clin Invest* 1992; **22**: 396-402 [PMID: 1353022 DOI: 10.1111/j.1365-2362.1992.tb01480.x]
62 **Chen H**, Wilkins LM, Aziz N, Cannings C, Wyllie DH, Bingle C, Rogus J, Beck JD, Offenbacher S, Cork MJ, Rafie-Kolpin M, Hsieh CM, Kornman KS, Duff GW. Single nucleotide polymorphisms in the human interleukin-1B gene affect transcription according to haplotype context. *Hum Mol Genet* 2006; **15**: 519-529 [PMID: 16399797 DOI: 10.1093/hmg/ddi469]
63 **Santtila S**, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. *Scand J Immunol* 1998; **47**: 195-198 [PMID: 9519856 DOI: 10.1046/j.1365-3083.1998.00300.x]
64 **Mosser DM**, Zhang X. Interleukin-10: new perspectives on an old cytokine. *Immunol Rev* 2008; **226**: 205-218 [PMID: 19161426 DOI: 10.1111/j.1600-065X.2008.00706.x]
65 **Loh M**, Koh KX, Yeo BH, Song CM, Chia KS, Zhu F, Yeoh KG, Hill J, Iacopetta B, Soong R. Meta-analysis of genetic polymorphisms and gastric cancer risk: variability in associations according to race. *Eur J Cancer* 2009; **45**: 2562-2568 [PMID: 19375306 DOI: 10.1016/j.ejca.2009.03.017]
66 **Taguchi A**, Ohmiya N, Shirai K, Mabuchi N, Itoh A, Hirooka Y, Niwa Y, Goto H. Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 2487-2493 [PMID: 16284368 DOI: 10.1158/1055-9965.epi-05-0326]
67 **Liu L**, Zhuang W, Wang C, Chen Z, Wu XT, Zhou Y. Interleukin-8 -251 A/T gene polymorphism and gastric cancer susceptibility: a meta-analysis of epidemiological studies. *Cytokine* 2010; **50**: 328-334 [PMID: 20363644 DOI: 10.1016/j.cyto.2010.03.008]
68 **Orosz P**, Echtenacher B, Falk W, Rüschoff J, Weber D, Männel DN. Enhancement of experimental metastasis by tumor necrosis factor. *J Exp Med* 1993; **177**: 1391-1398 [PMID: 8478614 DOI: 10.1084/jem.177.5.1391]
69 **Wu S**, Boyer CM, Whitaker RS, Berchuck A, Wiener JR, Weinberg JB, Bast RC. Tumor necrosis factor alpha as an autocrine and paracrine growth factor for ovarian cancer: monokine induction of tumor cell proliferation and tumor necrosis factor alpha expression. *Cancer Res* 1993; **53**: 1939-1944 [PMID: 8385577]
70 **Moore RJ**, Owens DM, Stamp G, Arnott C, Burke F, East N, Holdsworth H, Turner L, Rollins B, Pasparakis M, Kollias G, Balkwill F. Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. *Nat Med* 1999; **5**: 828-831 [PMID: 10395330 DOI: 10.1038/10552]
71 **Xu Z**, Shi R, Zhang R, Zhang D, Wang L. Association between tumor necrosis factor β 252 A/G polymorphism and risk of gastric cancer: a meta-analysis. *Tumour Biol* 2013; **34**: 4001-4005 [PMID: 23904263 DOI: 10.1007/s13277-013-0989-3]
72 **zur Hausen A**, Crusius JB, Murillo LS, Alizadeh BZ, Morré SA, Meijer CJ, van den Brule AJ, Peña AS. IL-1B promoter polymorphism and Epstein-Barr virus in Dutch patients with gastric carcinoma. *Int J Cancer* 2003; **107**: 866-867 [PMID: 14566840 DOI: 10.1002/ijc.11468]
73 **Grivennikov S**, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, Karin M. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell* 2009; **15**: 103-113 [PMID: 19185845 DOI: 10.1016/j.ccr.2009.01.001]
74 **Liu RY**, Song X, Chen P, Lei Z, Miao JC, Yi N, Zhang K, Pasche B, Zhang HT. Association between IL6 -174G/C and cancer: A meta-analysis of 105,482 individuals. *Exp Ther Med* 2012; **3**: 655-664 [PMID: 22969947 DOI: 10.3892/etm.2012.454]
75 **Niu YM**, Yuan H, Zhou Y. Interleukin-17 gene polymorphisms contribute to cancer risk. *Mediators Inflamm* 2014; **2014**: 128490 [PMID: 25147431 DOI: 10.1155/2014/128490]
76 **Jackson CB**, Judd LM, Menheniott TR, Kronborg I, Dow C, Yeomans ND, Boussioutas A, Robb L, Giraud AS. Augmented gp130-mediated cytokine signalling accompanies human gastric cancer progression. *J Pathol* 2007; **213**: 140-151 [PMID: 17724739 DOI: 10.1002/path.2218]
77 **Sun Z**, Cui Y, Jin X, Pei J. Association between IL-4 -590C& gt; T polymorphism and gastric cancer risk. *Tumour Biol* 2014; **35**: 1517-1521 [PMID: 24072495 DOI: 10.1007/s13277-013-1209-x]
78 **Barnes PJ**, Karin M. Nuclear factor-kappaB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997; **336**: 1066-1071 [PMID: 9091804 DOI: 10.1056/NEJM199704103361506]

79 **Ghosh S**, Karin M. Missing pieces in the NF-kappaB puzzle. *Cell* 2002; **109** Suppl: S81-S96 [PMID: 11983155 DOI: 10.1016/S0092-8674(02)00703-1]

80 **Iizasa H**, Nanbo A, Nishikawa J, Jinushi M, Yoshiyama H. Epstein-Barr Virus (EBV)-associated gastric carcinoma. *Viruses* 2012; **4**: 3420-3439 [PMID: 23342366 DOI: 10.3390/v4123420]

81 **Sousa H**, Pinto-Correia AL, Medeiros R, Dinis-Ribeiro M. Epstein-Barr virus is associated with gastric carcinoma: the question is what is the significance? *World J Gastroenterol* 2008; **14**: 4347-4351 [PMID: 18666324 DOI: 10.3748/wjg.14.4347]

82 **Gridley G**, McLaughlin JK, Ekbom A, Klareskog L, Adami HO, Hacker DG, Hoover R, Fraumeni JF. Incidence of cancer among patients with rheumatoid arthritis. *J Natl Cancer Inst* 1993; **85**: 307-311 [PMID: 8426374 DOI: 10.1093/jnci/85.4.307]

83 **Correia M**, Machado JC, Ristimäki A. Basic aspects of gastric cancer. *Helicobacter* 2009; **14** Suppl 1: 36-40 [PMID: 19712166 DOI: 10.1111/j.1523-5378.2009.00696.x]

84 **Keller G**, Höfler H, Becker KF. Molecular medicine of gastric adenocarcinomas. *Expert Rev Mol Med* 2005; **7**: 1-13 [PMID: 16156904 DOI: 10.1017/S1462399405009592]

85 **David S**, Meltzer SJ. Stomach - Genetic and epigenetic alterations of preneoplastic and neoplastic lesions. *Cancer Biomark* 2010; **9**: 493-507 [PMID: 22112492 DOI: 10.3233/CBM-2011-0169]

86 **Panani AD**. Cytogenetic and molecular aspects of gastric cancer: clinical implications. *Cancer Lett* 2008; **266**: 99-115 [PMID: 18381231 DOI: 10.1016/j.canlet.2008.02.053]

87 **Milne AN**, Carneiro F, O'Morain C, Offerhaus GJ. Nature meets nurture: molecular genetics of gastric cancer. *Hum Genet* 2009; **126**: 615-628 [PMID: 19657673 DOI: 10.1007/s00439-009-0722-x]

88 **Buffart TE**, Louw M, van Grieken NC, Tijssen M, Carvalho B, Ylstra B, Grabsch H, Mulder CJ, van de Velde CJ, van der Merwe SW, Meijer GA. Gastric cancers of Western European and African patients show different patterns of genomic instability. *BMC Med Genomics* 2011; **4**: 7 [PMID: 21226972 DOI: 10.1186/1755-8794-4-7]

89 **Grabsch HI**, Askham JM, Morrison EE, Pomjanski N, Lickvers K, Parsons WJ, Boecking A, Gabbert HE, Mueller W. Expression of BUB1 protein in gastric cancer correlates with the histological subtype, but not with DNA ploidy or microsatellite instability. *J Pathol* 2004; **202**: 208-214 [PMID: 14743503]

90 **Martin SA**, Hewish M, Lord CJ, Ashworth A. Genomic instability and the selection of treatments for cancer. *J Pathol* 2010; **220**: 281-289 [PMID: 19890832 DOI: 10.1002/path.2631]

91 **Kang JU**, Kang JJ, Kwon KC, Park JW, Jeong TE, Noh SM, Koo SH. Genetic alterations in primary gastric carcinomas correlated with clinicopathological variables by array comparative genomic hybridization. *J Korean Med Sci* 2006; **21**: 656-665 [PMID: 16891809 DOI: 10.3346/jkms.2006.21.4.656]

92 **Morohara K**, Nakao K, Tajima Y, Nishino N, Yamazaki K, Kaetsu T, Suzuki S, Tsunoda A, Kawamura M, Aida T, Tachikawa T, Kusano M. Analysis by comparative genomic hybridization of gastric cancer with peritoneal dissemination and/or positive peritoneal cytology. *Cancer Genet Cytogenet* 2005; **161**: 57-62 [PMID: 16080958 DOI: 10.1016/j.cancergencyto.2005.01.007]

93 **Weiss MM**, Kuipers EJ, Postma C, Snijders AM, Pinkel D, Meuwissen SG, Albertson D, Meijer GA. Genomic alterations in primary gastric adenocarcinomas correlate with clinicopathological characteristics and survival. *Cell Oncol* 2004; **26**: 307-317 [PMID: 15623941]

94 **Matysiak-Budnik T**, Mégraud F. Helicobacter pylori infection and gastric cancer. *Eur J Cancer* 2006; **42**: 708-716 [PMID: 16556496]

95 **Pérez de Castro I**, de Cárcer G, Malumbres M. A census of mitotic cancer genes: new insights into tumor cell biology and cancer therapy. *Carcinogenesis* 2007; **28**: 899-912 [PMID: 17259655 DOI: 10.1093/carcin/bgm019]

96 **Gollin SM**. Mechanisms leading to chromosomal instability. *Semin Cancer Biol* 2005; **15**: 33-42 [PMID: 15613286]

97 **Duesberg P**, Li R, Rasnick D, Rausch C, Willer A, Kraemer A, Yerganian G, Hehlmann R. Aneuploidy precedes and segregates with chemical carcinogenesis. *Cancer Genet Cytogenet* 2000; **119**: 83-93 [PMID: 10867141 DOI: 10.1016/S0165-4608(99)00236-8]

98 **Schmit TL**, Ahmad N. Regulation of mitosis via mitotic kinases: new opportunities for cancer management. *Mol Cancer Ther* 2007; **6**: 1920-1931 [PMID: 17620424 DOI: 10.1158/1535-7163.MCT-06-0781]

99 **Duesberg P**, Li R, Fabarius A, Hehlmann R. The chromosomal basis of cancer. *Cell Oncol* 2005; **27**: 293-318 [PMID: 16373963]

100 **Iovino F**, Lentini L, Amato A, Di Leonardo A. RB acute loss induces centrosome amplification and aneuploidy in murine primary fibroblasts. *Mol Cancer* 2006; **5**: 38 [PMID: 16987420 DOI: 10.1186/1476-4598-5-38]

101 **Enjoji M**, Iida S, Sugita H, Ishikawa T, Uetake H, Inokuchi M, Yamada H, Kojima K, Sugihara K. BubR1 and AURKB overexpression are associated with a favorable prognosis in gastric cancer. *Mol Med Rep* 2009; **2**: 589-596 [PMID: 21475871 DOI: 10.3892/mmr_00000142]

102 **Osaki M**, Inoue T, Yamaguchi S, Inaba A, Tokuyasu N, Jeang KT, Oshimura M, Ito H. MAD1 (mitotic arrest deficiency 1) is a candidate for a tumor suppressor gene in human stomach. *Virchows Arch* 2007; **451**: 771-779 [PMID: 17674037 DOI: 10.1007/s00428-007-0470-z]

103 **Wang L**, Yin F, Du Y, Chen B, Liang S, Zhang Y, Du W, Wu K, Ding J, Fan D. Depression of MAD2 inhibits apoptosis and increases proliferation and multidrug resistance in gastric cancer cells by regulating the activation of phosphorylated survivin. *Tumour Biol* 2010; **31**: 225-232 [PMID: 20440596 DOI: 10.1007/s13277-010-0036-6]

104 **Dar AA**, Zaika A, Piazuelo MB, Correa P, Koyama T, Belkhiri A, Washington K, Castells A, Pera M, El-Rifai W. Frequent overexpression of Aurora Kinase A in upper gastrointestinal adenocarcinomas correlates with potent antiapoptotic functions. *Cancer* 2008; **112**: 1688-1698 [PMID: 18311783 DOI: 10.1002/cncr.23371]

105 **Dar AA**, Belkhiri A, El-Rifai W. The aurora kinase A regulates GSK-3beta in gastric cancer cells. *Oncogene* 2009; **28**: 866-875 [PMID: 19060929 DOI: 10.1038/onc.2008.434]

106 **Zheng L**, Wang L, Ajani J, Xie K. Molecular basis of gastric cancer development and progression. *Gastric Cancer* 2004; **7**: 61-77 [PMID: 15224192]

107 **Guo T**, Lee SS, Ng WH, Zhu Y, Gan CS, Zhu J, Wang H, Huang S, Sze SK, Kon OL. Global molecular dysfunctions in gastric cancer revealed by an integrated analysis of the phosphoproteome and transcriptome. *Cell Mol Life Sci* 2011; **68**: 1983-2002 [PMID: 20953656 DOI: 10.1007/s00018-010-0545-x]

108 **Palli D**, Polidoro S, D'Errico M, Saieva C, Guarrera S, Calcagnile AS, Sera F, Allione A, Gemma S, Zanna I, Filomena A, Testai E, Caini S, Moretti R, Gomez-Miguel MJ, Nesi G, Luzzi I, Ottini L, Masala G, Matullo G, Dogliotti E. Polymorphic DNA repair and metabolic genes: a multigenic study on gastric cancer. *Mutagenesis* 2010; **25**: 569-575 [PMID: 20817763 DOI: 10.1093/mutage/geq042]

109 **Hudler P**, Repše S, Juvan R, Komel R. A genomic approach to investigate expression profiles in Slovenian patients with gastric cancer. *Oncol Lett* 2011; **2**: 1003-1014 [PMID: 22866164 DOI: 10.3892/ol.2011.362]

110 **Kim HK**, Choi IJ, Kim CG, Kim HS, Oshima A, Michalowski A, Green JE. A gene expression signature of acquired chemoresistance to cisplatin and fluorouracil combination chemotherapy in gastric cancer patients. *PLoS One* 2011; **6**: e16694 [PMID: 21364753 DOI: 10.1371/journal.pone.0016694]

111 **Wang P**, Tang JT, Peng YS, Chen XY, Zhang YJ, Fang JY. XRCC1 downregulated through promoter hypermethylation is involved in human gastric carcinogenesis. *J Dig Dis* 2010; **11**: 343-351 [PMID: 21091896 DOI: 10.1111/j.1751-2980.2010.00459.x]

112 **Wu F**, Shirahata A, Sakuraba K, Kitamura Y, Goto T, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y, Hibi K. Down-regulation of Mus81 as a potential marker for the malignancy of gastric cancer. *Anticancer Res* 2010; **30**: 5011-5014 [PMID:

21187482]
113 **Kang B**, Guo RF, Tan XH, Zhao M, Tang ZB, Lu YY. Expression status of ataxia-telangiectasia-mutated gene correlated with prognosis in advanced gastric cancer. *Mutat Res* 2008; **638**: 17-25 [PMID: 17928013 DOI: 10.1016/j.mrfmmm.2007.08.013]
114 **Toller IM**, Neelsen KJ, Steger M, Hartung ML, Hottiger MO, Stucki M, Kalali B, Gerhard M, Sartori AA, Lopes M, Müller A. Carcinogenic bacterial pathogen Helicobacter pylori triggers DNA double-strand breaks and a DNA damage response in its host cells. *Proc Natl Acad Sci USA* 2011; **108**: 14944-14949 [PMID: 21896770 DOI: 10.1073/pnas.1100959108]
115 **Baek HY**, Lim JW, Kim H, Kim JM, Kim JS, Jung HC, Kim KH. Oxidative-stress-related proteome changes in Helicobacter pylori-infected human gastric mucosa. *Biochem J* 2004; **379**: 291-299 [PMID: 14711373 DOI: 10.1042/BJ20031208]
116 **Ding SZ**, O'Hara AM, Denning TL, Dirden-Kramer B, Mifflin RC, Reyes VE, Ryan KA, Elliott SN, Izumi T, Boldogh I, Mitra S, Ernst PB, Crowe SE. Helicobacter pylori and H2O2 increase AP endonuclease-1/redox factor-1 expression in human gastric epithelial cells. *Gastroenterology* 2004; **127**: 845-858 [PMID: 15362040 DOI: 10.1053/j.gastro.2004.06.017]
117 **Farinati F**, Cardin R, Russo VM, Busatto G, Franco M, Rugge M. Helicobacter pylori CagA status, mucosal oxidative damage and gastritis phenotype: a potential pathway to cancer? *Helicobacter* 2003; **8**: 227-234 [PMID: 12752735 DOI: 10.1046/j.1523-5378.2003.00149.x]
118 **Machado AM**, Figueiredo C, Touati E, Máximo V, Sousa S, Michel V, Carneiro F, Nielsen FC, Seruca R, Rasmussen LJ. Helicobacter pylori infection induces genetic instability of nuclear and mitochondrial DNA in gastric cells. *Clin Cancer Res* 2009; **15**: 2995-3002 [PMID: 19383819 DOI: 10.1158/1078-0432.CCR-08-2686]
119 **Slomiany BL**, Slomiany A. Helicobacter pylori Induces Disturbances in Gastric Mucosal Akt Activation through Inducible Nitric Oxide Synthase-Dependent S-Nitrosylation: Effect of Ghrelin. *ISRN Gastroenterol* 2011; **2011**: 308727 [PMID: 21991502 DOI: 10.5402/2011/308727]
120 **El-Omar EM**. The importance of interleukin 1beta in Helicobacter pylori associated disease. *Gut* 2001; **48**: 743-747 [PMID: 11358884 DOI: 10.1136/gut.48.6.743]
121 **Crabtree JE**, Shallcross TM, Heatley RV, Wyatt JI. Mucosal tumour necrosis factor alpha and interleukin-6 in patients with Helicobacter pylori associated gastritis. *Gut* 1991; **32**: 1473-1477 [PMID: 1773951]
122 **Figueiredo C**, Machado JC, Pharoah P, Seruca R, Sousa S, Carvalho R, Capelinha AF, Quint W, Caldas C, van Doorn LJ, Carneiro F, Sobrinho-Simões M. Helicobacter pylori and interleukin 1 genotyping: an opportunity to identify high-risk individuals for gastric carcinoma. *J Natl Cancer Inst* 2002; **94**: 1680-1687 [PMID: 12441323 DOI: 10.1093/jnci/94.22.1680]
123 **Ottini L**, Falchetti M, Lupi R, Rizzolo P, Agnese V, Colucci G, Bazan V, Russo A. Patterns of genomic instability in gastric cancer: clinical implications and perspectives. *Ann Oncol* 2006; **17** Suppl 7: vii97-vi102 [PMID: 16760303 DOI: 10.1093/annonc/mdl960]
124 **Buermeyer AB**, Deschênes SM, Baker SM, Liskay RM. Mammalian DNA mismatch repair. *Annu Rev Genet* 1999; **33**: 533-564 [PMID: 10690417 DOI: 10.1146/annurev.genet.33.1.533]
125 **Ottini L**, Falchetti M, Saieva C, De Marco M, Masala G, Zanna I, Paglierani M, Giannini G, Gulino A, Nesi G, Mariani Costantini R, Palli D. MRE11 expression is impaired in gastric cancer with microsatellite instability. *Carcinogenesis* 2004; **25**: 2337-2343 [PMID: 15319296 DOI: 10.1093/carcin/bgh257]
126 **Yamamoto H**, Perez-Piteira J, Yoshida T, Terada M, Itoh F, Imai K, Perucho M. Gastric cancers of the microsatellite mutator phenotype display characteristic genetic and clinical features. *Gastroenterology* 1999; **116**: 1348-1357 [PMID: 10348818]
127 **Kang DH**, Han ME, Song MH, Lee YS, Kim EH, Kim HJ, Kim GH, Kim DH, Yoon S, Baek SY, Kim BS, Kim JB, Oh SO. The role of hedgehog signaling during gastric regeneration. *J Gastroenterol* 2009; **44**: 372-379 [PMID: 19291354 DOI: 10.1007/s00535-009-0006-1]
128 **García I**, del Casar JM, Corte MD, Allende MT, García-Muñiz JL, Vizoso F. Epidermal growth factor receptor and c-erbB-2 contents in unresectable (UICC R1 or R2) gastric cancer. *Int J Biol Markers* 2003; **18**: 200-206 [PMID: 14535591]
129 **García I**, Vizoso F, Martín A, Sanz L, Abdel-Lah O, Raigoso P, García-Muñiz JL. Clinical significance of the epidermal growth factor receptor and HER2 receptor in resectable gastric cancer. *Ann Surg Oncol* 2003; **10**: 234-241 [PMID: 12679307]
130 **Gencer S**, Şen G, Doğusoy G, Bellı AK, Paksoy M, Yazicioğlu MB. β-Catenin-independent noncanonical Wnt pathway might be induced in gastric cancers. *Turk J Gastroenterol* 2010; **21**: 224-230 [PMID: 20931424]
131 **Hayashi M**, Inokuchi M, Takagi Y, Yamada H, Kojima K, Kumagai J, Kawano T, Sugihara K. High expression of HER3 is associated with a decreased survival in gastric cancer. *Clin Cancer Res* 2008; **14**: 7843-7849 [PMID: 19047113 DOI: 10.1158/1078-0432.CCR-08-1064]
132 **Katoh M**, Kirikoshi H, Terasaki H, Shiokawa K. WNT2B2 mRNA, up-regulated in primary gastric cancer, is a positive regulator of the WNT- beta-catenin-TCF signaling pathway. *Biochem Biophys Res Commun* 2001; **289**: 1093-1098 [PMID: 11741304 DOI: 10.1006/bbrc.2001.6076]
133 **Kim MA**, Lee HS, Lee HE, Jeon YK, Yang HK, Kim WH. EGFR in gastric carcinomas: prognostic significance of protein overexpression and high gene copy number. *Histopathology* 2008; **52**: 738-746 [PMID: 18397279 DOI: 10.1111/j.1365-2559.2008.03021.x]
134 **Liang B**, Wang S, Zhu XG, Yu YX, Cui ZR, Yu YZ. Increased expression of mitogen-activated protein kinase and its upstream regulating signal in human gastric cancer. *World J Gastroenterol* 2005; **11**: 623-628 [PMID: 15655810 DOI: 10.3748/wjg.v11.i5.623]
135 **Nabais S**, Machado JC, Lopes C, Seruca R, Carneiro F, Sobrinho-Simões M. Patterns of beta-catenin expression in gastric carcinoma: clinicopathological relevance and mutation analysis. *Int J Surg Pathol* 2003; **11**: 1-9 [PMID: 12598910 DOI: 10.1177/106689690301100102]
136 **Song MS**, Park YK, Lee JH, Park K. Induction of glucose-regulated protein 78 by chronic hypoxia in human gastric tumor cells through a protein kinase C-epsilon/ERK/AP-1 signaling cascade. *Cancer Res* 2001; **61**: 8322-8330 [PMID: 11719466]
137 **Sun Y**, Gao X, Liu J, Kong QY, Wang XW, Chen XY, Wang Q, Cheng YF, Qu XX, Li H. Differential Notch1 and Notch2 expression and frequent activation of Notch signaling in gastric cancers. *Arch Pathol Lab Med* 2011; **135**: 451-458 [PMID: 21466361 DOI: 10.1043/2009-0665-OA.1]
138 **Velho S**, Corso G, Oliveira C, Seruca R. KRAS signaling pathway alterations in microsatellite unstable gastrointestinal cancers. *Adv Cancer Res* 2010; **109**: 123-143 [PMID: 21070916 DOI: 10.1016/B978-0-12-380890-5.00004-1]
139 **Yk W**, Cf G, T Y, Z C, Xw Z, Xx L, Nl M, Wz Z. Assessment of ERBB2 and EGFR gene amplification and protein expression in gastric carcinoma by immunohistochemistry and fluorescence in situ hybridization. *Mol Cytogenet* 2011; **4**: 14 [PMID: 21689422 DOI: 10.1186/1755-8166-4-14]
140 **Soutto M**, Belkhiri A, Piazuelo MB, Schneider BG, Peng D, Jiang A, Washington MK, Kokoye Y, Crowe SE, Zaika A, Correa P, Peek RM, El-Rifai W. Loss of TFF1 is associated with activation of NF-κB-mediated inflammation and gastric neoplasia in mice and humans. *J Clin Invest* 2011; **121**: 1753-1767 [PMID: 21490402 DOI: 10.1172/JCI43922]
141 **Hsu PI**, Hsieh HL, Lee J, Lin LF, Chen HC, Lu PJ, Hsiao M. Loss of RUNX3 expression correlates with differentiation, nodal metastasis, and poor prognosis of gastric cancer. *Ann Surg Oncol* 2009; **16**: 1686-1694 [PMID: 19290488 DOI: 10.1245/s10434-009-0428-2]
142 **Byun DS**, Cho K, Ryu BK, Lee MG, Park JI, Chae KS, Kim HJ, Chi SG. Frequent monoallelic deletion of PTEN and its reciprocal associatioin with PIK3CA amplification in gastric carcinoma. *Int J*

Cancer 2003; **104**: 318-327 [PMID: 12569555]
143 **Deng JY**, Sun D, Liu XY, Pan Y, Liang H. STAT-3 correlates with lymph node metastasis and cell survival in gastric cancer. *World J Gastroenterol* 2010; **16**: 5380-5387 [PMID: 21072904]
144 **Merchant JL**. What lurks beneath: IL-11, via Stat3, promotes inflammation-associated gastric tumorigenesis. *J Clin Invest* 2008; **118**: 1628-1631 [PMID: 18431518 DOI: 10.1172/JCI35344]
145 **Fan XY**, Hu XL, Han TM, Wang NN, Zhu YM, Hu W, Ma ZH, Zhang CJ, Xu X, Ye ZY, Han CM, Pan WS. Association between RUNX3 promoter methylation and gastric cancer: a meta-analysis. *BMC Gastroenterol* 2011; **11**: 92 [PMID: 21867527 DOI: 10.1186/1471-230X-11-92]

P- Reviewer: Alshehabi Z, Kir G
S- Editor: Ji FF **L- Editor**: A **E- Editor**: Jiao XK

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com