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Helicobacter pylori infection and gastric cancer

Tamara Matysiak-Budnik[a], *Francis Mégraud*[b,*]

[a]INSERM U793, Faculté Necker – René Descartes, Paris, France
[b]INSERM-ERI 10, Université Victor Segalen Bordeaux 2, Laboratoire de Bacteriologie, 146 rue Leo Saignat, 33076 Bordeaux, France

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ABSTRACT

The pathogenesis of gastric cancer (GC) includes a sequence of events that begins with *Helicobacter pylori*-induced chronic superficial gastritis, progressing towards atrophic gastritis, intestinal metaplasia, dysplasia and eventually GC. The association between *H. pylori* and GC is supported by experimental data showing a capacity of *H. pylori* to induce GC in animals, and the results of interventional studies showing that *H. pylori* eradication can lower the risk of GC and prevent development of pre-cancerous lesions in humans and in experimental animals. The 'driving force' of gastric carcinogenesis is a chronic gastric inflammation, whose intensity and localisation depending on bacterial, host and environmental factors, determines the risk of GC. The mechanisms by which chronic inflammation lead to epithelial and pre-cancerous lesions include induction of oxidative stress, perturbation of the epithelial cells proliferation/apoptosis ratio, and cytokine secretion. Several molecular alterations associated with gastric carcinogenesis have also been described.

1. Introduction

Despite the overall decline in incidence, gastric cancer (GC) remains the fourth most common cancer and the second most common cause of cancer-related death worldwide, killing more than 700,000 people every year. Although a marked decline in the incidence of non-cardia GC has been noted over the last 60 years, it is predicted that due to the ageing of the world's population its incidence may in reality increase, especially in developing countries, to represent in some countries a major public health problem during the next decades.[1] Once clinical manifestations appear, GC has an extremely poor prognosis since a 5-year survival rate using currently available treatments, surgery and radio-chemotherapy, is less than 20%. Therefore, the challenge in GC, as in many other cancers, is to prevent its development by detection and treatment of pre-cancerous lesions and elimination of known risk factors. In order to achieve this goal, it is necessary to understand the pathomechanisms of gastric carcinogenesis. Although stomach cancer is an ancient disease, probably affecting man for several millennia, its pathogenesis remains obscure. Epidemiological studies of migrant populations suggest that GC is associated with exposure to some environmental factor early in life.[2] In the early 1970s, a multistep model of gastric carcinogenesis was proposed by Correa.[3] According to this model, the sequence of events leading to GC begins with chronic superficial gastritis, which progresses to atrophic gastritis, intestinal metaplasia, dysplasia and eventually GC. Although at the time when this model was proposed, chronic inflammation was already considered the initial and indispensable stage of gastric carcinogenesis, the factor inducing this inflammation was not known. Our understanding of gastric carcinogenesis underwent a marked shift with the discovery of *Helicobacter pylori*, the bacterium capable of colonising the stomach and surviving there for years, inducing chronic inflammation, which fulfilled the missing link in Correa's cascade. The association between *H. pylori* and GC was proven by numerous case control studies nested

* *Corresponding author*: Tel.: +33 5 56 79 59 77; fax: +33 5 56 79 60 18.
E-mail address: francis.megraud@chu-bordeaux.fr (F. Mégraud).
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in large cohorts which could prospectively examine the *H. pylori* status of GC patients.[2,4–6] This association was considered sufficient by the Working Group of the International Agency for Research on Cancer/World Health Organization to recognise *H. pylori* as a Group I carcinogen for humans in 1994.[7]

Since then, data from several epidemiological, interventional and experimental studies have been gathered, confirming the causal link between *H. pylori* and GC. Ecological studies mostly confirm the geographical association between the prevalence of *H. pylori* and prevalence of GC, showing a declining incidence of GC in countries with falling rates of *H. pylori* infection. The results obtained with an animal model, the Mongolian gerbil, mimicking the gastric carcinogenesis steps after *H. pylori* infection, is another strong argument.[8] This model led to intervention studies aiming at the prevention of GC by eradication of *H. pylori* in humans. In one of these studies, performed in China, while globally no statistically significant benefit was found, a subgroup analysis clearly showed that no GC developed in subjects who did not have pre-malignant lesions at the time of eradication. These results indicate that there is a point of no return beyond which *H. pylori* eradication cannot lead to a complete prevention of GC.[9] Furthermore, interventional studies have shown that *H. pylori* eradication can prevent the development of pre-cancerous lesions in humans as well as in experimental animals. The last decade has also brought to the forefront new and fascinating data from more basic studies, giving insight into the molecular and cellular mechanisms involved in gastric carcinogenesis. The aim of this article is to review the data concerning the mechanism by which *H. pylori* and its associated chronic gastric inflammation can lead to the development of GC.

GC can be divided into two main histological types: the intestinal (well-differentiated) type and the diffuse type.[10] Intestinal type GC is more common, tends to occur in older patients and is more closely linked to environmental and dietary factors. Diffuse type GC may have a stronger genetic background, such as familial diffuse GC associated with mutations in the E-cadherin gene,[11] is more poorly differentiated and tends to be more aggressive. Recent studies indicate that both types are strongly linked to *H. pylori* infection.[5,12] This review will focus mainly on intestinal type GC, which is more frequently observed and whose association with *H. pylori* and chronic inflammation is well documented.

2. Gastritis as a precursor of gastric cancer

Atrophic gastritis and intestinal metaplasia are well-known histological precursors of intestinal type distal gastric adenocarcinoma.[5,13,14] The gastritis phenotype also correlates with the risk of GC. *H. pylori*-positive individuals with pangastritis, and with corpus-predominant gastritis, are at a markedly increased risk of developing GC. The presence of a corpus-predominant gastritis is associated with the highest risk of GC (relative risk (RR) = 34.5).[5] The causal relationship between *H. pylori* and gastritis has been confirmed by the results of intervention studies showing that bacterial eradication leads to healing of gastritis (disappearance or marked decrease of neutrophilic and lymphocytic infiltration),[15] and more importantly, after a sufficiently long follow-up, even to the regression of atrophy and intestinal metaplasia.[16,17]

H. pylori colonises the gastric epithelium, inducing a chronic inflammatory reaction that may persist throughout the patient's lifetime in spite of a strong local immune reaction.[18] The extent and severity of gastric mucosal inflammation, as well as the clinical outcome of the infection, depend on a number of factors, including the virulence of the bacterium, host genetic susceptibility, immune response, age at which the infection was acquired, and environmental factors, especially dietary. The complex interplay between these factors may explain why only a minority (<1%) of infected individuals ultimately develop GC.[13]

3. Role of bacterial virulence factors

It is now clear that the particular virulence of a given bacterial strain is one of the factors that determines the outcome of infection. The most studied virulence factor of *H. pylori* is the *cag* pathogenicity island (*cag* PAI). It is a segment of genome comprising 31 genes, six of which are thought to encode a type IV secretion system. CagA is a protein encoded by one of the PAI genes (*cagA*) which has been the most extensively studied. The relationship between infection with CagA-positive *H. pylori* strains and a higher risk of GC is well established.[19] The patients infected with CagA-positive strains are at a higher risk of developing peptic ulcer and GC than those infected with CagA-negative *H. pylori* strains.[20] This is in agreement with epidemiological data showing that in Eastern Asian countries, known for a high prevalence of GC, most of *H. pylori* strains are CagA-positive, while in Western countries, where the incidence of GC is low, only 50% of the strains isolated from gastritis patients possess the *cag* PAI.[21] The molecular basis for the pathogenic activity of CagA on gastric epithelial cells has been, in part, elucidated. After attachment of the *cag* positive *H. pylori* strain to gastric epithelial cells, the CagA protein is injected by the type IV secretion system into the cells.[22,23] Translocated CagA undergoes host-mediated phosphorylation on tyrosine residues within the five-amino-acid motif EPIYA sequence repeats by Src family kinases (SFKs)[24,25] and stimulates cell signalling through its interaction with Src homology (SH) 2-containing protein trypsine phosphatase-2 (SHP-2), a molecule involved in mitogenic signal transduction, cell migration and adhesion,[26,27] and with other proteins like Grb2, COOH-terminal Src kinase, hepatocyte growth factor (HGF) receptor/c-Met, and zonula occludens-1 (ZO-1).[26,28–31] Phosphorylated CagA binds Crk adaptor proteins (Crk-II, Crk-I and Crk-L) and this interaction may lead to disruption of the E-cadherin/catenin-containing adherent junctions.[32] Non-phosphorylated CagA also interacts with host proteins, such as Grb2 and ZO-1, inducing cell responses.[30,31,33] Higuchi and colleagues have shown, using small interfering RNA, that SHP-2 plays a critical role in CagA-mediated morphological changes of epithelial cells ('hummingbird' phenotype).[34] Some studies suggest that CagA properties differ among the different strains. For instance, after tyrosine phosphorylation, stronger SHP-2 binding and transforming activities are more common in Eastern Asian strains than in Western strains. This could be related to the presence of specific sequences in the 3′ region

in the former but not in the latter. A Japanese study indicated that East Asian *H. pylori* strains are associated with a greater degree of gastric inflammation and atrophy than Western strains, and GC is found only in those East Asian strains that exhibit greater SHP-2 binding activity than in Western strains.[35] Similarly, an in vitro study of clinical isolates from South African patients shows that strains secreting CagA with more phosphorylation motifs induce higher levels of CagA phosphorylation in epithelial cells and more cytoskeletal changes, and are more likely to be associated with GC.[36]

However, *cag* PAI does not seem to be indispensable for inducing GC. *Helicobacter* species that do not possess *cag*, such as *H. felis*, also appear to promote gastric carcinogenesis. It seems that induction of pre-neoplastic lesions correlates to a greater extent with the type or importance of inflammatory reaction than with the degree of bacterial colonisation.

Another virulence factor susceptible to play a role in gastric carcinogenesis is the vacuolating cytotoxin VacA. Recent studies indicate that, in addition to its well-known effects on epithelial cells, i.e., induction of apoptosis, VacA may be a potent immunomodulatory toxin, targeting the adapted immune system. VacA has been reported to: (i) affect B lymphocyte antigen presentation; (ii) inhibit the activation and proliferation of T lymphocytes; and (iii) modulate the T cell-cytokine response.[37–41] It is possible that the immunosuppressive activities of VacA may contribute to gastric carcinogenesis by favouring bacterial survival in the stomach and maintenance of inflammation. Moreover, strains bearing the more cytotoxic VacA genotypes (s1m1), have been associated with more severe forms of gastritis, pre-malignant lesions (atrophy and intestinal metaplasia),[42] as well as GC.[43]

Other bacterial products including BabA,[44] an adhesin interacting with Lewis antigens on gastric epithelial cells, and OipA, an outer membrane protein,[45] have been studied, but it is not clear whether they have an independent role in bacterial virulence related to gastric carcinogenesis. Recent work indicated, however, that at least in vitro, *H. pylori* adherence to gastric epithelial cells is associated with alterations of bacterial gene expression.[46]

An important discovery is that delivery of *H. pylori* peptidoglycan to host cells may occur via the type IV secretory system. The molecule involved (muramyl dipeptide) links to Nod1, an intracellular pathogen-recognition molecule specific for Gram-negative peptidoglycans, and induces the production of pro-inflammatory mediators via the NF-κB pathway. Mice deficient in Nod1 are more susceptible to infection by *cag* PAI positive strains of *H. pylori* than are wild-type mice, suggesting a physiological role for this interaction.[47] The potential role of *H. pylori* interaction with Nod1 in gastric carcinogenesis remains to be established, but the proposed function of NF-κB as a tumour promoter in inflammation-associated cancer must be taken into account.[48]

4. Role of host genetic factors

It has been postulated for a long time that host genetic factors are important determinants in gastric carcinogenesis. Of particular interest are those related to the host response to infection because of the positive association existing between the intensity of chronic inflammation and GC. The study of GC families showed that in most of these families the development of GC was associated with *H. pylori* infection and that relatives of cancer patients who were also infected had an increased prevalence of pre-cancerous lesions, such as gastric atrophy.[49] In the same study, the genetic polymorphism of interleukin (IL)-1β, a pro-inflammatory cytokine that also bears a strong anti-secretory power, was studied in GC patients and their relatives and the authors demonstrated a positive association between the most pro-inflammatory genotypes of IL-1β and GC.[49] Polymorphisms of other cytokine genes have also been identified, including IL-1RN receptor antagonist, IL-10 and tumour necrosis factor (TNF)-α.[50] Studies on single nucleotide polymorphisms (SNPs) within pro-inflammatory genes showed that a combination of IL-1β, IL-1 RN, TNFα and IL-10 SNPs, which potentially results in elevated levels of IL-1β and TNFα and low levels of IL-10, conferred a 27-fold increased risk of GC to those infected with *H. pylori*, but not to those who were not infected.[51] Some studies even proposed to define a specific host genetic profile,[50,51] which combined with the bacterial genotyping would identify precisely those patients at higher risk of GC.[52–54] Although the association between cytokine genetic polymorphism and risk of GC has not been confirmed in all of the studies, these data indicate the critical role of host factors in determining the outcome of *H. pylori* infection.

The adaptive immune response to *H. pylori* may also play a role. The mechanisms responsible for the induction of the immune response against *H. pylori* and for the development of antigen-specific immunity to this bacterium are largely unknown. Monocytes and macrophages possibly recognise *H. pylori* via toll-like receptor-2 (TLR-2).[55] The role of the other toll-like receptors (TLR 4, 5 and 9) is less well-documented.[56,57] Activation of TLRs leads to the induction of an adaptive immune response, typically with a Th1-polarised cytokine pattern. Its importance in the pathogenesis of GC is well recognised, mostly based on the studies on animal models. In immune-deficient ([RAG2] or [SCID]) mice infected with *Helicobacter*, despite a high level of colonisation, only very mild epithelial- or pre-neoplastic lesions are observed.[58,59] In order to determine which population of immune cells may be important in disease development, mice deficient in specific cell populations have been studied. *Helicobacter*-infected B-cell deficient mice (which retain a normal T-cell response) develop severe atrophy and metaplasia, while T-cell deficient mice are largely protected against these lesions, suggesting a role for T cells in disease initiation and progression to GC.[60] To determine the mechanism by which T cells induce disease, cytokine profiles have been compared in susceptible (C57BL/6) and resistant (BalbC) mice strains.[59–61] Mice that are susceptible to atrophy/metaplasia (such as C57BL/6) have a strong Th1 type (IFN-γ) response, while mice which are resistant to atrophy/metaplasia (such as BalbC) have a predominant Th2 type response.[61] In C57BL/6 mice in which the immune response was shifted from Th1 toward a Th2-polarised response (through infection with an intestinal helminth), a protection from *Helicobacter*-induced atrophy and metaplasia was noted.[62] Conversely, shifting the immune response of resistant mice (BalbC) into a Th1-polarised response via co-infection with *Toxoplasma gondii*, led to progressive atrophy and intestinal metaplasia.[63] Based on these

results, it can be speculated that variations in the prevalence of parasitic and/or other infections could explain geographical discrepancies between the prevalence of *H. pylori* and the incidence of GC, e.g., the African enigma. The association between a more Th2 polarised immune response to *H. pylori* and a lower risk of GC has been confirmed by a study carried out in South Africa.[64]

5. Role of dietary factors

A number of dietary factors have been shown to increase the risk of GC, in particular agents, e.g., nitrates and proteins, which promote nitrosation, while decreased risks were found for consumption of fresh fruit and vegetables which contain nutrients (ascorbic acid and alpha-tocopherol) which inhibit the process.[65] A systematic review and meta-analysis of cohort studies confirmed the protective role of fruit and vegetable consumption against GC. When the follow-up period was 10 years or more, the RR were 0.66 and 0.71 for fruit and vegetable intake, respectively.[66] Conversely, a high-salt diet increases the risk of GC with a RR of approximately twofold. A recent population-based prospective study from Japan indicates that high salt intake is associated in a dose-dependent manner with GC in men but not in women.[67] Studies in mice infected with *H. pylori* and fed a high-salt diet support a synergistic interaction between salt and *H. pylori*, with more severe gastric lesions found in mice exposed to both environmental factors.[68] The mechanisms involved may include effects on colonisation, activation of the p38 MAPK stress pathway, and up-regulation of gastrin or IL-1β. A high-salt diet could be a cofactor in *H. pylori*-associated GC in the INS-GAS mouse model.[69] or could be an independent factor promoting gastric carcinogenesis as shown in *H. pylori*-infected Mongolian gerbils.[70] Tobacco smoking is the other environmental factor that has been linked with a higher risk of GC, but only in men (RR = 1.6),[71] while no association was found with alcohol consumption.

6. What is the link between inflammation and gastric cancer?

The notion of an association between inflammation and GC dates back to Virchow who, in 1863, hypothesised that cancer originates from the sites of chronic inflammation.[72] Today, although the link between inflammation and GC is formally recognised, the mechanisms remains unclear. Chronic inflammation leads to oxidative stress and production of leukocytes and other phagocytic cells generating reactive oxygen species which, in turn, can increase the risk of mutations in proliferating cells. In a model of transgenic mice (Big blue) *H. pylori* infected during 6 months, a significant increase in the mutation rate has been shown.[73]

6.1. Role of oxidative stress

Oxidative stress, associated with inflammation, may play a role in carcinogenesis through its numerous effects on the cells, including damage to protein, lipid and DNA, which can in turn lead to alterations in cell turnover and enhanced cell death. Different factors associated with *H. pylori*-induced inflammation may lead to oxidative stress, such as (i) bacterial production of superoxide;[74] (ii) bacterial induction of reactive oxygen species by gastric epithelial cells;[75,76] and (iii) reduced vitamin C level, a natural antioxidant associated with *H. pylori* infection.[77] Inflammation provides several sources of oxidants including leukocytes, which release reactive oxygen species and reactive nitrogen species; cytokines, some of which, particularly TNF-α, act via increasing reactive species; and even lymphocytes activated by *H. pylori*. Studies in humans have shown an accumulation of reactive oxygen species in the gastric mucosa of infected subjects, which was associated with increased levels of oxidative DNA damage.[78] In vitro studies indicate that oxidative stress induced by the infection leads to proteome changes related to cell proliferation, carcinogenesis, cytoskeletal function, and cellular defence mechanisms.[79] An important determinant of the epithelial response to oxidative stress is the DNA repair enzyme, AP endonuclease-1. Recent studies demonstrate that its expression is increased in *H. pylori* infection as well as in the case of experimentally induced oxidative stress. This enzyme plays a role in *H. pylori*-induced cell signalling.[80]

An indirect epidemiological argument indicating that oxidative stress plays a role in gastric carcinogenesis is that a high antioxidant diet (fresh fruit and vegetables) leads to lower rates of GC and dysplasia.[81] Moreover, an interventional trial in Columbia of antioxidant supplementation has shown the beneficial effect of antioxidants on gastric mucosa, equal to that obtained by *H. pylori* eradication.[16] A similar study from Japan suggests that vitamin C supplementation may protect against the progression of gastric atrophy.[82]

6.2. Gastric cell proliferation

Chronic inflammation promotes apoptosis,[83] which can lead to a compensatory proliferative response by the remaining tissue.[84] The dynamic balance between cell proliferation and apoptosis is essential for maintaining mucosal homeostasis. Decreased apoptosis as well as increased proliferation may favour the carcinogenetic process. Prolonged survival of abnormal cells can favour the accumulation of sequential genetic mutations, which can result in tumour promotion.[85,86] Both an increased rate of cell proliferation and a decreased apoptotic index of the gastric epithelial cells have been reported in *H. pylori* infection.[86] The expression of c-fos, which regulates the transcription of genes related to cell cycle control, was higher in *H. pylori*-infected gastric mucosa than in normal mucosa or in pre-cancerous lesions.[87] Cyclo-oxygenase 2 (COX-2), which interferes in the balance between proliferation and apoptosis is also abnormally expressed in *H. pylori*-infected mucosa.[88,89] Overexpression of COX-2 has been observed in *H. pylori*-positive gastritis, but also in pre-cancerous lesions, atrophic gastritis and intestinal metaplasia, and in GC, suggesting an early role of COX-2 in gastric carcinogenesis.[88,89]

6.3. Molecular events leading to gastric cancer

The end-stage of the multistep process of gastric carcinogenesis corresponds to accumulation of molecular alterations involving either the suppressor pathway (defect in tumour

suppressor genes) or the mutator pathway (defect in DNA mismatch repair genes).[90]

Numerous genes have been implicated in gastric malignant transformation. The earliest alterations in the gastric mucosa involve epigenetic changes, e.g., hypermethylation leading to gene inactivation.[91] Gastric epithelial cells are particularly susceptible to aberrant methylation of CpG islands because of a direct contact with the exterior environment. The mechanisms by which *H. pylori*-induced inflammation stimulates DNA methyltransferases are not completely understood, but may involve stimulation by nitric oxide leading to epigenetic gene silencing.[92,93] Methylation of CpG islands of multiple genes, e.g., APC, COX-2, DAP-kinaze, E-cadherin, GSTP1, hMLK1, MGMT, p16, p14 RASSF1A, THBS1 and TIMP3, in pre-cancerous gastric lesions has been investigated and it was shown that aberrant CpG island methylation tends to accumulate along the multistep process of gastric carcinogenesis.[93,94] In fact, pre-cancerous lesions, such as gastric atrophy or intestinal metaplasia, are typical abnormalities of cell differentiation, which depend on epigenetic modifications.[86] More importantly, elimination of *H. pylori* may lead to regression of epigenetic alterations and restoration of a normal phenotype.[15,93,95–97]

Candidate gene analysis and microarray studies of human tumours have revealed a number of genetic markers.[98] Moreover, several transgenic mouse models have confirmed a significant contribution from growth regulatory genes, exclusive of *Helicobacter*, like TFF1, the IL-6 receptor subunit *gp130*, and *Runx3*.[99] The difficulty is to associate the genetic events with the particular phenotypic changes occurring in gastric mucosa during the carcinogenetic process, such as atrophy, intestinal metaplasia and dysplasia. Atrophic lesions have been shown to be associated with the loss of gastric specific genes encoding H+, K+-ATPase, Muc5AC and intrinsic factor, and with the appearance of intestine-related genes, such as Cdx2, Muc2, TFF3 and villin.[100]

In vitro study where GC cell lines were co-cultured with *H. pylori*, indicated that *H. pylori* infection leads to a decrease in DNA mismatch repair proteins, related at least in part to the bacteria-induced decrease in messenger RNA levels of repair genes. These data suggest that *H. pylori* infection may lead to a deficiency of DNA mismatch repair in gastric epithelial cells that may in turn increase the risk of mutation accumulation in gastric mucosa cells and the risk of GC.[101]

7. Gastric carcinogenesis in animal models

As previously mentioned in this article, studies in animal models have brought three important arguments supporting a causal relationship between *H. pylori*, *H. pylori*-induced inflammation and GC. These data especially concern: (i) the gradual progression of *Helicobacter*-induced lesions from superficial gastritis to atrophy and intestinal metaplasia which are regarded as pre-cancerous lesions; (ii) the development of GC in different animals including ferrets, gerbils and mice; and (iii) the lower risk of GC after *Helicobacter* eradication in experimentally infected animals. The first studies in ferrets infected with *Helicobacter mustelae* used a known carcinogen, *N*-methyl-*N*-nitro-*N*′-nitrosoguanidine (MNNG) and showed the co-carcinogenic role of *Helicobacters* in the development of GC. Later studies, however, reported spontaneous gastric adenocarcinoma in Mongolian gerbils infected with *H. pylori*[8] or aged ferrets naturally infected with *H. mustelae*[102] in the absence of exposure to MNNG. Studies in mice and Mongolian gerbils allowed the reproduction of Correa's multistep model of gastric carcinogenesis by showing the appearance of gastric atrophy, intestinal metaplasia, and even dysplasia and invasive gastric carcinoma in animals infected with *H. felis* or with *H. pylori*. These studies also allowed the tracing of the cellular events occurring successively within the gastric epithelial cells during the carcinogenetic process. Initial infection of C57BL/6 with either *H. felis* or *H. pylori* leads to acute and chronic inflammatory cell infiltrates, and increased apoptosis followed by an increased proliferation. As infection continues, a progression to atrophy and metaplasia is observed.[103] In the *H. felis* model, progression to dysplasia and finally invasive cancer occurs consistently over a period of 12–16 months and large polypoid antral tumours, mimicking lesions found in humans, after 2 years.[104–106] These models, which in many aspects reproduce Correa's multistep model of human gastric carcinogenesis, allowed the study of some of the mechanisms involved in this process. Several characteristics have been consistently observed in the mouse models: parietal cell loss and development of achlorhydria which precede cancer formation in humans, increased apoptosis followed by the rebound of hyperproliferation, and the appearance of metaplasia. Metaplasia begins in general as a TFF2-positive expressing mucous cell lineage, which has been termed spasmolytic polypeptide expressing metaplasia (SPEM).[107] It is now clear in the murine model that the combination of chronic inflammation and achlorhydria and the progression through stages of increased apoptosis, cell proliferation, and metaplasia are associated with the emergence of dysplasia and GC. The most important arguments, however, come from an intervention study showing that bacterial eradication in mice lowers the risk of GC.[105,108]

However, a breakthrough in our concept of gastric carcinogenesis comes from the study of Houghton and colleagues using a mouse model. This study points out the role of mesenchymatous stem cells originating from the bone marrow and repopulating the altered gastric mucosa, as a target for cancer development.[109] The authors investigated the role of bone marrow-derived cells (BMDC) in the metaplasia/dysplasia/carcinoma sequence associated with inflammation in the model of C57BL/6 mice infected with *H. felis*. C57BL/6J mice were irradiated and underwent transplantation with bone marrow from transgenic C57BL/6JGtrosa26 mice, which express a non-mammalian beta-galactosidase enzyme, or C57BL/6J-beta-actin-EGFP mice, which express green fluorescent protein (GFP). They showed that chronic *H. felis*-induced inflammation led to the development of gastric adenocarcinoma originating from the stem cells recruited by the chronically injured gastric mucosa. In the early stages, increased inflamed BMDC could be found within the gastric tissue, but no engraftment and differentiation as epithelial cells was observed. Engraftment was seen at 20 weeks of infection as rare glands were entirely replaced by BMDC. Chronic infection increased apoptosis within the stem cell region, followed by an increase in proliferation and appearance of metaplasia. In the chronically infected mice, a large population of BMDC within the gas-

tric mucosa expressed TFF2 and/or intestinal-type mucins and had the classic phenotype of the metaplastic lineage. Epithelial dysplasia increased in severity over time and by 1-year post-inoculation, resulted in carcinoma in situ or high-grade gastrointestinal intraepithelial neoplasia. All of the intraepithelial neoplasia observed in mice infected for 12–16 months arose from donor marrow stem cells, strongly suggesting that this population of cells is inherently vulnerable to malignant progression. These data confirm a crucial role of chronic inflammation in gastric carcinogenesis and underline the role of BMDC in this process.

8. Conclusion

Data accumulated during the last decade has considerably increased our knowledge on the mechanisms involved in gastric carcinogenesis. They all point towards the prerequisite role of chronic inflammatory reaction in the carcinogenetic process. Now the challenge for the medical and scientific community is to use this knowledge to determine how we can interfere with this process to prevent the development of GC.

Conflict of interest statement

None declared.

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