Significant Factors on Gastric Carcinogenesis Revealed by Experimental Animal Models

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Abstract: The pathological and molecular biochemical analyses in the experimental animal models are important for the solutions of human disorders, including stomach cancer. Stomach cancers are induced experimentally by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea (MNU) in rats and mice. Helicobacter pylori (H. pylori) is one of the most important factor in the human stomach disorders, and the H. pylori infected and chemical carcinogen treated Mongolian gerbil (MG) has thus proved very useful for the analysis of human stomach carcinogenesis. Intestinal metaplasia is important not as a precancerous lesion but as a paracancerous cord from such studies of clonality of stomach cancers and of phenotypic expression of each intestinal metaplastic or stomach cancer cell, while the pepsinogen altered pyloric glands can be regarded as a common change in rodents, acting as a precursor for a variety of adenocarcinoma types. As the results of the analyses of the MG model, H. pylori is a strong promoter of gastric carcinogenesis rather than an initiator. The dose-dependent enhancing effects of salt on stomach carcinogenesis are demonstrated in the MGs treated with MNU and H. pylori, although high salt intake has a minor influence compared to H. pylori. Bile reflux is not an initiator, but rather an important promoter in the carcinogenesis of gastric stump after partial gastrectomy. Stomach cancers develop from single cells, based on data from clonality analysis in C3H/HeNe⇔BALB/c chimeric mice. Intestinalization progresses from gastric, through gastric-and-intestinal mixed, to intestinal phenotypes in non-cancerous and cancerous tissue independently. The chemopreventive effects of H. pylori eradication and reduction of salt intake against stomach cancer are confirmed in the MG models.

Key words: stomach, carcinogenesis, animal models

Introduction

Human gastric cancers histologically present with various morphological structures, which is one of the most characteristic features compared with other digestive apparatus carcinomas. Many pathological and biological analyses of gastric carcinomas, including precancerous lesions, have been performed with human samples. In general, spontaneous adenocarcinomas of the glandular stomach are very rare in experimental small animals. Since the 1930’s, attempts to experimentally induce stomach cancers in animal have been performed by many researchers using several carcinogens such as benzo[a]pyrene, 3-methylcholanthrene, and 2-acethylaminofluorene¹. However, the incidences of experimentally induced stomach cancer were low in all animal models.

Experimental Animals and Gastric Carcinomas

In 1967, Sugimura et al. were able to report good yields of adenocarcinomas in the glandular stomachs of rat treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)². Tumors were selectively produced with very high frequency when MNNG was continuously administered as a solution in drinking water². In hamsters and dogs, MNNG also proved to be a gastric carcinogen. Oral administration of the carcinogen to hamsters at a concentration of 50–83 µg/ml in the drinking water resulted in a high incidence of tumors in the glandular stomach³. Similarly, production of stomach cancer in dogs by MNNG has been well documented⁴. The presence of surfactants, such as alkylbenzenesulfonate, enhances the effect of carcinogens in the stomach of animals⁵.

Stomach cancers also occur in experimental animals such as the rat, hamster, and dog given N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG)⁶. Oral administration of 4-nitroquinoline 1-oxide (4-NQO) and 4-hydroxyaminoquinoline 1-oxide (4-HAQO) similarly induces carcinomas in the stomach as well as the various other tissues⁷. However, the yields of gastric carcinomas using these chemical carcinogens are lower than with in
MNNG.

The glandular stomach of mice has generally been found to be relatively resistant to carcinogen action, and administration of MNNG in the drinking water to BRSUNT/NJms mice over the life span only resulted in adenomatous hyperplasia of gastric epithelium. In 1992, induction of adenocarcinomas in the glandular stomach of BALB/c mice treated with N-methyl-N-nitrosourea (MNU) was reported. Reasonable yields were also obtained in C3H mice treated with MNU in the drinking water.

Based on the epidemiological findings, *Helicobacter pylori* (*H. pylori*) was defined as a “definite biological carcinogen” by WHO/IARC in 1994. In 1996, Hirayama et al. reported a Mongolian gerbil (MG) model of human *H. pylori* infection, with the bacteria detectable throughout a 12-month study period. MGs can be easily infected with *H. pylori*, and the resultant chronic active gastritis, peptic ulcers, and intestinal metaplasia resemble lesions apparent in man. Then, in 1998, we described establishment of an animal model of stomach carcinogenesis using MGs with MNU and MNNG as the carcinogens. *H. pylori* infection increases the incidence of both MNU- and MNNG-induced adenocarcinomas of all histological types in the MG glandular stomach. The *H. pylori* infected and chemical carcinogen treated MG has thus proved very useful for the analysis of gastric carcinogenesis.

**Precancerous Lesions for Gastric Carcinomas**

**Intestinal metaplasia**

Intestinal metaplasia (IM) has been extensively studied as a possible premalignant condition in human stomach. However, many questions remain regarding its pathogenesis as well as the actual relationship to gastric cancers. The present widely applied classification, into complete and incomplete types, was first proposed by Kawachi and colleagues. However, this classification is only based on intestinal properties and do not take into account the gastric properties that are still preserved in association. With recent developments in mucin histochemistry and immunohistochemistry, intestinal metaplastic cells can now be readily classified into a gastric epithelial cell type, encompassing pyloric gland cells and surface mucous cells, and an intestinal epithelial cell type, like goblet and intestinal absorptive cells, on analysis of phenotypic expression. Concerning gastric phenotypic markers, the surface mucous cell type contains galactose oxidase-Schiff (GOS) and sialidase-GOS reactive mucin. Cells of pyloric gland cell type contain class III mucin, and show pepsinogen reactivity. Regarding intestinal epithelial markers, the goblet cell type contains mucin that is GOS-negative and sialidase-GOS reactive. Cells of intestinal absorptive cell type demonstrate sucrase and intestinal type alkaline phosphatase activity (I-ALP).

We have proposed a new IM classification based upon the cell differentiation status using both gastric and intestinal cell phenotypic markers. Division is into two major types; a gastric and intestinal (GI) mixed type, and a solely intestinal (I) type (Fig. 2). Experimentally, a phenotypic shift from GI-IM to I-IM could be clearly observed on sequential observation of rat stomach treated with X-rays. Heterotopic proliferative glands (HPGs) frequently develop with *H. pylori* infection in the glandular stomach of infected MGs, with a slightly dysplastic change of constituent cells. When they often resemble differentiated carcinomas, but are not malignant in character. HPGs also show a phenotypic shift from G type to GI type or I type with appearance of Paneth cells during the overall course of *H. pylori* infection.

**Pepsinogen Altered Pyloric Glands (PAPG)**

Pepsinogen isozymes are divided into pepsinogen isozyme (Pg) 1, Pg2, Pg3, and Pg4 in the normal rat glandular stomach. Three isozymes (Pg1, 3, 4) occur in the pyloric mucosa, and all four (Pg 1–4) in the fundic mucosa. Of the three pepsinogen isozymes which have been separated from the pyloric mucosa by polyacrylamide gel electrophoresis, Pg1 disappears or preferentially decreases in areas of pyloric mucosa during the early stages of MNNG-induced rat glandular stomach carcinogenesis and before morphologically distinct preneoplastic histological changes appear. This altered pepsinogen isozyme pattern has been also been consistently observed in gastric tumors. More recent immunohistochemical studies have demonstrated individual pyloric glands low in Pg1 (thus termed Pg1 altered pyloric glands, PAPG) in otherwise normal appearing pyloric mucosa after MNNG-treatment and have furthermore revealed that cells of pyloric gland cell type within gastric tumors contain little or no Pg3. In addition, induction of PAPG has been found to be dependent on the dose of MNNG administered and numbers increase with time. The susceptibility of different rat strains to induction of gastric carcinomas by MNNG also correlates with their susceptibility to induction of PAPG, and the constituent cells demonstrate a degree of independence from the surrounding pyloric glands with regard to proliferation kinetics. Therefore, PAPG detected immunohistochemically are considered to be putative preneoplastic lesions in the glandular stomach of rats. An experimental protocol consisting of the following four components: (i) PAPG as the endpoint marker lesion; (ii) single dose of MNNG as the initiator; (iii) test chemical administration for 14 weeks; and (iv) administration of saturated sodium chloride solution during the test chemical exposure, has been used effectively for the detection of gastric carcinogens as well as promoters of gastric carcinogenesis in relatively short time period. The altered methylation of the Pg1 gene observed in stomach cancer is acquired early in the carcinogenesis process and progressive methylation changes occur with tumor development. In mice, PAPG are also detectable immunohistochemically, suggesting possible use as a preneoplastic lesion for gastric chemical carcinogenesis in this species, independent of the strain (Fig. 3). Thus, PAPG can be regarded as a common
Fig. 1. GI-IM (arrow) and I-IM (arrow head) in the rat stomach treated with X-rays. (A) The cytoplasm of columnar and goblet cells in GI-IM is stained red and blue, respectively, with AB-PAS. In the I-IMs, the only cytoplasm of goblet cells is stained with blue. (B) I-ALP is present at the luminal surface of absorptive cells in the I-IMs, not the GI-IMs. (GI-IM, gastric-and-intestinal mixed phenotype intestinal metaplasia; I-IM, solely intestinal phenotype intestinal metaplasia; AB-PAS, alcian blue-periodic acid Schiff staining; I-ALP, intestinal type alkaline phosphatase).

Fig. 2. Direction of cell migration and differentiation of gastric and intestinal phenotypic cells in human GI-IM. (A) Isolated GI mixed intestinal metaplastic gland from pyloric mucosa. The isolated gland has both goblet cells stained with Alcian blue (blue) as intestinal exocrine cell phenotypic expression, and pyloric glands stained with PCS (brown) as gastric counterpart. (B) Isolated GI mixed intestinal metaplastic glands incubated in the presence of 10 mg/ml BrdU for 2 h. Proliferating cells are visualized with BrdU (dark brown) immunohistochemistry, and goblet cells with Alcian blue (blue). (C) Schematic view of cell differentiation (left panel) and direction of cell migration in GI mixed intestinal metaplastic glands consisting of both gastric (highlighted red) and intestinal (highlighted blue) phenotypic cells (right panel). (Original magnification, A and B, × 100; GI-IM, gastric-and-intestinal mixed phenotype intestinal metaplasia; PCS, paradoxical concanavalin A staining; BrdU, bromodeoxyuridine). Modified from ref. 21 with permission.

Fig. 3. Double immunohistochemical staining of BrdU, (brown), and Pg1 (red) 60 min after a single injection of BrdU in the glandular stomach of mice. (A) Pyloric mucosa of a control mouse at week 10. (B) Pyloric mucosa after treatment with 120 ppm MNU at week 10. PAPG consisting of cells with low or Pg1 content are apparent (arrows). (BrdU, bromodeoxyuridine; Pg1, pepsinogen isozyme 1; MNU, N-methyl-N-nitrosourea; PAPG, Pg1 altered pyloric glands). Reproduced from ref. 35 with permission.
Modification of Gastric Carcinogenesis

Salt

Salt and salted foods are probable risk factors for gastric cancer, based on evidence from a large number of case-control and ecological studies. In experimental animals, we found sodium chloride to enhance the carcinogetic effects of MNNG and 4-NQO in the rat glandular stomach. Possibly, sodium chloride decreases the viscosity of the gastric mucus and so reduces the protective mucous barrier. When given alone, it has no apparent carcinogeticity in rats but, when administered with MNNG or 4-NQO, it promotes gastric carcinogenesis in the rat glandular stomach, in a dose-dependent fashion. A high concentration of sodium chloride causes initial tissue damage and consequent regenerative cell proliferation. Furthermore, in 2002, Nozaki et al. demonstrated that a high-salt diet enhances the effects of *H. pylori* infection on gastric carcinogenesis, and these two factors act synergistically to promote the development of stomach cancers in the MG model, while high salt intake has a minor influence compared to *H. pylori*. The available data from experimental animal models clearly supports the concept that salt-preserved foods and salt increase the risk of stomach cancer in man.

Endocrine hormones

Tatsuta et al. have shown that prolonged administration of gastrin to rats after MNNG initiation results in a significant increase in acid production and a concomitant reduction in the incidence of stomach adenocarcinomas. Histamine is similarly associated with decreased incidences of adenocarcinomas in the rat glandular stomach, but only gastrin affects the histological type. Prolonged administration of gastrin to rats after MNNG exposure also suppresses development of gastric cancer precursors, but administration of a small dose during MNNG treatment results in the development of so-called undifferentiated adenocarcinomas.

Estrogen and progesterone receptors have been detected in adenocarcinomas of the stomach. Somatostatin, thyroxine, neurotensin, vaso-active intestinal peptide (VIP) and Substance P have been reported to enhance gastric carcinogenesis after MNNG treatment in rats.

Bile

An increased risk of gastric cancer in stomach remnants long after partial gastrectomy and possibility of effects of reflux of bile and/or pancreatic juice have been reported. Promoting effects of bile on experimental stomach tumorigenesis in the rat have also been suggested and it has been shown that the incidence of gastric cancers induced by carcinogens is increased after partial gastrectomy, with tumor development related to the promotional effect of bile reflux. Since gastrectomized rats not receiving any unequivocal carcinoegen also frequently demonstrate cancers in the regions of anastomosis, however, not only tumor-promoting but also initiating activity is conceivable on the gastric stump. While the cancer incidence is very low in gastric stumps after partial stomach resection with transit reconstruction by the Billroth II technique, the proportion of human patients with cancer in the gastric stump after surgical procedures has been reported to range from 23 to 42%.

Helicobacter pylori

From epidemiological findings, there is little a room for doubt that *H. pylori* infection has “positive correlation” with stomach cancer development. Mongolian gerbils can be easily infected with *H. pylori*, and the resultant chronic active gastritis, peptic ulcers, and IM resemble lesions apparent in man. Thus, MGs appear to be an ideal experimental animal for detailed analysis of the roles of *H. pylori* in gastric disorders. All histological types of gastric cancer development can be observed in the glandular stomach of MGs treated with the chemical carcinogens MNU or MNNG, and *H. pylori* infection markedly enhances the yields of lesions. As noted above a high-salt diet enhances the effects of *H. pylori* infection on gastric carcinogenesis, and the two factors act synergistically to promote the development of stomach cancers in the MG model. To evaluate variation in susceptibility to stomach carcinogenesis in relation to age of acquisition of *H. pylori* infection, Cao et al. designed an experiment involving inoculation of *H. pylori* followed by MNU exposure at different time points in the MG lifespan. Early acquisition of *H. pylori* significantly increases gastric chemical carcinogenesis with MNU, as compared to the case with later infection, possibly because of differences in host gastric mucosal factors and immunologic responses. This would imply that childhood *H. pylori* infection must not be overlooked in approaches to the prevention of stomach
Several studies based on histopathology showed no carcinomas in animals treated only with *H. pylori* infection. However, two reports concluded that *H. pylori* infection alone can induce well-differentiated adenocarcinomas at very high incidences in the glandular stomach of MGs, while another study resulted in only one poorly-differentiated adenocarcinoma. The incidences and histological patterns of the lesions differed greatly in these three papers. After *H. pylori* infection, glands in the stomach of MGs start to proliferate into submucosa, disrupting the lamina muscularis mucosa. Submucosal HPGs develop in the glandular stomach of MGs with *H. pylori* infection alone, often resembling differentiated carcinomas. The characteristics of the HPGs include: 1) organized polarity of their component cells; 2) differentiation from G type HPGs into I type HPGs with mature Paneth cells; 3) formation of large cystic dilatations containing mucin, often with calcification; 4) shedding of epithelial cells and necrosis at the tips of lesions; 5) high-grade inflammation with infiltration of inflammatory cells; and 6) organized polarity of proliferating zones. These characteristics are quite different from those of well-differentiated adenocarcinomas, which are characterized by obvious cellular atypia. After eradication, HPGs are obviously reduced, and gastric lesions in mucosa also differ from those seen in association with *H. pylori* infection alone.
decrease with few remnants of the former injury (Fig. 4). Reversible HPGs are frequently induced solely by *H. pylori* infection in this species, and are related to severe gastritis, rather than being malignant in character. Thus, distinguishing reversible lesions from true neoplasms is necessary in investigating the relationship of *H. pylori* infection with gastric carcinogenesis in the MG model.

Taking into account all the available data, we conclude that *H. pylori* is a strong promoter of gastric carcinogenesis rather than an initiator.

### The Characteristics and Differentiation of Gastric Cancer Cells

#### Monoclonal growth of gastric cancers

Gastric cancer cells show heterogeneity in terms of both the histological and phenotypic types, raising the question of clonality. Recently, numerous histological markers have been used for analysis of mosaicism in chimeric mice, and establishment of antibodies strictly recognizing a C3H strain-specific antigen (CSA) by Kusakabe *et al.*, has enabled immunohistochemical discrimination of C3H cells in histological sections of C3H/HeN⇔BALB/c chimeric mice. In normal gastric and intestinal mucosa of chimera adult mice, each individual gland is composed entirely of CSA-positive or negative cells and no mixed glands are apparent, indicating that each is derived from a single progenitor cell (Fig. 5A). Surface mucous cells (foveolar epithelial cells), mucous neck cells, parietal cells and chief cells in the fundic glands thus all arise from the same cell. Similarly, surface mucous cells and pyloric gland cells in each pyloric gland are from a single progenitor cell. MNU in the drinking water selectively induces neoplastic lesions in the glandular stomach of BALB/c and C3H mice, and gastric cancers in C3H/HeN⇔BALB/c chimeric mice treated with MNU were also found to be composed of only one parental type (Fig. 5B). Thus, individual gastric cancers are derived from single cells with multi-potential activity.

#### The phenotypic classification and the shift from gastric to intestinal phenotype with progression in gastric cancers

**Markers of gastric and intestinal epithelium:** The phenotypic expression of malignant cells is widely thought...
to resemble that of the tissue of origin. Using gastric and intestinal epithelial cell markers, it is possible to analyze the phenotypic expression of each gastric cancer cell, independent of the histological type\textsuperscript{22,30,64-70\textsuperscript{1}}. MUC5AC, human gastric mucin (HGM), GOS, MUC6, paradoxical concanavalin A staining (PCS) class III mucin are markers of the human gastric epithelial cell phenotype, whereas MUC2, sialidase-GOS, sialyl-Tn antigen, small intestinal mucinous antigen (SIMA), sucrase, intestinal type alkaline phosphatase (I-ALP), CD10, villin are typical of the human intestinal epithelial cell phenotype. The gastric epithelial cell phenotypic markers are consisted of surface mucous (MUC5AC, HGM, and GOS) and pyloric gland (MUC6 and PCS) cell ones. The intestinal epithelial cell phenotypic markers are also consisted of goblet (MUC2, sialidase-GOS, sialyl-Tn antigen, and SIMA) and absorptive (sucrase, I-ALP, CD10, and villin) cell ones. Some of these are positive in the animal gastric and intestinal epithelial cells\textsuperscript{22,30,64,69,70\textsuperscript{1}}.

A shift from gastric to intestinal phenotypic expression of gastric cancer cells: Gastric cancers comprising epithelial elements presenting only gastric or intestinal phenotypic expression are classified as of gastric (G type), or intestinal (I type) phenotype, respectively. Those with both gastric type cells and intestinal type cells are classified as having a gastric and intestinal mixed phenotype (GI type), while the remainder exhibiting neither are grouped as unclassified (N type)\textsuperscript{22,67,71\textsuperscript{1}} (Figs. 6 and 7). In the rat glandular stomach, experimentally induced adenocarcinomas consist mainly of G type cancer cells, with I type cancer cells appearing later in larger tumors\textsuperscript{22,64,65,69,70\textsuperscript{1}}. A shift from gastric to intestinal phenotypic expression is in fact observed with progression of each histological type of human gastric cancer\textsuperscript{68,72\textsuperscript{1}}. In humans and animals, gastric cancers at early stages, independent of the histological type, mainly consist of G type cancer cells, and a phenotypic shift from gastric to intestinal phenotypic expression is clearly observed with progression\textsuperscript{21,64,65,69,70\textsuperscript{1}} (Fig. 8).

**IM is a paracancerous lesion:** Regarding the histogenesis of gastric cancers, it would be logical if those originating from IM should be of the I type. Even if the phenotypic expression of I type gastric cancer cells is unstable, the incidence of I type cancer cells in small gastric cancers should then be higher than in large gastric cancers, although expression in fact appears to be stable\textsuperscript{65\textsuperscript{1}}. Sequential and quantitative analysis of the appearance of IM and I type gastric cancer cells during gastric carcinogenesis induced by

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**Fig. 7:** Histology and phenotype of poorly differentiated adenocarcinomas in MGs- G type (A, B) and I type (C, D). (A) H&E staining of the G type tumor. (B) PCS is present in the cytoplasm of tumor cells. (C) The cytoplasm of tumor cells was stained blue by AB-PAS. (D) SIMA is evident in the cytoplasm of cancer cells. (Original magnification, A, B, and C, ×200; D, ×640; MG, Mongolian gerbil; PCS, paradoxical concanavalin A staining; AB-PAS, Alcian blue-periodic acid Schiff staining; SIMA, small intestinal mucinous antigen). Modified from ref. 71 with permission.
chemical carcinogens in rats has clearly demonstrated the following: (i) Adenomatous hyperplasias totally consisting of G type cells appear first. (ii) All adenocarcinomas mainly consist of G type cancer cells and in more than 50% of cases they are composed entirely of cells of G type. No tumors consisting only of I type cells are found. (iii) The incidence of I type cancer cells increases significantly in gastric lesions with progression from adenomatous hyperplasia through small well-differentiated adenocarcinomas to large well-differentiated adenocarcinomas. (iv) Tumor cells of G and I types may be present in the same acini in adenocarcinomas. (v) Adenocarcinomas with I type cancer cells occasionally develop in pyloric mucosa in the absence of IM, and tumors without I type tumor cells sometimes occur in pyloric mucosa with IM. In humans, also, there is no consistent phenotypic expression between gastric cancers and surrounding gastric mucosa between with or without IM. We have concluded that IM is not a preneoplastic change in gastric carcinoma, but rather that cells of the I type may appear independently in the gastric mucosa in IM or in gastric cancers (Fig. 8).

### The Prevention of Gastric Carcinomas

#### Polyphenols

Epidemiological studies have shown a lower risk of gastric cancer among people who consume a large amount of green tea or vegetables. These contain various polyphenols, (–)-epigallocatechin (EGCG) being a major constituent of green tea. Several experimental studies have revealed that green tea polyphenols and EGCG can inhibit chemical carcinogenesis in the duodenum, colon, skin, and lung, and in one study, EGCG reduced MNNG-induced carcinogenesis of the glandular stomach in rat-associated with a significant decrease in the BrdU labeling index of the mucosa.

#### Eradication of H. pylori

Shimizu et al. have provided direct evidence that *H. pylori* eradication may be useful as a prevention approach against gastric cancer. In the *H. pylori* infected MGs treated with MNU, the incidences of gastric cancers after curative treatment for *H. pylori* were thus significantly lower than without *H. pylori* eradication. For further evaluation, an experimental model with eradication in the early, middle, late period was studied using *H. pylori*-infected and MNU-treated MGs. *H. pylori* infection was found to strongly enhance gastric carcinogenesis initiated with the chemical carcinogen, and following eradication at an early period this effect was effectively reduced.

#### Restriction of salt and salted food

Intake of salt and salty food is known as a risk factor for gastric carcinogenesis. With regard to experimental animal models, we already reported in the 1970s that sodium chloride enhances the carcinogenic effects of MNNG and 4-
NQO in the rat glandular stomach. In 2002, Nozaki et al. further demonstrated that a high-salt diet acts to promote effects of *H. pylori* infection on gastric carcinogenesis, and these two factors act synergistically to drive the development of stomach cancers in the MGs model. And Study of Kato et al. showed dose-dependent enhancing effects of salt on gastric carcinogenesis in MGs treated with MNU and *H. pylori*. In gerbils infected with *H. pylori*, a high salt diet was associated with elevation of anti-*H. pylori* antibody titers, serum gastrin levels, and inflammatory cell infiltration in a dose-dependent fashion. Ten percent NaCl diet upregulated the amount of surface mucous cell mucin, suitable for *H. pylori* colonization, despite no increment of MUC5AC mRNA, while *H. pylori* infection itself had an opposing effect, stimulating transcription of MUC6 and increasing the amount of gland mucous cell mucin. High salt diet, in turn, decreased the amount of gland mucous cell mucin, which acts against *H. pylori* infection. Reduction of salt intake could thus be one of the most important chemopreventive methods for human gastric carcinogenesis, especially in the patients with *H. pylori* infection.

**Conclusion**

We can conclude that IM is important not as a precancerous lesion but as a paracancerous cord from such studies of clonality of gastric cancers and of phenotypic expression of each intestinal metaplastic or stomach cancer cell. Intestinalization progresses from G, through GI, to I types in non-cancerous and cancerous tissue independently, accompanied by homeotic transformation of underlying control factors. *H. pylori* is not an initiator, but rather a strong promoter in gastric carcinogenesis, whose eradication, together with reduction in salt intake, might effectively prevent gastric cancer development.

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