Review Article

Role of *Helicobacter pylori*-induced Antralization in Gastric Carcinogenesis and its Implications in Clinical Practice

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Abstract

The aim of this article was to review the roles of *Helicobacter pylori*-induced antralization in gastric carcinogenesis and its implications in clinical practice. A search of PubMed/PubMed Central, Web of Science, and China National Knowledge Infrastructure was performed in December 2018 to retrieve all literature related to antralization (or antralisation), pyloric (or pseudopyloric) glands, pyloric (or pseudopyloric) metaplasia, or pyloric (or pseudopyloric) gland metaplasia, and spasmolytic polypeptide-expressing metaplasia (or SPEM). Among the synonyms, antralization and SPEM, which are derived in the same mechanisms at the molecular, cellular and tissue levels, are more commonly used in recent studies. Antralization (or SPEM) is associated with *H. pylori* infection, atrophic gastritis and intestinal metaplasia, while *H. pylori* eradication may reverse antralization. It is proposed that *H. pylori* infection leads to inflammation in the gastric mucosa and apoptosis of the epithelial cells of the proximal stomach, including gastric incisura, body and fundus. The stem cells proliferate and transform into mucous cells and form antral-type mucosa (*i.e.* antralization). Subsequently, *H. pylori*-induced antralization, if not controlled, may develop into atrophic gastritis, intestinal metaplasia, dysplasia, and early intestinal-type gastric cancer. Although many biomarkers, including the spasmolytic polypeptide and mucin 6, are specifically expressed in the gastric mucosa with antralization, none of them are evaluated for the clinical diagnosis of antralization. *H. pylori*-induced antralization (or SPEM) is believed to be an initiating and reversible stage of gastric carcinogenesis. Identification of antralization would help make an early intervention to cease or even reverse the process toward the development of gastric cancer. Currently, the "gold standard" for diagnosing antralization is pathology, which is invasive and time consuming. A non-invasive and convenient method that accurately and specifically diagnoses antralization is urgently required.

Introduction

According to the latest global cancer data released by the World Health Organization (commonly known as WHO), gastric cancer was responsible for over 1,000,000 new cases in 2018 and an estimated 783,000 deaths (equating to 1 in every 12 deaths globally), making it the fifth most frequently diagnosed cancer and the third leading cause of cancer death.[1] It has been established by WHO that *Helicobacter pylori* infection is a class I carcinogen of gastric cancer, and contributes to nearly 90% of non-cardiac cancer cases.[2] Although the incidence of gastric cancer has declined over the past recent decades, following due improvements in the environment and control of *H. pylori* infection in clinical practice, gastric cancer is still a major threat to human health.

Currently, there are still no curative therapeutic modalities for advanced gastric cancer, and screening of early gastric cancer is critical for improvement of its outcomes. Histologically, there are two major types of gastric cancer: intestinal-type and diffuse-type.[3] The international consensus on the prevention and treatment of gastric cancer recommends the detection and monitoring of precancerous lesions, including atrophic gastritis, intestinal metaplasia, and dysplasia and diagnosis of early gastric cancer,[4] which is virtually based on the theory on intestinal-type gastric cancer pub-

Keywords: Antralization; *Helicobacter pylori*; Gastritis; Precancerous lesions; Gastric carcinogenesis.

Abbreviations: CagA, cytotoxin-associated gene A; CDX2, caudal-related homeobox transcription factor 2; HtrA, high temperature requirement A; ME-NBI, magnifying endoscopy with narrow band imaging; MUC6, mucin 6; NKX6.1, NK6 homeobox 1; PAX6, paired box 6; PDX1, pancreatic duodenal homeobox 1; PGI, pepsinogen I; SCF, stem cell factor; SPEM, spasmolytic polypeptide-expressing metaplasia; STAT3, signal transduction and activator of transcription 3; TFF2, trefoil factor 2; T4SS, a type IV secretion system encoded in the *cag*-pathogenicity island; VacA, vacuolating cytotoxin A; WHO, World Health Organization.

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lished by Correa *et al*. In 1990, Correa and colleagues[5] proposed a model of gastric carcinogenesis,[5] typically for intestinal-type gastric cancer, with the following sequential stages: from normal gastric mucosa to acute and chronic superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and subsequently early gastric cancer.

Correa's cascade of gastric carcinogenesis is now widely accepted, with *H. pylori* infection being recognized as the major initiating factor in the process. Eradication of *H. pylori* infection is shown to reduce the risk of developing gastric cancer, and thus considered to be a strategy to prevent gastric cancer.[3] However, the preventive efficacy is limited in the presence of precancerous lesions, such as atrophic gastritis, intestinal metaplasia, and dysplasia.[6] In fact, there is no consensus on whether atrophic gastritis and intestinal metaplasia are reversible, although it is generally agreed that dysplasia is irreversible. Whereas some studies have demonstrated that *H. pylori* eradication decreases the degree of atrophic gastritis and intestinal metaplasia, others have failed to confirm the findings.[7–15] Therefore, there is not sufficient evidence indicating the reversibility of atrophic gastritis and intestinal metaplasia, and identification of a reversible pathological change that occurs immediately prior to these precancerous lesions is crucial to developing an early intervention strategy that will cease or even reverse the development of gastric cancer.

In 1998, with the in-depth investigation of gastric mucosal histology, Xia *et al*.[16] observed that *H. pylori* infection was associated the presence of antral-type mucosa in gastric incisura, which was further associated with atrophic gastritis and intestinal metaplasia. They coined a term "antralization" for this histological change. In addition, the authors reported later that antralization was present at the edge of proximal gastric ulcers and disappeared after *H. pylori* eradication in a substantial proportion of patients.[17] In 1999, Schmidt *et al*.[18] reported a metaplasia lineage in the stomach that specifically expresses spasmolytic polypeptide (also called trefoil factor 2 or TFF2) that is associated with gastric cancer. The authors defined this lineage as spasmolytic polypeptide-expressing metaplasia (SPEM), which is believed to share the same histological origin, *i.e.* they are derived in the same mechanisms at the molecular, cellular and tissue levels. It is hypothesized that *H. pylori*-induced antralization, or SPEM, may play a key role in the process of gastric carcinogenesis. More importantly, it may be a reversible point prior to the precancerous lesions after eradication of *H. pylori* infection,[17] which may subsequently slow down, cease, or even reverse the process of gastric carcinogenesis; although, convincing evidence is required to prove this hypothesis.

The aim of this article was to review the role of *H. pylori*-induced antralization (or SPEM), in gastric carcinogenesis, specifically for intestinal-type gastric cancer, and its implications in clinical practice. In this review, we use "antralization", where appropriate, to emphasize the carcinogenic roles and clinical implications of this histological change, as we believe that antralization is more suitable to specifically describe the transition of the specialized glands in the gastric body or at the body-antrum junction by mucous-secreting glands.

Literature search

A search of PubMed/PubMed Central, Web of Science, and China National Knowledge Infrastructure was performed in December 2018 to retrieve all literature related to the terms antralization (or antralisation), pyloric (or pseudopyloric) glands, pyloric (or pseudopyloric) metaplasia, pyloric (or pseudopyloric) gland metaplasia, and SPEM. As the present review mainly focuses on antralization and SPEM, we tried to include articles with the highest relevance to the topic of this review. As a result, a total of 40 articles were identified, 13 on antralization (or antralisation), 9 on pyloric (or pseudopyloric) glands, pyloric (or pseudopyloric) metaplasia, pyloric (or pseudopyloric gland) metaplasia, and 18 on SPEM.

Antralization and its synonymous terms

Normal gastric biopsy mucosa can be divided into three types: antral-type (also called pyloric type), body-type (also called fundic, acid-secreting or oxyntic type), and transitional-type (also called junctional or intermediate type).[16,17,19,20] Antral-type mucosa is characterized by coiled and branching antral glands, which are arranged by mucous cells scattered with endocrine cells (mainly G cells and D cells), and a few parietal cells (Fig. 1a). The glands in body-type mucosa are straight tubes, forming parietal cells that produce hydrochloric acid, and there are scattered mucous cells in their upper portion and mainly endocrine cells in their lower portion (Fig. 1b). Transitional-type mucosa is a mixture of structural characteristics and cell types found in the antral and body type mucosa. "Antralization", which was first officially coined in 1998 by Xia *et al.*,[16,17,20] refers to the change of gastric mucosa from the transitional or even body-type to the antral-type, especially in the gastric incisura angularis[16] (Fig. 1c).

This histological change can also be observed at the endocrine level. Rubio *et al.*[19] found that the positive rate of gastrin-producing cells in the mucosa with antralization in the incisura was similar to that in the gastric mucosa of the antrum, but significantly higher than that in the transitional and body-type mucosa in the incisura, and confirmed that antralization was a form of transformation or metaplasia. Indeed, Nookaew *et al.*[21] found that antralization of the corpus mucosa with atrophy was characterized by increased gastrin expression and decreased expression of corpus-related genes, such as those associated with acid production, energy metabolism, and blood clotting. In fact, over the past 5 decades prior to the introduction of antralization or SPEM, a few terms, including pyloric/pseudopyloric glands, pyloric/pseudopyloric metaplasia, and pyloric/pseudopyloric gland metaplasia, have been used to describe the change.[22–26]

As early as in 1949, Hebbel *et al*.[22] described the presence of pseudopyloric glands in the gastric mucosa of some patients with gastritis and pointed out that there were pseudopyloric glands near the healed gastric ulcer. Similar to true pyloric glands, pseudopyloric glands consist of two different types of cells: mucin-containing cells, which lack the characteristics of typical mucous cells but with characteristics between mucous and serous cells, and endocrine cells, which are mainly composed of enterochromaffin cells, pancreatic A cell-like cells, and A cell-like cells (a subtype of gastric A cells).[27] The same phenomenon was found in animal stomach tissue. Hunt[28] found that the ulcerated area of the gastric fundus mucosa was first covered by regenerated epithelium containing mucous glands, which are pseudopyloric glands. Van der Gaag also found antral-like tissue in the nonantral region of canine stomach and found that it was associated with gastritis.[29] Whitehead *et al*.[30] analyzed more than 2,500 biopsy specimens and found that pseudopyloric gland metaplasia often occurred in patients with atrophic gastritis, which may be reversible. These studies demonstrate that pyloric/pseudopyloric glands, pyloric/pseudopyloric metaplasia, and pyloric/pseudopyloric gland metaplasia are associated with chronic gastric injuries. However, these studies are also limited by their observational nature, with little

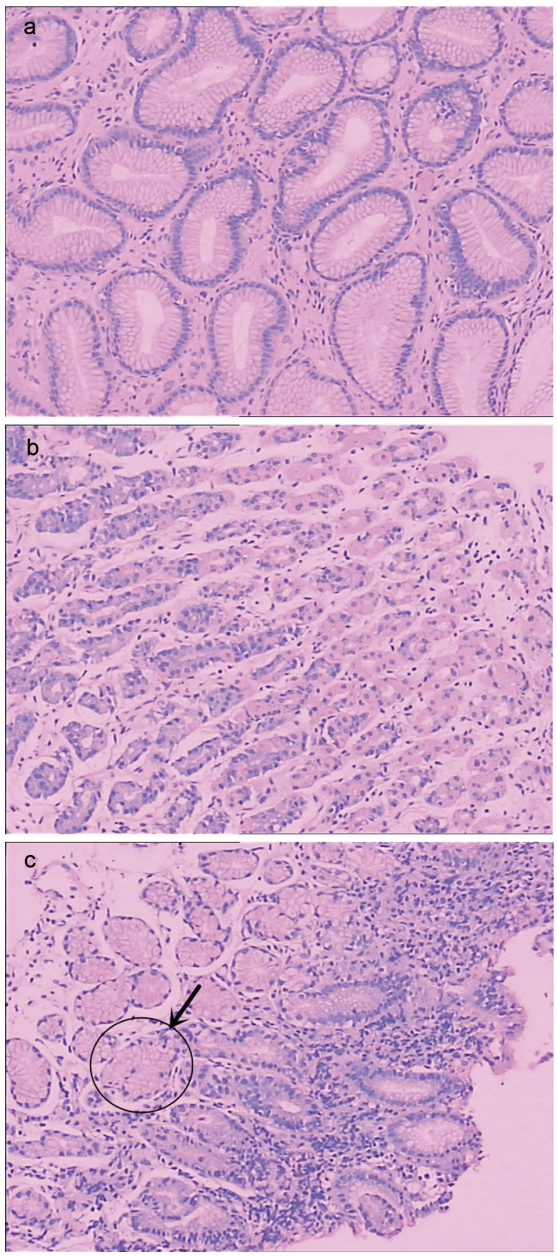

Fig. 1. Gastric glands and antralization. (a) Pyloric glands under light microscope, ×100, H&E staining. (b) Fundus glands under light microscope, ×100, H&E staining. (c) Antralization (arrow) in the gastric incisura under light microscope, ×100, H&E staining. The pictures were provided by Department of Pathology, the First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, China. H&E, hematoxylin and eosin.

exploration on the etiological and pathological significance and the underlying molecular mechanisms.

In 1999, Schmidt *et al.*[18] reported SPEM and its association with gastric cancer. Then, Yamaguchi *et al.*[31] identified SPEM in remnant gastric cancer and considered SPEM as a potential precursor lesion of gastric cancer. Current studies have shown that SPEM is associated with the occurrence of gastric cancer, and it has been proposed that SPEM may develop into intestinal metaplasia, then further into gastric cancer or directly into gastric cancer. However, this hypothesis still needs to be confirmed.[32]

Although all the synonymous terms described above are believed to share the same histological origin, *i.e.* they are derived in the same mechanisms at the molecular, cellular and tissue levels. The terms antralization and SPEM are more commonly used in recent years in various extensive clinical and experimental studies. To emphasize the carcinogenic roles and clinical implications of this histological change, we use "antralization", where appropriate, in this review, as we believe that the term antralization is more suitable to specifically describe the transition of the specialized glands in the gastric body or at the body-antrum junction by mucous-secreting glands.

Role of *H. pylori*-induced antralization (or SPEM) in gastric carcinogenesis

H. pylori-induced antralization (or SPEM) is believed to play an important role in the development of intestinal-type gastric carcinoma, and thus is a critical step of the Correa's cascade. Several mechanisms are proposed.

Colonization of *H. pylori* in the gastric mucosa is a key step in the occurrence of antralization. However, whether or not antralization, and subsequent precancerous lesions, occurs largely depends on the presence of virulence factors of *H. pylori*, including CagA (an effector protein encoded by the cytotoxin-associated gene A), T4SS (a type IV secretion system encoded in the *cag*-pathogenicity island), VacA (vacuolating cytotoxin A), γ-glutamyl transpeptidase, high temperature requirement A (HtrA, a serine protease), and cholesterol glycosyl-transferase, *etc*.[33] A recent study found that *H. pylori* penetrated deep within the body glands in mice with SPEM induced by high-dose tamoxifen, by combining its adhesin, SabA, with the SPEM-related sialyl-Lewis X, and that *H. pylori* colonization further strengthened the expansion of SPEM in the gastric mucosa, which progressed to more serious lesions.[34]

After *H. pylori* colonization, a series of protective reactions occur in the gastric mucosa. Generally, there are two kinds of protective reactions, including superficial reaction, which occurs after acute mucosal injury, and protects the stomach from the endogenous acid and glandular reaction that occurs during chronic infection and leads to the loss or damage of parietal cells and subsequent glandular regeneration, and adapts to the loss or damage of the gastric acid source.[35]

It has been postulated that both apoptosis and cell proliferation increase in *H. pylori*-induced gastritis.[29] The increased apoptosis and proliferation in *H. pylori* infection are regulated by over-expression of tumor suppressor genes, such as *p53*, *bax* or *bak*, and down-expression of the oncogenes, such as *bcl-2*, to maintain their balance. However, when precancerous lesions such as atrophic gastritis, intestinal metaplasia and dysplasia develop, expression of antiapototic genes is increased and pro-apoptotic genes become decreased, resulting in a decrease in apoptosis, whereas proliferation remains increased. Therefore, the balance between apoptosis and proliferation is altered (i.e. in favor of proliferation), thereby increasing the risk of developing gastric carcinoma.[36–38]

Indeed, Xia *et al.*[39] found that apoptosis and proliferation of the epithelial cells in the gastric antrum, incisura, and body mucosa were significantly higher in patients with *H. pylori* infection than in those without infection. These findings suggest that significantly increased cell proliferation and apoptosis exist simultaneously in the process of *H. pylori* infection, which is associated with the process of antralization. Moreover, the authors reported that Ki-67 (a biomarker for cell proliferation) and Bcl-2 expression was

increased, whereas Bax expression was decreased in the presence of atrophic gastritis and intestinal metaplasia,[40] further supporting the hypothesis that the balance between apoptosis and proliferation is altered (*i.e.* in favor of proliferation) in precancerous lesions, leading to increased risk of developing gastric carcinoma.

H. pylori infection is likely to interfere with the balance of cell proliferation and apoptosis by stimulating stem cells, as a previous study found that *H. pylori* infection stimulates the expression of mast cell growth factor (stem cell factor, SCF) in gastric mucosal cells,[41] thus promoting the proliferation and differentiation of stem cells in gastric mucosa. Gastric stem cells are known to play an important role in glandular regeneration and carcinogenesis of gastric cancer.[42–44] At present, most researchers believe that gastric stem cells capable of inducing glandular adaptation mainly exist in the isthmus of gastric gland.[42,43,45–47] Engevik *et al*.[48] found that SPEM hardly occurred in old-age healthy mice, but was induced in old-age mice after the organ transplantation of gastric tissues from the younger mice, suggesting that SPEM-related stem cells are probably derived from the gastric tissue. Matsuo *et al.*[46] discovered that isthmic stem cells were the main stem cells for induction of foveolar hyperplasia and antralization in the gastric corpus in eR1-CreERT2 and Rosa-LSL-td Tomato mouse models, which can be used to cultivate organoids *in vitro*.[46] Nam *et al.*[49] found that SPEM was induced by DMP-777, L-635 and *H. felis* infection in mice, and which evolved from differentiated chief cells. The authors proposed that mature gastric chief cells are able to act as cryptic progenitors and reacquire proliferative capacity in the presence of mucosal injury and inflammation. However, these researchers also showed that in Lgr5-EGFP-IRES-Cre ERT2/+ mice, Lgr-5-expressing chief cells were not the major stem cells for SPEM[50]; although, they were present along the lesser curvature of the gastric oxyntic mucosa. Whether these cells play a role in the subsequent deterioration after antralization still needs to be elucidated.

Many gene expression pathways and biological factors have been demonstrated to be involved in the process of *H. pylori*-induced antralization or SPEM and the subsequent carcinogenesis. Scotti *et al*.[51] found that L-asparaginase of *H. pylori* interferes with the cell cycle of epithelial cells and alters the normal balance between cell proliferation and apoptosis. Ishii *et al.*[52] found that loss of IL-6-mediated activation of signal transduction and activator of transcription 3 (STAT3) signaling significantly reduced the incidence of epithelial cell proliferation, atrophy and metaplasia in *H. pylori* infected mice, and that the inhibition of STAT3 significantly reduced the expression level of the antralization-associated marker TFF2, indicating that the STAT3 signal plays an important role in *H. pylori*-induced antralization or SPEM and gastric carcinogenesis. Huang *et al.*[14] found that *H. pylori*-induced inflammation leads to subclonal mutation, DNA methylation and telomere length changes, thus promoting the development of intestinal metaplasia in the gastric mucosa of infected patients and suggesting that antralization may also be related to the above-mentioned changes.

In addition, other pathways, such as Notch signal, Wnt signal, epidermal growth factor signal, transforming growth factor-β signal and bone morphogenetic protein signal, have also been found to play an important role in the proliferation of stem cells and may be involved in the process of *H. pylori*-induced antralization or SPEM.[43,53,54] It is believed that these biological factors promote gastric stem cells to differentiate into neck mucinous cells and endocrine cells, which makes them more similar in the physiological characteristics of antral glands, until they are completely "antralized" (or covered by SPEM). However, antralization or SPEM does not necessarily develop further to more serious precancerous lesions, such as atrophic gastritis, intestinal metaplasia, dysplasia, or atypical hyperplasia; it may, however, further develop into more serious lesions in the inflammatory environment or may regress in the absence of inflammation.[55] Indeed, Weis *et al*.[55] found that SPEM with inflammation in mice expressed multiple intestinal transcription factors, such as cystic fibrosis transmembrane conductance regulator, similar to the profile of specific transcription factors for intestinal metaplasia in humans, suggesting that inflammation could promote the development of SPEM to intestinal metaplasia. It is, thus, likely that *H. pylori* infection induces chronic inflammation, leading to further progress of antralization (or SPEM) towards atrophic gastritis and intestinal metaplasia. Many inflammatory factors, such as IL-1β, TNF-α and IL-4, have been found to be significantly increased in the course of *H. pylori* infection and to play an important role in *H. pylori*-induced SPEM.[50] A previous study showed that macrophages were the main cells that induce the inflammatory response through IL-1β and play an important role in SPEM and the subsequent deterioration process.[56]

A few studies have reported *H. pylori*-associated changes in gastrointestinal microbiota. Ge *et al*.[57] reported that the bacterial composition in the gastrointestinal tract of C57BL/6 (B6) mice changed significantly after *H. pylori* colonization. The investigators also observed that the colonization ability of *H. pylori* was different between C57BL/6 (B6) mice obtained from different laboratories. Accordingly, *H. pylori*-infected mice from Jackson Laboratory had decreased abundance of Bifidobacteriaceae (stomach, colon and feces), Anaeroplasmataceae (stomach and feces), Clostridiaceae (stomach), Coribacteriaceae (colon), and Turicibacteriaceae (feces). *H. pylori*-infected mice from Taconic Sciences had increased abundance of Helicobacteraceae (stomach), Lactobacillaceae (colon and feces), and Mogibacteriaceae (feces). Maldonado-Contreras *et al.*[58] found that the gastric microbiota in *H. pylori*-negative patients had greater abundance of Actinobacteria and Firmicutes, whereas *H. pylori*-positive subjects had greater abundance of Proteobacteria and Acidobacteria. Gao *et al*.[59] showed that Bacteroidetes, Firmicutes and Proteobacteria in fecal microbiota were significantly correlated with *H. pylori* infection. These findings indicate that *H. pylori* colonization may affect the structure and composition of gastrointestinal bacteria in humans, and thus in the gut microbiota.

It is believed that *H. pylori* infection induces antralization of the incisura and body and consequently affects gastric acid secretion, resulting in hypohydrochloria. The anhydrochloric environment is in favor of many bacteria, leading to changes in the microbiota of the stomach. In the meantime, *H. pylori*-induced hypohydrochloria also forms an unfriendly or hostile living environment for *H. pylori*.[60] The abundance of *H. pylori* gradually decreases or even disappears following the development of precancerous lesions.[61,62] This phenomenon indicates that *H. pylori* infection per se is not necessarily required to contribute to the subsequent development of gastric cancer in the presence of precancerous lesions. Then, what causes the further development of more severe precancerous lesions and gastric cancer after the disappearance of *H. pylori*?

One of the potential carcinogenic mechanisms is a series of pathological and inflammatory reactions derived from the original *H. pylori* infection, i.e. *H. pylori* further influencing the progression of gastric cancer from precancerous lesions after its disappearance, indirectly through these reactions.[62] Another possible carcinogenic mechanism is altered gastric microbiota, *i.e.* some gastrointestinal bacteria during *H. pylori* infection or after its disappearance acting as an "accomplice or successor" of *H. pylori* and playing an important carcinogenic role after *H. pylori* disappearance. Indeed, it has also been demonstrated that there are significant differences in the composition of gastrointestinal microbiota among patients with nonatrophic gastritis, intestinal metaplasia, and gastric cancer.[63] This suggests that gut microbiota, especially those in the stomach, may play an important role in the progression of *H. pylori*-related

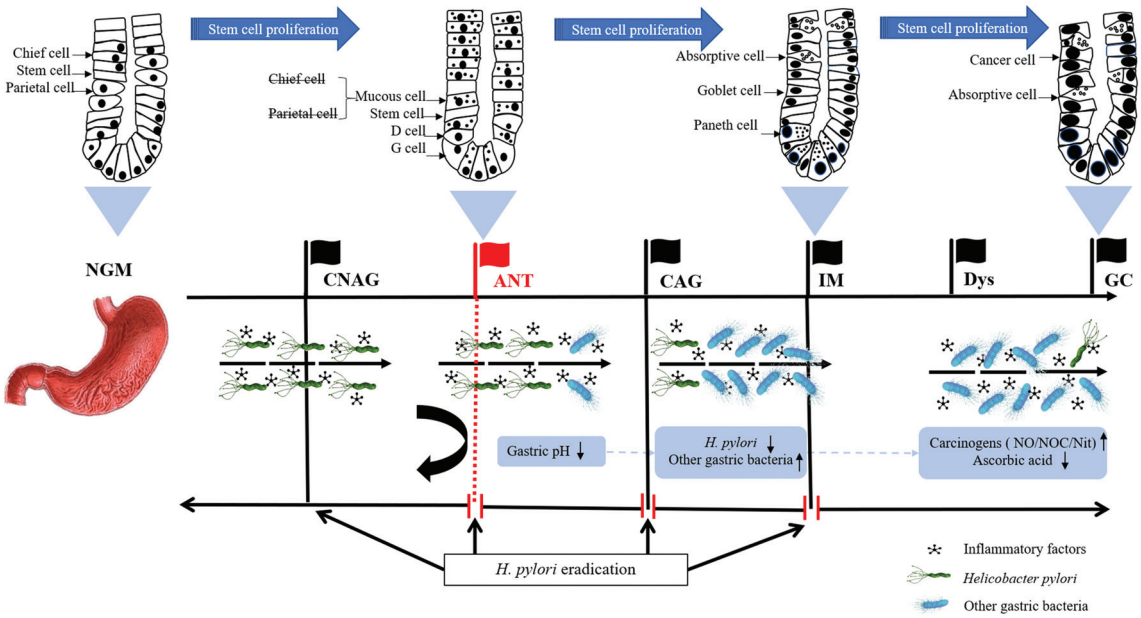

Fig. 2. Proposed role of *Helicobacter pylori*-induced antralization in gastric carcinogenesis. *H. pylori* infection leads to inflammation in the gastric mucosa and apoptosis of the epithelial cells. The stem cells proliferate and transform into mucous cells and form antral-type mucosa (*i.e.* antralization). *H. pylori*-induced antralization may participate in the development of atrophic gastritis, intestinal metaplasia, dysplasia and early gastric cancer, with the presence of carcinogens and gastric bacteria. *H. pylori* eradication may reverse antralization and cease the process from antralization to gastric cancer. ANT, antralization; CAG, chronic atrophic gastritis; CNAG, chronic non-atrophic gastritis; Dys, dysplasia; GC, gastric cancer; IM, intestinal metaplasia; NGM, normal gastric mucosa.

gastric lesions, including antralization, atrophic gastritis, intestinal metaplasia and gastric cancer; although, more experimental evidence is required to confirm the hypothesis. The gastric microbiota is known to cause gastric cancer through chronic inflammation, immune regulation, and microbial metabolites.[64] Therefore, it is conceivable that chronic *H. pylori* infection causes an alteration in gastric or gut microbiota, which plays a critical role in gastric carcinogenesis of *H. pylori*-induced antralization during *H. pylori* infection and in the "post-*H. pylori*" era.

Based on the evidence and findings above, we postulate that in the persistence of *H. pylori* infection the stem cells in the proliferative zone at the proximal stomach, especially the gastric incisura (where acid-secreting gastric body type glands are normally predominant), shift to generate antral-type glands that produce gastric mucins involved in defense and repair mechanisms.[16,65] Chronic insults by *H. pylori* infection thus lead to the replacement of body-type mucosa by antral-type mucosa at the proximal stomach. Antral-type mucosa is believed to be more predisposed to the development of gastric precancerous lesions, including atrophic gastritis, intestinal metaplasia and dysplasia, compared with body-type mucosa.[16] In addition, increased cell proliferation stimulated by persistent *H. pylori* infection presumably facilitates the development of precancerous lesions and intestinal-type gastric carcinoma in antralized mucosa.[36–38] Thus, antralization (or SPEM) may play a critical role in gastric carcinogenesis, and it may also be a reversible step (Fig. 2).[17]

Diagnosis of antralization and its implications in clinical practice

As a reversible pathological step occurring immediately prior to the "point-of no-return" precancerous lesion development during gastric carcinogenesis, antralization can be used as an important indicator for screening individuals with high-risk of gastric cancer for early intervention. Currently, the only diagnostic method of antralization is histological examination of gastric tissue biopsies, which can be used as the golden standard for diagnosis of antralization. The diagnostic criterion is the change of gastric mucosa from transitional-type or body-type to antral-type, histologically characterized by the loss of parietal cells and the substitution proliferation of mucus neck cells and endocrine cells.[16]

There are some critical issues for biopsy-based diagnosis of antralization. First, histological examination of gastric biopsies is associated with upper endoscopy, which is an invasive procedure that is not feasible for screening a population on large scale. Second, previous studies have demonstrated that antralization often occurs at the gastric incisura, and then gradually extends up to the gastric body or even the fundus.[20] Due to the importance of the gastric incisura in the pathological changes, including gastric inflammation and precancerous lesions, the updated Sydney system suggests that endoscopists take four biopsy specimens, two each from the gastric antrum and body, respectively, with an additional biopsy from the incisura angularis.[66] However, this protocol is hardly followed in clinical practice, particularly in developing countries, including China where the upper endoscopic workload is huge and patients usually cannot afford the cost for histological examination of five biopsy specimens. In addition, histological examination is a tedious procedure that may take days to produce results.

Clinically, the combination of magnifying endoscopy with narrow band imaging (commonly known as ME-NBI) may be used to detect antralization, although its performance needs to be further validated. Li *et al*.[67] reported that under ME-NBI, the body-type mucosa presents as a honeycomb-like subepithelial capillary network (dark brown), with an oval dark glandular opening in the

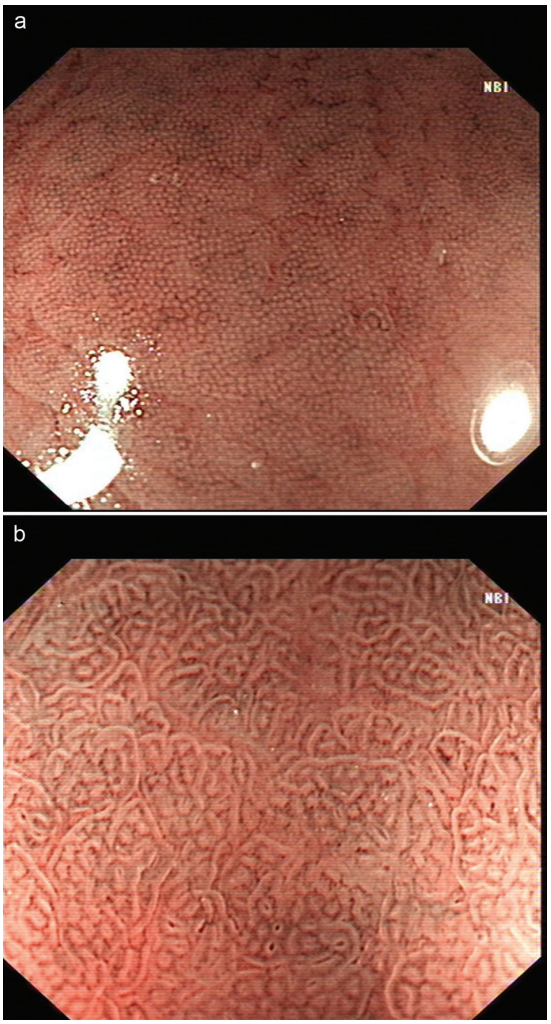

Fig. 3. Normal gastric mucosa. (a) Body-type mucosa under ME-NBI. (b) Antral-type mucosa under ME-NBI. The pictures were provided by Department of Digestive Endoscopy, the First Affiliated Hospital of Guangdong Pharmaceutical University, Guangzhou, China. ME-NBI, magnifying endoscopy with narrow band imaging.

center and interspersed with spider-like collecting veins (Fig. 3a), whereas the antral-type mucosa presents as a curly or wavy subepithelial capillary network, with the spider-like collecting veins being rarely observed (Fig. 3b). Therefore, antralization is considered when the appearance of antral-type mucosa is shown in gastric incisura, body, and fundus under ME-NBI. However, traditional endoscopy is still an invasive operation, and thus not appropriate for screening in the population. Therefore, search for surrogate non-invasive methodologies, such as serological tests of biomarkers, that are simple, rapid and cheap, and which can accurately diagnose antralization of the proximal stomach, are required.

Several biomarkers have been explored for their ability to diagnose antralization. For example, the regenerated antral-like mucosa is often characterized by the antral glands;[16] moreover, the TFF2 and mucin 6 (MUC6) expressed in antral glands are specific indicators of antralization.[65] Sakai *et al*.[68] reported co-expression of MUC6 and pepsinogen I (PGI) as a characteristic feature of the pseudopyloric gland. The authors also demonstrated that the pancreatic duodenal homeobox 1 (PDX1), one of ParaHox gene family members, was expressed in antralization and intestinal metaplasia but not in advanced lesions. Moreover, they also found that the ratio of MUC6 to MUC5AC was higher in patients with antralization and lower in patients with intestinal metaplasia. Xia *et al*.[40] found that in the antralized mucosa, chronic atrophic gastritis and intestinal metaplasia mucosa at the gastric incisura, cell proliferation and Bcl-2 increased, while Bax expression decreased. Moreover, this group of researchers also demonstrated that the expression of PDX1, NK6 homeobox 1 (NKX6.1) and paired box 6 (PAX6) was lower in the cytoplasmic chamber of antralized mucosa with *H. pylori* infection than that in transitional-type or body-type mucosa; whereas, caudal-related homeobox transcription factor 2 (CDX2) expression was up-regulated in the intestinal metaplasia group.[69] Therefore, biological factors like TFF2 and MUC6 are specifically expressed in the antralized mucosa, while other biological factors like MUC5AC, PAX6, PDX1, PG I, BAX, NKX6.1, BCl-2 and Ki-67 and inflammatory markers like IL-1β, TNF-α, IL-4 and macrophages can also be used to assist in the diagnosis of antralization.[20,65–69] However, a complete prediction system that is based on these specific and general biological factors and evaluates the pathological state of the gastric mucosa for antralization is required to be established and used as an important index for screening and diagnosis of precancerous lesions and early gastric cancer.

Over the past decades, the biological markers for precancerous lesions, such as atrophic gastritis, intestinal metaplasia and early gastric cancer, have been explored extensively, producing some conflicting results. For example, Urita *et al*.[70] reported that the observed lower PG I/II ratios and lower PG I values could be used to identify intestinal metaplasia. This contrasted with the report from Stemmermann *et al*.[71] that there was no notable specificity of PG I and PG I/PG II in predicting gastric cancer. Another study showed that a gastric cancer prediction tool based on a panel of serum biomarkers including PG I, PG II, gastrin-17 (g-17) and anti-*H. pylori* IgG antibody had good performance in identifying high-risk patients.[72] Huang *et al*.[73] further found that serum TFF3 levels were significantly higher in patients with gastric cancer than in their control group, and suggested that the combined detection of serum PG and TFF3 could improve the efficacy of gastric cancer screening. Similarly, Aikou *et al*.[74] found that serum TFF3 levels in patients with gastric cancer were significantly higher than those in the normal group. In addition, the serum levels of TFF1 and TFF2 in patients with differentiated gastric cancer were lower than in those with undifferentiated gastric cancer, and suggesting that this feature may reflect the replacement of foveolar hyperplasia and SPEM with intestinal metaplasia. Finally, other researchers found that TFF2 can predict the severity of SPEM in familial relatives of gastric cancer patients with corpus gastritis index.[75]

Based on the above findings and data, we propose a series of factors that are potential biomarkers specific for antralization of the proximal stomach, as well as intestinal metaplasia and gastric cancer, according to Correa's cascade, in order to provide ideas for further exploring noninvasive methods for the diagnosis of gastric antralization (Table 1).

Future research directions

As mentioned above, *H. pylori*-induced antralization may play a key role in the process of gastric carcinogens. Future research will focus on the specific mechanisms by which it develops into gastric

Table 1. Biomarkers of antralization, intestinal metaplasia and gastric cancer

Biomarker	Change in the expression		
	Antralization	Intestinal metaplasia	Gastric cancer
TFF1	NR	Down	Down
TFF2	Up*	Down	Down
TFF3	NR	Up	Up*
MUC6	Up*	NR	Down
MUC6:MUC5AC	Up	Down	NR
PDX1	Up	Up	Up
CDX2	NR	Up*	Up
PGI	Down	Down	Down
PGII	Up	Up	Up
PGI/PGII	Down	Down	Down
Bcl-2	Up	Up	Up
Bax	Down	Down	Down
Ki-67	Up	Up	Up
PAX6	Down	Down	Down
NKX6.1	Down	Down	Down

*Specific alteration. NR, not reported.

cancer. In addition, a noninvasive method that specifically identifies antralization is currently lacking, and thus further investigation is required to develop a simple, noninvasive diagnostic method specifically for the detection of antralization in order to conveniently identify high-risk gastric cancer patients.

Conclusions

H. pylori-induced antralization (or SPEM) may be an initiating and reversible stage of gastric carcinogenesis.[17] Identification of antralization would help to develop an early intervention (*e.g.* eradication of *H. pylori* infection) to cease or even reverse the process toward development of gastric cancer, and more importantly to identify patients with high-risk for precancerous lesions so that gastric cancer can be detected earlier. Currently, the "gold standard" for diagnosing antralization is pathology, which is invasive and time consuming. Thus, a noninvasive and more convenient diagnostic method is urgently required.

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Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Writing the main part of the article (ZNY), providing some figure and related information (RZ), providing revision suggestions (XXH), guiding writing and revising the article (HHX).

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