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Review Article

The role of non-*H. pylori* bacteria in the development of gastric cancer

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Abstract: There is a complex ecosystem of bacteria and other microorganisms inside and outside the human body, which play an intricate role in maintaining health. In recent years, many researches focused on the relationship between microorganisms and cancer. Studies have identified that numerous microbes are presented in human stomach, which are closely linked to the development of gastric cancer (GC). *Helicobacter pylori (H. pylori*) is the mostly well-studied bacterial pathogen in the stomach, which account for the vast majority of GC. However, recent studies have found that microflora dysbiosis was occurred in mucosa of GC patients, and evidences have potentially proved that microbes other than *H. pylori* are also contribute to the development of GC, while the overall knowledge is still limited. In this review, we summarized the role of gastric flora in GC, especially the possible role of non-*H. pylori* bacteria in the development of GC. These knowledges and awareness may open doors for new therapeutic strategies of GC.

Keywords: Gastric cancer, *H. pylori,* microbiota, carcinogenesis

Epidemiology and environmental and genetic factors of gastric cancer

Gastric cancer (GC) is a common gastrointestinal tumor and ranged as the third leading cause of cancer related deaths worldwide [1], with nearly one million new patients were diagnosed each year, which represent a great health problem [2]. In China, the incidence rate of GC is second in men and fourth in women, and nearly 300 thousand people died of GC in 2011 [3]. Early diagnosis of GC is helpful to improve the therapeutic effect and prognosis. However, a considerable number of patients are in the advanced stage at the time of discovery. Therefore, the early prevention and treatment of GC is particularly important. At present, studies have found that the occurrence of GC is a multi-factor and multi-step process, which is characterized by complex interaction between host and environmental factors.

Among the environmental and dietary factors, living habits and occupational environment are particularly important. It is a commonly accepted that intake of nitrosamine would lead to GC, the concentration of nitrosamine in gastric juice is significantly correlated with the incidence rate of GC [4]. In addition, high salt diet and low intake of fresh fruits and vegetables are also related to GC [5]. Drinking are also risk factors [6], and the World Health Organization International Cancer Research Institute recently listed acetaldehyde produced by alcoholic beverages and endogenous ethanol as a class I carcinogen [7].

The genetic instability of the host is an important feature of GC, involving the genetic and epigenetic changes of host oncogene, tumor suppressor gene, DNA repair gene, cell cycle regulatory factor and signal molecule. Abnormal activation or amplification of oncogenes such as K-ras, HOTTIP, KIF26B can promote the proliferation, migration and metastasis of GC cells [8-10]. While the inactivation of tumor suppressor genes is also closely related to GC. For example, tripartite motif 59 (TRIM59), which encodes ubiquitin ligase, interacts with p53, promotes ubiquitination and degradation of

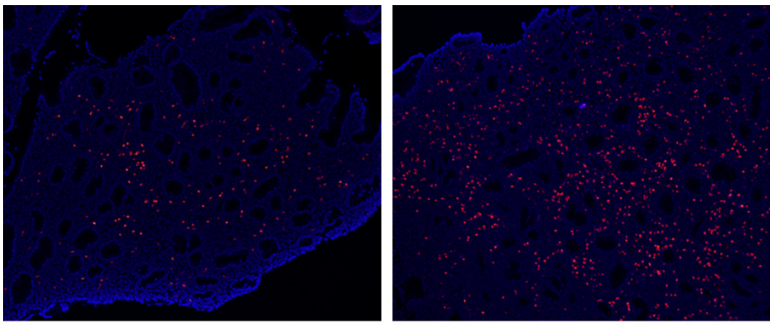

Figure 1. Gut microbiota in gastric mucosa. A large number of bacteria in gastric mucosa was detected by fluorescence in situ hybridization (FISH), ×100 magnification.

p53, and thus promoting the occurrence of GC [11].

Microbe and cancer

It has been identified that a large number of microbiota lives symbiotically in human body and comprise an important microecosystem, which coevolves with host and has a great effect on human health [12]. Scientists have shown that a diverse microbial flora inside in human gastrointestinal tract, which are responsible for maturation and regulation of host metabolism and immunity [13, 14]. The microbial diversity in human body is site-specific, which changes according to the position of the body, and may be related to human health. Dysbiosis of those bacteria may contribute to diseases in multiple systems of human body, including cancer, which provides a new perspective for cancer research and treatment.

Although cancer is often thought to be closely linked to genetic and environmental factors, evidences proved that gut microbiota has emerged as a critical environmental factor for some cancers, such as colorectal cancer (CRC), hepatocellular carcinoma, pancreatic cancer and so on [15, 16]. For example, *Bacteroides fragili, Fusobacterium nucleatum, certain strains of Escherichia coli* and other opportunistic pathogens, which have been investigated to have the potential to participate in the occurrence and progression of CRC [17, 18]. *Fusobacterium* species has been well established that it can promote the carcinogenesis of pancreatic cancer [19]. The mechanism of tumor formation promoted by microbiota can be divided into the following three categories: firstly, affect the balance of host cells between proliferation and death; secondly, regulate the immune function of host; thirdly, participate the metabolic process of host [20, 21].

Complex microbiota in the stomach

Studies have found that GC is an inflammation-associated disease, thus the factors affecting the mucosal immune response may play a role in the development of GC [22]. The stomach is considered a sterile organ due to the acid-producing function of the stomach, the return of bile acids in the stomach, the thickness of the mucus layer, and the effectiveness of gastric motility, which hinders colonization of bacteria in the stomach. Consequently, the complexity of the microbiota in the stomach is usually much lower when compared to that in the intestinal and oral [23]. The development of methods based on 16S rRNA genes, such as spot hybridization with rRNA targeting probes, fluorescence in situ hybridization, denaturing gradient gel electrophoresis, cloning and sequencing of rDNA, temperature gradient gel electrophoresis, these methods have gradually made people aware that a complex bacteria are present in the stomach (**Figure 1**) [24]. The microbiome in stomach of human mainly contains five phyla, they are Bacteroides, Actinomycetes, Firmicutes, Proteobacteria, and Fusobacteria respectively [24]. Besides, many bacteria are isolated from the stomach of patients with hypochlorhydria, which contains *Lactobacillus*, *Streptococcus, Pseudomonas, Xanthomonas, Proteus, Klebsiella, Neisseria, E. coli* and *Campylobacter jejuni* [25].

As a part of tumor microenvironment, microbiome may play an important role in the development, progression and metastasis of GC. Studies have found that the initiation and progression of GC are highly related to the changes of microbial structure [26]. Consequently, the gut microbiota was also considered as an important factor for progression of GC.

The relationship between gastric cancer and *Helicobacter pylori*

It is generally recognized that persistent infection of *Helicobacter pylori (H. pylori*) triggers the inflammatory cascade, followed by atrophic gastritis and increased risk of developing GC [27]. First, *H. pylori* can penetrates the mucus layer and colonizes the gastric mucosa [28], and produces ammonia and $HCO3^{2-}$ by using urease and α-carbonic anhydrase, thereby reducing gastric acidity and resulting high pH [29]. By influencing the expression of mucins Muc1, Muc4, and Muc5b, *H. pylori* could change the mucus barrier [30]. In addition, the virulence factors of *H. pylori* are closely related to GC, CagA and VacA are well-studied. Phosphorylated CagA could regulate the structure and function of cytoskeleton and intercellular connections, thereby destroying mucosal integrity [31]. CagA could also promotes the epithelial mesenchymal transition (EMT) of GC by triggering oncogenic YAP pathway [32]. Recent studies also identified that CagA correlated with autophagy in the progression of GC [33, 34]. As far as another virulence factor VacA is concerned, it can regulate the metabolism of host cells by inhibiting mTORC1 [35], and promotes apoptosis of gastric epithelial cells by interfering with function of mitochondria [36]. Autophagy induced by VacA is another mechanism by which VacA induces gastric inflammation and promotes GC [37]. Besides, VacA can also bind to CD4+ T cells and inhibit antigen-dependent T cell proliferation by inactivating dephosphorylation of the transcription factor nuclear factor of activated T cells (NFAT) [38].

The role of *H. pylori* in the development of GC has been widely studied. However, many studies have found that other bacteria in the stomach also play an indispensable role in the development of GC.

The role of non-*H. pylori* bacteria in gastric cancer

A recent report investigated that the incidence of GC has increased in some regions despite a reduction in *H. pylori* infection [39], which may inspire us that some bacteria other than *H. pylori* in the stomach also contribute to the development of GC. Although *H. pylori* colonization rates exceed 50% worldwide, only 1-3% of individuals infected with *H. pylori* will develop GC [40], and eradication of *H. pylori* could not completely prevent the development of GC [41]. In *Helicobacter*-free INS-GAS mouse model of spontaneous GC, the onset of tumorigenesis was delayed in mouse infected with *H. pylori* alone when compare with those infected with *H. pylori* and other gastric microbiota, which indicating that *H. pylori* may not acting alone to promote GC [42]. Those evidences indicate that bacteria other than *H. pylori* also play a potential role in the carcinogenesis and progression of GC in mice [43, 44].

Dysbiosis of gastric microbiota in gastric cancer

In addition to *H. pylori* colonization, there are also many other bacteria colonized in the gastric mucosa or in the gastric cavity. In human beings, chronic *H. pylori* infection or the use of drugs such as proton pump inhibitor can reduce gastric acid secretion and result in hypochlorhydria (pH between 4-7) or achlorhydria (pH 7), thereby creating a favorable environment for colonization and reproduction of other bacteria [45, 46]. Those microbes could also interact with gastric mucosa.

In the last few years, scientists have detected that the flora of GC mucosa has changed compared with that of healthy people, that is, the dysbiosis occurred, and those researches show that the characteristics of this dysbiosis can distinguish gastric cancer from other diseases [47, 48]. A study found that the microbes in gastric cardia adenocarcinoma (GCA) tissues are mainly composed of *Firmicutes, Bacteroidetes and Proteobacteria* at the phylum level [47]. In addition, there is a significant difference in composition of microbiota between non-atrophic gastritis (NAG) and GC, of which the bacterial diversity is gradually reduced from NAG to intestinal metaplasia (IM) and then to GC, and the abundance of *Helicobacteria* was decreased while intestinal commensals were enhanced [49, 50]. At genus level, remarkable increases in abundance of *Achromobacter, Citrobacter, Phyllobacterium, Clostridium, Rhodococcus* and *Lactobacillus* in GC were found when compared with chronic gastritis [50], and the relative abundance of *Prevotella, Streptococcus, Veillonella, Haemophilus* and *Neisseria* were increased in GCA tissues when compared with non-tumor tissues [47]. At species level, *Prevotella melaninogenica, Streptococcus angino-*

sus and *Propionibacterium acne* were increased, while *H. pylori* and *Bacteroides uniformis* were deceased in tumor tissues [51]. Besides, changes in microbial composition at various stages of gastric cancer development were also identified in a recent study, in which *Peptostreptococcus stomatis, Streptococcus anginosus, Parvimonas micra, Slackia exigua* and *Dialister pneumosintes* were found to have potentially important roles in GC progression [52]. There are still many studies have found that the flora of GC patients has changed, and the significantly changed flora in the cancer tissue at different levels of classification have been identified [53, 54]. From the above evidences, we may conclude that bacteria with high abundance in the microenvironment may play an essential role in the development of GC. Dysbiosis of GC flora are summarized in **Table 1**.

The differences of changed microbiota in the above explorations may be caused by different regions, levels of flora and other factors. However, another relevant point that needs to be considered is that whether those microbiota act alone or in combination with *H. pylori* to promote carcinogenesis of GC. Since several studies have drawn our attention to that the abundance of *H. pylori* was decreased in gastric cancer tissues, which may demonstrate a critical role of bacteria other than *H. pylori* in promoting the progression rather than initiation of GC, yet further researches are needed to prove that.

Non-H. pylori bacteria may promote gastric cancer via inducing inflammatory response

A cohort study consist of 268 GC patients and 288 controls, indicating that individuals with higher relative abundance of *Propionibacterium acnes* (*P. acnes*) and *Prevotella copri* (*P. copri*) had a significantly higher risk of GC than non-carriers [55]. Previous study shown that lymphocytic gastritis could be caused by high abundance of *P. acnes*. Furthermore, *P. acnes*-related lymphocytic gastritis produce proinflammatory cytokines, such as IL-15, to promote the development of GC [56]. As for *P. copri*, it has the ability to induce several inflammatory responses by producing redox protein in human body, which result in the presence of several diseases including GC [57, 58]. Therefore, we may hypothesis that *P. acnes* and *P. copri* promote GC through inducing inflammatory condition, but the detailed mechanism still need to be further investigated.

Non-H. pylori bacteria may promote gastric cancer by influencing the function of immune cells in TME

A recent study found correlations between gastric microbiota and immune cells: the number of BDCA2+ plasmacytoid dendritic cell was positively correlated with *Stenotrophomonas,* and the number of Foxp3+ regulatory T cell was positively correlated with *Selenomonas* in the microenvironment of GC. They concluded that those immune cells may be modulated by the changed microbiota, which participated in the formation of immunosuppressive microenvironment [59]. Nevertheless, they did not do experiments to study the mechanism to obtain a convincing evidence. Plasmacytoid dendritic cells (pDCs) and Regulatory T cells (Tregs) have been found to suppress functions of effector cells, thus facilitating tumor cells of GC and other cancers escape from immune surveillance [60, 61]. Therefore, we could suspect that these two bacteria may act with these two immune cells respectively to promote GC, but further experiments are needed.

Another study shown that *Clostridium, Fusobacterium,* and *Lactobacillus* species were abundant in GC. *Clostridium colicanis* and *Fusobacterium nucleatum* were shown to exhibited a diagnostic ability in GC by using a receiver operating characteristic curve analysis [62]. *Fusobacterium nucleatum* has been demonstrated to have potential role in the carcinogenesis of CRC, pancreatic cancer and so on. In our previous study, we had summarized the role of *Fusobacterium nucleatum* in the tumor microenvironment (TME) of CRC [63]. It can affect the function and phenotype of immune cells, such as macrophages, T cells, NK cells, dendritic cells, tumor-associated Neutrophils. Thus, shaping an immunosuppressive environment which is benefit for tumor growth. Therefore, *Fusobacterium nucleatum* may also favors the development of GC.

Non-H. pylori bacteria may promote gastric cancer through the production of metabolites

It should be noted that recent reports have shown a sustained increase in the abundance

Table 1. Dysbiosis of gastric microbiota in gastric cancer

Year	Sample size	Results	Reference
2009	10 patients with GC, 5 dyspeptic controls.	Gastric cancer microbiota was dominated by different species of the genera *Streptococcus, Lactobacillus, Veillonella* and *Prevotella*, while the abundance of *H. pylori* was relatively low.	[53]
2014	5 patients each of NAG, IM and intestinal-type GC.	Bacterial diversity ranged from 8 to 57 and steadily decreased from NAG to IM to GC ($p = 0.004$). A significant microbiota difference was observed between NAG and GC.	[49]
2014	11 noncardia GC patients, 10 IM patients, and 10 chronic gastritis patients.	The relative abundance of *Streptococcaceae* family significantly increased, while the relative abundance of *Helicobacteraceae* family was significantly lower in the gastric cancer group compared with chronic gastritis and intestinal metaplasia groups.	[54]
2016	A total of 15 patients with hp(-), and 13 healthy people with hp(-) as control.	The number of nitrate-reducing bacteria (NB) other than HP (non-HP-NB) was two times higher in the cancer groups than in the control groups, but it did not reach statistical significance.	[70]
2016	A total of 315 patients, including 212 patients with chronic gastritis and 103 patients with gastric cancer, were enrolled in the study.	Five genera of bacteria with potential cancer-promoting activities were enriched in gastric cancer, including *Lactobacillus, Escherichia Shigella, Nitrospirae, Burkholderia fungorum* and *Lachnospiraceae*.	[64]
2017	54 patients with GC and 81 patients with chronic gastritis.	*Citrobacter* was proved to be abundant in GC, and significant increases in abundance of *Achromobacter, Citrobacter, Phyllobacterium, Clostridium, Rhodococcus* and *Lactobacillus*.	[50]
2018	21 superficial gastritis, 23 atrophic gastritis, 17 IM and 20 GC.	*Peptostreptococcus stomatis*, *Streptococcus anginosus, Parvimonas micra, Slackia exigua* and *Dialister pneumosintes* were significantly increased in GC.	[52]
2019	230 normal, 247 peritumoral and 229 tumoral tissues.	*Prevotella melaninogenica, Streptococcus anginosus* and *Propionibacterium acne* were increased, while *H. pylori, Prevotella copri* and *Bacteroides uniformis* were deceased in tumor tissues.	[51]
2019	The study participants included 268 GC patients and 288 controls.	Family: The relative abundance of *Helicobacteraceae*, *Propionibacteriaceae*, and *Prevotellaceae* higher in GC patients. Genus: The relative abundances of *Helicobacter, Propionibacterium,* and *Prevotella* were higher in GC patients, while *Lactococcus* was decreased. Species: The GC patients had higher relative abundances of *H. pylori*, *Propionibacterium acnes (P. acnes),* and *Prevotella copri (P. copri)* than the controls, while the relative abundance of *Lactococcus lactis (L. lactis)* was higher in the healthy controls than in the patients.	[55]
2019	36 GCA tissue samples and paired nontumor tissue.	The relative abundance of *Prevotella, Streptococcus, Veillonella*, *Haemophilus* and *Neisseria* were increased in GCA tissues when compared with non-tumor tissues.	[47]

GC, gastric cancer; GCA, gastric cardia adenocarcinoma; NAG, non-atrophic gastritis; IM, intestinal metaplasia.

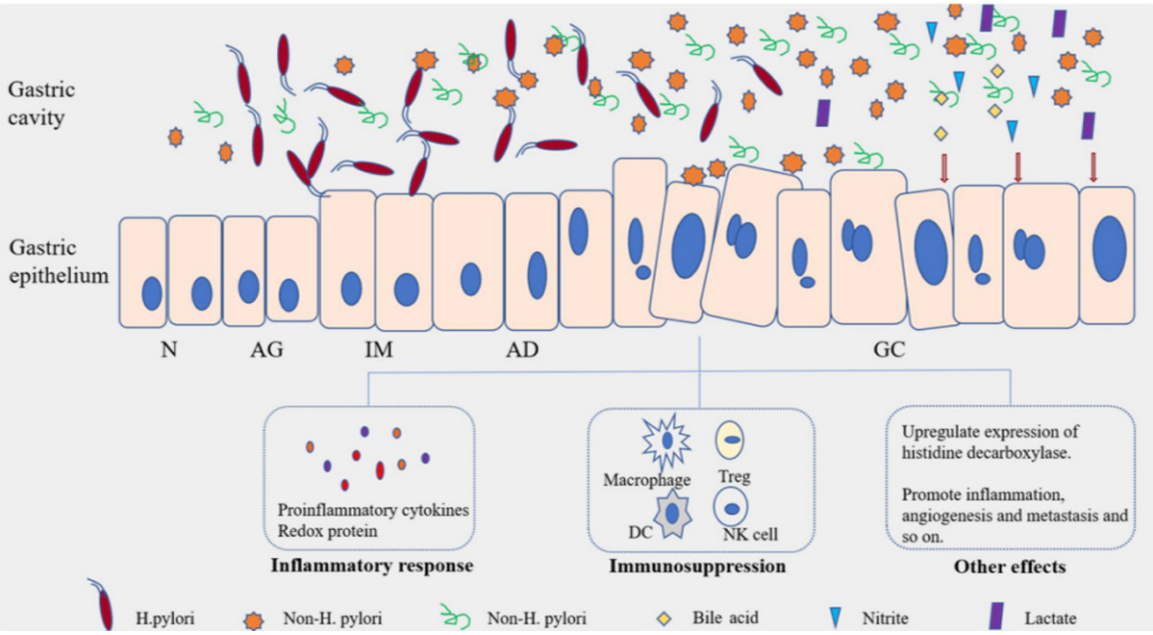

Figure 2. The possible roles of non-*H. pylori* bacteria in the development of gastric cancer. Non-*H. pylori* bacteria promote GC by inducing inflammatory response and producing an immunosuppressive microenvironment. Besides, they could also promote the development of GC through their metabolites such as lactate, nitrite and bile acid. AD, adenoma; AG, atrophic gastritis; GC, gastric cancer; IM, intestinal metaplasia; N, normal.

of lactic acid bacteria (LAB) in GC patients, including *Streptococcus* [52], *Lactobacillus* [50, 64], *Bifidobacterium* and *Lactococcus* [65]. Those LABs were identified to have the ability to promote GC through a number of mechanisms, such as the production of reactive oxygen species (ROS) and N-nitroso compounds, the supply of exogenous lactic acid [66]. The most powerful evidence to prove the role of LABs in development of GC was performed in the Insulin-Gastrin (INS-GAS) transgenic mouse model. Scientists reported that male INS-GAS mice colonized with specific microbiome (including *Lactobacillus* murinus ASF361, *Clostridium* ASF356 and Bacteroides ASF519) could promote gastrointestinal intraepithelial neoplasia, which is associated with a strong upregulation of pro-inflammatory and cancer-related genes [44]. In addition, it has been proved that nitrate can be reduced to nitrite to form a large number of N-nitroso compounds by LABs [67], which is closely related to GC. Moreover, evidence to date clearly shows lactate, the metabolite of LABs, could promote inflammation, angiogenesis, metastasis, and regulate immune response [68, 69], which may influence the outcome of GC.

Besides, the frequency of nitrate-reducing bacterial species was increased in GC, including *Neisseria, Clostridium, Staphylococcus* [70], and *Clostridium colicanis* [62]. Compared with that in chronic gastritis, metagenomes analysis indicated that the functional composition of GC microbiota increased the functions of nitrate reductase and nitrite reductase, those nitrate-reducing bacteria would increasing the concentrations of nitrite and N-nitroso compounds [50]. Animal studies had drawn a convincing conclusion that exogenous nitroso compounds are the cause of GC and several cancers [71]. Therefore, these nitrate-reducing bacteria may also have a close relationship with the occurrence of GC. And as the product of bacteria, nitroso compounds may play an indispensable role in promoting GC. However, there are still no researches about the direct effect of these bacteria on GC.

The imbalance of bile acid (BA) was discovered to be directly associated with GC, affect the carcinogenesis of stomach through upregulation of histidine decarboxylase (HDC) [72]. BAs are produced by host cells and intestinal microorganisms. Primary BAs are produced in the human liver, and secondary BAs are produced from primary BAs by some bacteria in the gastrointestinal tract (GI) including *Clostridium* [73]. When the pylorus sphincter is damaged or dysfunctional, the BAs could flow back to the

Table 2. The possible roles of non-*H. plori* bacteria in the develpoment of gastric cancer

Effects on the development of GC	Microbiota	Mechanisms	References
Promote inflammatory response	*Propionibacterium acnes (P. acnes)*	*P. acnes*-related lymphocytic gastritis produce proinflammatory cytokines, such as IL-15, to promote the development of GC.	[55, 56]
Promote inflammatory response	*Prevotella copri (P. copri)*	It has the ability to induce inflammatory responses by producing redox protein in human body, which result in the presence of several diseases including GC.	[55, 57, 58]
Promote inflammatory response	*Lactobacillus murinus*	In the transgenic INS-GAS mouse model, mice colonized with *Lactobacillus murinus* ASF361 could promote gastrointestinal intraepithelial neoplasias, which is associated with a strong upregulation of pro-inflammatory and cancer-related genes.	[44]
Influencing the function of immune cells in TME	*Stenotrophomonas*	pDCs has been found to suppress functions of immune effector cells, which was positively correlated with *Stenotrophomonas* in GC tissues. Thus, *Stenotrophomonas* may facilitate tumor cells of GC by promoting cancers escape from immune surveillance.	[59, 60]
Influencing the function of immune cells in TME	*Selenomonas*	Tregs was repoted to have immunosuppressive effect, which was positively correlated with *Selenomonas*. Therefore, *Selenomonas* may promote GC by immunosuppressive effect of Tregs.	[59, 61]
Influencing the function of immune cells in TME	*Fusobacterium nucleatum (Fn)*	It can affect the function and phenotype of immune cells, such as macrophages, T cells, NK cells, dendritic cells, tumor-associated neutrophils. Thus, shaping an immunosuppressive environment which is benefit for tumor growth.	[62, 63]
Promote GC through the production of metabolites	*lactic acid bacteria (LAB)*	Lactate, the metabolite of LABs, could promote inflammation, angiogenesis, metastasis, and regulate immune response, which may influence the outcome of GC.	[68, 69]
Promote GC through the production of metabolites	*lactic acid bacteria (LAB)*	LABs promote GC by producing reactive oxygen species (ROS) and N-nitroso compounds.	[66, 67]
Promote GC through the production of metabolites	*Neisseria, Clostridium, and Staphylococcus*	Those nitrate-reducing bacteria would increase the concentrations of nitrite and N-nitroso compounds.	[50]
Promote GC through the production of metabolites	*Clostridium colicanis*	*Clostridium colicanis* can reduce nitrate to nitrite.	[62]
Promote GC through the production of metabolites	*Clostridium*	*Clostridium* could produce Secondary bile acid, which was discovered to be directly associated with GC by affecting the carcinogenesis of stomach through upregulation of histidine decarboxylase (HDC).	[72-74]

GC, gastric cancer; INS-GAS, Insulin-Gastrin; NK, natural killer; pDCs, Plasmacytoid dendritic cells; Tregs, regulatory T cell.

stomach by bile reflux [74]. Previous study had identified that the abundance of *Clostridium* was increased in GC tissues [50], thus *Clostridium* may utilize the BAs refluxed to stomach to produce secondary BAs and promote the development of GC.

Conclusion

Evidences have indicated that alterations in microbiota are potentially associated with occurrence and progression of cancer [75]. In the stomach of healthy people, the acid environment in the stomach is not suitable for bacterial colonization, which can kill many microorganisms entering the gastrointestinal tract, and provide an effective barrier to prevent microorganisms from entering the body [76]. There is overwhelming evidence supporting the notion that *H. pylori* play a vital role in GC, while few studies identified the mechanisms of non-*H. pylori* bacteria that also play an important role in the development of GC. In this paper, we discussed the possible roles of non-*H. pylori* bacteria in GC, including induction of inflammatory response, creation of an immunosuppressive TME and production of some metabolites that is responsible for tumorigenesis (**Figure 2**; **Table 2**). Therefore, various *in vitro* and *in vivo* experiments are still needed, which will provide a better strategy for GC prevention and therapy.

Disclosure of conflict of interest

None.

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