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MINIREVIEWS

Participation of microbiota in the development of gastric cancer

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Abstract

There are a large number of bacteria inhabiting the human body, which provide benefits for the health. Alterations of microbiota participate in the pathogenesis of diseases. The gastric microbiota consists of bacteria from seven to eleven phyla, predominantly *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria*. Intrusion by *Helicobacter pylori* (*H. pylori*) does not remarkably interrupt the composition and structure of the gastric microbiota. Absence of bacterial commensal from the stomach delays the onset of *H. pylori*-induced gastric cancer, while presence of artificial microbiota accelerates the carcinogenesis. Altered gastric microbiota may increase the production of N-nitroso compounds, promoting the development of gastric cancer. Further investigation of the carcinogenic mechanisms of microbiota would benefit for the prevention and management of gastric cancer.

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Key words: Microbiota; *Helicobacter pylori*; Gastric cancer; Nitrite; Metagenomics

Core tip: The gastric microbiota consists of bacteria from seven to eleven phyla, predominant with *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Actinobacteria* and *Fusobacteria*. Absence of bacterial commensal from the stomach delays the onset of *Helicobacter pylori*-induced gastric cancer, while presence of artificial microbiota accelerates the carcinogenesis. Altered gastric microbiota may increase the production of N-nitroso compounds, promoting the development of gastric cancer. Further investigations of the carcinogenic mechanisms of microbiota would benefit for the prevention and management of gastric cancer.

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INTRODUCTION

The surface of the human gastrointestinal mucosa is inhabited by a huge number of microbes of diverse species[1,2]. They interact with each other, constituting an integrated and functional ecosystem, the gastrointestinal microbiota. It provides immune, nutritional and energetic benefits for its host[3]. Disruption of the microbiota may lead to the development of diabetes mellitus, asthma, colorectal cancer and inflammatory bowel disease[4-7].

Gastric cancer is the fourth most common malignant carcinoma and the second leading cause of cancer-related death[8,9]. It is estimated that 989000 new cases of gastric

cancer occur each year[10]. In East Asia, the incidence of gastric cancer is much higher than that in the other regions[11]. The gastric microbiota has long been considered an important factor contributing to the development of cancer[12,13]. Secretion of gastric acid drops in patients with mucosal atrophy. This reduces the acid inhibition of bacterial growth, resulting in the overgrowth of bacteria in the stomach. Under the influence of bacterial enzymes, the production of N-nitroso compounds in the stomach is increased[14]. The latter causes DNA damages and methylation of epithelial cells, promoting the carcinogenesis of gastric mucosa[15-17]. With the advance of the sequencing technique, it is possible to examine the microbiota in details. The role played by microbiota in the gastric carcinogenesis has been re-evaluated. We searched for publications related to gastric cancer and microbiota in PubMed using key words including gastric cancer, microbiota, pH and nitrite. Publications pertinent to carcinogenesis associated with microbiota were selected. The current knowledge of the gastric microbiota and its carcinogenic potentials is reviewed in this paper.

COMPOSITION AND BIODIVERSITY OF THE GASTRIC MICROBIOTA

The median pH of the stomach is 1.4. The high acidity inhibits the survival and proliferation of bacteria in the stomach. However, the gastric mucus forms a pH gradient, thus providing protection of bacteria from acid attack[18]. The presence of non-*Helicobacter pylori* (*H. pylori*) bacteria in the gastric mucosa has been demonstrated using histological methods[19]. A number of bacteria have been isolated from gastric juice[20-22]. The bacterial counts, however, appear to be lower in the stomach than in the other parts of the gastrointestinal tract[23]. It is estimated that there are 10^{2-4} cfu/mL of bacteria in the gastric juice, but 10^{10-12} cfu/mL in the colon. The results using bacterial culture methods show that gastric microbiota is mainly composed of bacteria present in the upper respiratory tract, oropharyngeal and intestinal microbiota. In healthy individuals, *Veillonella* sp., *Lactobacillus* sp. and *Clostridium* sp. are most frequently isolated bacteria from the gastric mucosa[24]. However, the compositions of gastric microbiota vary remarkably between individuals and studies. A study from Spain found that the most abundant bacteria isolated from stomach were *Propionibacterium*, *Lactobacillus*, *Streptococcus* and *Staphylococcus*[23]. Considering the limitations of the bacterial culture method, it is unattainable to thoroughly examine the compositions of the gastric microbiota.

With the advance of the sequencing technology, it is achievable to analyze the gastric microbiota in detail by sequencing the bacterial 16S rRNA gene. Molecular analyses reveal much more diverse microbial communities in the stomach. It harbors more than 130 phylotypes representing seven to thirteen bacterial phyla[25,26]. *Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria* and *Fusobacteria* are the major phyla in the gastric microbiota. The most abundant phyla was *Proteobacteria*, *Streptococcus* and *Prevotella* are the most abundant genus found in the stomach in *H. pylori*-negative subjects[26]. The compositions of gastric microbiota from gastric antrum and corpus are nearly identical. In preterm neonates, bacteria from gastric juice were mainly composed of *Firmicutes*, *Tenericutes*, *Actinobacteria* and *Proteobacteria* in the first week of life, but the abundance of *Proteobacteria* increased steadily, becoming the predominant bacteria by the fourth week of life[27]. Roles of the diverse and abundant gastric microbiota in the pathogenesis of gastric diseases have been explored in recent years.

INFLUENCE OF GASTRIC MICROBIOTA BY *H. PYLORI*

H. pylori is a Gram-negative carcinogenic bacterium colonizing the human stomach[28]. In addition, overgrowth of bacteria in the stomach has been considered to be another risk factor for gastric cancer[12,13]. It is, therefore, a great concern of whether there is an interaction between *H. pylori* and gastric microbiota.

Using a high density 16S rRNA gene microarray, the compositions of gastric microbiota have been analyzed from eight *H. pylori* infected patients and four *H. pylori* negative patients[29]. The relative abundance of *Proteobacteria* (excluding *H. pylori*) and *Acidobacteria* was higher in *H. pylori* infected patients, whereas, greater relative abundance of *Actinobacteria* and *Firmicutes* was found in the gastric microbiota from *H. pylori* negative patients[29]. In experimentally *H. pylori* infected BALB/c mice, the biodiversity of gastric microbiota was increased[30]. Vaccination against *H. pylori* prevented the alteration of gastric microbiota[30]. Therefore, it appears that *H. pylori* infection may alter the composition and biodiversity of the gastric microbiota.

Contradicting findings, however, have been reported. Acute or chronic infection of *H. pylori* did not alter the compositions of murine gastric microbiota[31]. There is no difference in the gastric microbiota between gerbils persistently infected with *H. pylori* and those uninfected[32]. A culture-based analysis of 29 healthy volunteers found that the composition of gastric microbiota was similar regardless of *H. pylori* status[23]. The composition and biodiversity of gastric microbiota were explored by analyzing 1833 sequences of 16S rDNA generated by broad-range bacterial PCR from the gastric mucosa of 23 individuals[26]. Double principal coordinate analysis and redundancy analysis revealed no significant association between phylotype distribution and *H. pylori* status. Hierarchical clustering found no distinct cluster between *H. pylori*-negative and -positive subjects. These findings suggest that the presence of *H. pylori* in the gastric mucosa does not affect the composition of the gastric community. Thus, it appears that *H. pylori* acts more like a commensal bacteria, rather than an intruder, to the gastric microbiota. Further studies are required to clarify the interactions between *H. pylori* infection and the gastric microbiota. This would

substantially enhance our understanding of the development of gastric pathologies, especially gastric cancer.

ROLES OF MICROBIOTA IN THE DEVELOPMENT OF GASTRIC CANCER

It has been proposed that gastric microbiota plays a role in the development of gastric cancer[12,13]. Lowered acid secretion due to gastric atrophy favors overgrowth of bacteria in the gastric fluid, enhancing the production of carcinogenic N-nitrosamine compounds. Recent studies on animal models strongly support the fundamental role of microbiota in the development of gastric cancer. Transgenic INS-GAS mice over-expressing human gastrin may spontaneously develop intramucosal carcinoma[33]. Gastric intraepithelial neoplasia developed in all specific pathogen-free male INS-GAS mice with a complex microbiota 7 mo after *H. pylori* infection[34]. For germ free male INS-GAS mice which were absent of microbiota, however, the incidence of gastric intraepithelial neoplasia was only 10.0%. The incidence merely increased to 44.4% 11 mo after *H. pylori* infection[34]. These results suggest a role of microbiota in the carcinogenesis of the stomach. Furthermore, colonization of the stomach by an artificial intestinal microbiota (Altered Schaedler's Flora, including ASF356 *Clostridium* species, ASF361 *Lactobacillus murinus* and ASF519 B*acteroids* species) increased the incidence of gastric intraepithelial neoplasia to 69.0% in male INS-GAS mice 7 mo after *H. pylori* infection[35]. Antibiotic treatments significantly delayed onset of gastric neoplasia in helicobacter-free and specific pathogen-free INS-GAS mice[36]. These findings indicate the involvement of microbiota in the development of gastric cancer.

Elevation of pH dramatically influences the bacterial growth. Treatments with acid inhibition drugs increase the luminal pH, and the total bacterial count is increased[19,37,38]. It returns to normal after discontinuation of the treatment. The increased pH and bacterial count correlate with the enhanced production of nitrite in the stomach[39]. This could be attributed to the increased abundance of nitrate-reducing bacteria[40], which catalyse the nitrite production from the nitrate reduction. *Haemophilia* and *Veillonella* reduce nitrate more rapidly than nitrite. They could be responsible for the accumulation of nitrite in the stomach[41,42]. An increased luminal pH is common in precancerous conditions and gastric cancer. This may lead to alterations of the compositions of gastric microbiota. In gastric cancer patients, gastric microbiota was predominated by *Veillonella*, *Haemophilus* along with *Streptococci*, *Lactobacillus*, *Prevotella* and *Neisseria*[43]. These studies suggest that alterations of gastric microbiota occur under the influence of pH. Further studies are required to investigate roles and mechanisms of these alterations in the development of gastric cancer. A recent study on *H. pylori* infected mice suggests that gastric microbiota promotes the carcinogenesis, but its composition does not influence the incidence of gastric cancer[34]. For the *H. pylori* infected INS-GAS mice, colonization of the stomach with an artificial Altered Schaedler's Flora including ASF356 *Clostridium* Species, ASF361 *Lactobacillus murinus* and ASF519 *Bacteroides* Species promoted the development of cancer[34]. However, the incidence of gastric cancer did not significantly differ from *H. pylori* infected INS-GAS mice fed under specific pathogen free conditions.

N-nitroso compounds, consisting of N-nitrosamines and N-nitrosamides, are potent carcinogens[35,44]. Humans are exposed to N-nitroso compounds from diet, tobacco smoke and other environmental sources. Increased exposure to these exogenous N-nitroso compounds has been linked to an increased incidence of gastric cancer[45]. The amount of endogenous formation of N-nitroso compounds, however, is much higher than that of exogenous formation[46]. The study on a population of more than a half million individuals revealed that endogenous N-nitroso compounds are significantly associated with gastric cancer[46]. Nitrite is a precursor of the endogenous N-nitroso compounds. Bacterial cytochrom-cd1-nitrite reductase catalyzes the conversion of nitrite to nitrosamines in the presence of secondary amines[47]. In gastric cancer patients, the concentration of nitrite in gastric juice may increase up to 107.6 μmol/L[48]. When the acid output reduces, bacterial overgrowth occurs in the stomach. These bacteria contain both nitrate reductase and nitrite reductase, which catalyze the reduction of nitrate and nitrite, respectively. However, some bacteria have a differential rate in nitrate reduction and nitrite reduction. *Veillonella parvula* and *Haemophilus parainfluenzae* have a higher capacity in nitrate reduction than nitrite reduction, thus increasing nitrite accumulation in the gastric juice[42]. In nature, many bacteria produce enzymes influencing the production of nitrite. Ammonia oxidizing bacteria possess ammonia monooxygenase and hydroxylamine oxidoreductase which catalyze the production of nitrite from ammonia under aerobic conditions[49,50]. Ammonia oxidizing bacteria mainly include species from the phylum of *Planctomycetes*[50]. The phylum of *Nitrospirae* is a group of nitrite oxidizing bacteria. They encode nitrite oxidoreductase which oxidizes the formation of nitrate from nitrite[51,52]. Thus, they tend to decrease the production of nitrite. These bacteria involved in the production of nitrite are widely present in soil, water and marine, where humans are frequently exposed to. Molecular analyses of the gastric microbiota suggest their potential presence in the stomach. Their participation in the accumulation of nitrite in the stomach remains to be studied in the future.

Findings from current studies support a role of microbiota in the development of gastric cancer. However, techniques used in many studies have limited powers in examination of composition, richness and biodiversity of gastric microbiota. With the application of the metagenomics and single cell genomics[53-55], we could further understand the properties of carcinogenic microbiota and mechanisms by which they participate in the genesis of gastric cancer.

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