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Microbial community reshaped in gastric cancer

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Abstract. – Patients with gastric cancer harbor distinct microbiota in the stomach. It features with lowered biodiversity, discrete structure, and varied composition. Some bacteria from gastric microbiota are potentially carcinogenic as they are enriched or depleted in gastric cancer. Distinct profile of microbial community in gastric cancer is possibly resulted from altered caused by pathophysiological and environmental factors. *H. pylori* is a carcinogen colonizing the human stomach. Although persisting for decades, it rarely causes compositional alteration of microbiota. Secretion of acid decreases gradually during the carcinogenic process. Increased pH results in overgrowth of bacteria in gastric fluid. The abundance of a particular taxon, but not the profile of microbiota, is altered in proton pump inhibitor users. Compositions of microbiota vary substantially between individuals, which may account for differential cancer risk. It has been demonstrated that genetic variations contribute to inter-individual variations in gut microbiota. However, their influence on the composition of gastric microbiota requires further exploration. Currently, it appears disrupted homeostasis and inter-individual variations of gastric microbiota are involved in cancer development. Clarifying factors responsible for these changes would reveal how microbiota induces carcinogenesis, benefiting the prevention of gastric cancer.

Key Words:
Gastric cancer, Microbiota, *Helicobacter pylori,* Genetic variations, Gastric acid.

Introduction

Gastric cancer is one of most common cancers[1]. Multiple factors including host genetics, environment, and *Helicobacter pylori* genetics play a role in the development of gastric cancer. *H. pylori* infection is a major risk factor for gastric cancer[2]. Genome-wide association studies have found many genetic variations are associated with gastric cancer[3-5]. Decreased production of gastric acid, smoking, alcohol and diet factors increase cancer risk.[6] Recent scholars[7,8] demonstrate a role of gastric microbiota in cancer development.

Transgenic insulin-gastrin (INS-GAS) mice have been used as a model for studying gastric cancer[9]. Germ-free INS-GAS mice develop minimal mucosal lesions through seven months of age[10]. In the presence of gastric microbiota, however, severe lesions including inflammation, atrophy, and dysplasia occur at seven months of age[10]. Thus, microbiota is likely to promote the development of precancerous lesions in susceptible hosts. Seven months after infection with *H. pylori*, only 10% gnotobiotic mice develop gastric neoplasia[11]. In contrast, cancer was found in all specific pathogen-free mice harboring a complex microbial community in the stomach. Therefore, the presence of microbiota in the stomach is capable of promoting the development of *H. pylori*-induced cancer. Treatments with antibiotics decrease the incidence of gastric cancer in mice[12]. Collectively, these findings demonstrate gastric microbiota is involved in cancer development in mice.

To understand how it participates in cancer development, gastric microbiota has been characterized in recent studies[13-16]. Findings from these studies demonstrate substantial alterations of gastric microbiota. This review was aimed to summarize the features of microbiota in gastric cancer and discuss roles of gastric acid, *H. pylori* and genetic variations in reshaping gastric microbiota.

Discrete Profiles of Cancer Associated Gastric Microbiota

Microbes in the stomach have long been considered as a factor contributing to the development of gastric cancer[17,18]. Overgrowth of bacteria in the gastric fluid may produce an increased amount of nitroso compounds causing DNA damages and promoting cancer development[19-21]. To understand its carcinogenic mechanisms, micro-

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biota in gastric cancer has been characterized[13-15]. The total number of bacteria in the stomach increased in gastric cancer compared to chronic gastritis[15], while it decreased when removing cancer with subtotal gastrostomy[22]. These findings suggest bacteria overgrowth occurs in gastric cancer. The α-diversity measures the number of species (species richness) and the evenness of the relative abundance[23]. Comparing with chronic gastritis, the species richness is reduced in gastric cancer, but increased after subtotal gastrectomy[22]. This indicates gastric microbiota consists of less bacterial species in cancer patients. The structure of microbiota is usually reflected with β-diversity. It measures the similarity between individual microbial community in the number, relative abundance and phylogenetic relatedness of taxon[23]. The structure of gastric cancer is distinct, varying from that in chronic gastritis. This has been repeatedly demonstrated. Overall, microbiota in gastric cancer shows a distinct profile featured with more bacteria, less species, and discrete structure.

In gastric cancer, the most abundant predicted functions of microbiota are membrane transport, replication and repair, carbohydrate, amino acid and energy metabolism[22,24].

Before surgical removal of cancer, genes related to denitrification and nitrosation are more abundant[22]. These genes are associated with the production of mutagenic N-nitroso compounds, which may increase the risk for gastric cancer.

Bacteria enriched or depleted in gastric cancer are of particular interest since they are potentially carcinogenic. These cancer-associated bacteria have been identified, including *Streptococcus*, *Escherichia*, *Staphylococcus*, *Lactobacillus*, nitrosating bacteria and nitrate-reducing bacteria[14,25-28]. *Escherichia coli* is closely associated with colorectal cancer[26,29]. The relative abundance of *E. coli* was higher in gastric cancer than that in chronic gastritis[15], suggesting a potential role in cancer development. *Staphylococcus* is a commensal in human stomach[14,27]. It was significantly more abundant in low cancer risk region of Tumaco[16], suggesting a role in cancer. *Haemophilia*, *Veillonella*, and *Nitrospirae* belong to nitrosating bacteria and nitrate-reducing bacteria[28,30]. The production carcinogenic of N-nitroso compounds is possibly enhanced by these bacteria[8]. These enriched bacteria likely participate in the carcinogenesis.

Causes of microbiota variations in gastric cancer remain unclear. Gastric microbiota in intestinal metaplasia shows a transient structure from chronic gastritis to gastric cancer, suggesting a gradual change in the carcinogenic process[14]. This may be caused by inter-individual variations of microbiota. That is to say, certain individuals harbor a cancer prone microbiota. Otherwise, pathophysiological changes during carcinogenesis possibly contribute to alterations of microbiota.

Gastric Acid, Proton Pump Inhibitors and Microbiota

Lowered gastric acid is a risk factor for gastric cancer[31]. Patients with family history of gastric cancer have a reduced acid output and increased incidence of precancerous lesions[32]. Interleukin-1β potently promotes secretion of gastric acid[32]. Decreased expression of this gene caused by genetic variations leads to decreased acid secretion and increased susceptibility to gastric cancer[32]. Reduction of gastric acid may enhance bacteria overgrowth in the stomach, thus promoting the development of gastric cancer.

Influence of acid on gastric microbiota has not been well characterized. Proton pump inhibitors (PPI) reduce acid output causing a sustained rise in gastric pH[33]. Characterization of gastric microbiota in PPI users may help in elucidating of the influence of acid on the microbiota. Bacterial overgrowth has been found in gastric fluid but not gastric mucosa during PPI treatments[34]. The structure of gastric microbiota is not significantly different in PPI users[35]. The species richness of gastric microbiota is not altered by PPIs, although it seems that their relative abundance is slightly changed[35]. These findings suggest increased pH could not cause substantial changes in gastric microbiota. Nonetheless, a higher relative abundance of *Firmicutes* has been found in PPI-treated patients[35]. At the genus level, the relative abundance of *Capnocytophaga*, *Granulicatella* and *Streptococcus* was increased[35]. In addition, *Lactobacillus* is enriched in gut microbiota during PPI treatments[36]. These findings demonstrate PPI treatments are capable of altering the relative abundance of some bacteria in gastric microbiota.

Causes of alterations of microbiota in gastric cancer remain largely unclear. *Lactobacillus* is enriched in gastric cancer[14]. This has been verified by various studies[14,15,37]. *Lactobacillus* is capable of tolerating acid and proliferating under weak acid conditions[38]. Elevated pH in the carcinogenic process of stomach probably facilitates the overgrowth of *Lactobacillus*, resulting its enrichment in gastric cancer. This is supported by the finding that the relative abundance of *Lactobacillus* is increased

during PPI treatments[36]. Enrichment of *Streptococcus* in PPI users suggests decreased gastric acid may contribute to its increased abundance in gastric cancer[35]. Therefore, certain enriched bacteria in gastric cancer are most likely caused by reduced gastric acid. At present, contributions of acid associated alterations of gastric microbiota to the development of gastric cancer remain largely unknown. Many species of *Lactobacillus* have been used widely as probiotics in the clinical setting[39-41]. Certain strains have been used in the treatment of *H. pylori* infection, modulation of the microbiota and alleviation of inflammation[42-44]. It seems very unlikely that *Lactobacillus* is of carcinogenic potentials. In contrast, *Streptococcus* was more frequently found in gastric tumor tissues compared to the surrounding nor-malignant tissues. *Streptococcus bovis* is associated with colorectal cancer[45,46], suggesting a close association with the development of gastric cancer. Thus, caution must be taken in explaining the carcinogenic role of the alterations of gastric microbiota.

Association of Helicobacter Pylori with Gastric Microbiota

Gut microbiota starts to establish soon after the birth of hosts and becomes usually matured before the age of three years[47]. Since then gut microbiota remains stable although fluctuation may occur under some environmental changes[48]. Acquisition of carcinogenic *H. pylori* appears to occur after the maturation of microbiota[49]. Infection by the pathogen usually lasts for decades unless eradicated. The influence of chronic infection of *H. pylori* on the indigenous gastric microbiota is of great concern.

The structure of gastric microbiota has been compared between *H. pylori*-infected and uninfected individuals. PCoA analyses demonstrate a distinct segregation of gastric microbiota in *H. pylori*-infected patients from that in uninfected patients[15,37]. This suggests gastric microbiota associated with *H. pylori* infection may be altered. However, these analyses are based on the combined sequences of *H. pylori* and indigenous gastric microbiota. When *H. pylori* sequences are excluded from analyses, gastric microbiota appears to be not altered significantly in *H. pylori*-infected patients. The relative abundance of bacterial genera in gastric microbiota showed no significant difference between *H. pylori*-infected and uninfected patients[13]. In *H. pylori* eradicated patients, the relative abundance of major bacteria phyla or genera were very similar to that in *H. pylori*-negative controls[50]. In Rhesus Monkey, infection by *H. pylori* did not alter the abundance of bacteria genera in indigenous gastric microbiota[51]. However, some studies argue that some component of gastric microbiota might be associated with *H. pylori* infection. *H. pylori* infection reshapes the composition of gastric microbiota in pediatric patients, but not in adults[52]. A recent study suggests that *H. pylori* abundance is correlated with the presence of *Campylobacter*, *Deinococcus*, and *Sulfurospirillum*[53]. Further studies are required to resolve this controversy.

The *cag* pathogenicity island is a major virulent determinant of *H. pylori*[54]. It encodes type IV secretion system that translocates CagA into epithelial cells[55]. The translocated CagA protein causes damages of epithelial cells and promotes malignant transformation[55,56]. Phylogenetic origin is another virulence determinant of *H. pylori*. Current strains of *H. pylori* evolve from different ancestral populations[57]. Cancer-associated *H. pylori* strains seem to have a different phylogenetic origin[58]. In the region of high incidence of gastric cancer, *H. pylori* has a different phylogenetic origin[59]. A recent study found no apparent association of the presence of *cag* pathogenicity island or phylogenetic types of *H. pylori* with microbiota composition[16]. This indicates gastric microbiota is not influenced by *H. pylori* virulence.

Spatial location in the mucus layer appears different between gastric commensals and *H. pylori* [60]. As a pathogen, *H. pylori* inhabited mostly within the inner mucus layer, which is closely attached to the epithelium. It is uncommonly located in the outer mucus layer. The inner mucus is very firm, restricting the penetration of gut commensals through the mucus layer to epithelial cells[61]. Localization of commensals in the inner layer is uncommon[62]. Differences with spatial location might be a determinant for the influence of *H. pylori* on the gastric microbiota.

Interpersonal Variations of Gastric Microbiota and Cancer Risk

Microbiota varies greatly among individuals and between different parts of human body[63]. These variations are not continuous but usually stratified. This is possibly caused by a limited number of host-microbial symbiotic states. Typing gut microbiota reveals three enterotypes in healthy individuals that are not associated with body mass index, age, gender or geographical origin[64]. *Bacteroides*, *Prevotella* and *Ruminococcus* are main contributors for each enterotype. Using Di-

richlet multinomial mixture models, human gut microbiota are partitioned into four metacommunity types. This further supports the stratification of gut microbiota variations[63]. Analyses of global data sets revealed the core fecal microbiota consisted of only 14 genera (present in more than 95% individuals). Whereas 664 bacterial genera were varied among healthy individuals. Huge compositional variations are associated with 69 covariants including medication and diseases[65]. Compositional differences of microbiota may thus account for individual risks for various diseases.

Resembling gut microbiota[66], inter-individual variations of gastric microbiota are large. Each individual has a distinct profile of gastric microbiota. Even for co-twins, the profile is not identical[67]. *Proteobacteria* is the most predominant phylum of gastric microbiota[13]. However, dominance by *Firmicutes* or other phyla has been seen in some healthy individuals[13]. A recent work on gastric microbiota demonstrated the species richness was reduced in patients with family history of gastric cancer, indicating decreased biodiversity may increase individuals' risk for gastric cancer[37]. Geographical variations in microbiota compositions occur frequently. A comparison of gastric microbiota from two regions with contrasting incidence of gastric cancer demonstrates marked differences in the structure of microbial community[16]. *Leptotrichia wadei* and *Veillonella sp.* are enriched in the high-risk region. They are candidate carcinogenic bacteria of gastric microbiota. Otherwise, they may only represent geographical variations of microbiota. Further validation in other high-risk regions is indicated. Inflammation of gastric mucosa is central to the development of cancer. Gastric microbiota varies greatly between C57BL/6N mice from two laboratories, manifesting as differential abundances in *Lactobacillus* species[68]. The degree of mucosal inflammation triggered by *H. pylori* is significantly different. Pretreatment with antibiotics dramatically alters the composition of gastric microbiota and dampens Th1 inflammatory responses of gastric mucosa to *H. pylori* infection. Furthermore, transplantation with normal gastric microbiota restores inflammatory response[68]. These findings demonstrate compositional variations of gastric microbiota contribute to the variations of cancer or inflammation risks.

Microbiota is developed and matured in the early life of hosts[68]. Genetic variations contribute to variations of microbiota[69,70]. Heritable components, defined as the phenotypic variance that can be attributed to genetic variance, have been identified in fecal microbiota[70]. Goodrich et al[71] reported that the taxon with the highest heritability in gut microbiota was *Christensenellaceae*. It is a family within the *Firmicutes* that forms a co-occurrence consortium with other heritable taxa including the dominant human gut methanogen *Methanobrevibacter smithii*. There was a strong signal in the association between unclassified *Clostridiaceae* and the SNP rs10055309 in the gene SLIT3[71]. Associations have been identified between members of the phylum *Firmicutes* and variants in or the expression of host genes involved in the Toll-like receptor and T-cell receptor pathways (IRAK4 and IRAK3, respectively) [72]. Microbiota compositions are affected by host genetics probably through modulating the abundance of individual microbial species, groups of related taxa, groups of distantly related organisms[73].

Gastric cancer risk is influenced by genetic variations of hosts. Some genetic variants are associated with gastric cancer. These include variants located in PSCA, MUC1, ASH1L, PLCE1, ZBTB20, PTGER4-PRKAA1, LRFN2, DNAH11 and ATM genes[4,5,74-78]. It remains poorly understood regarding how these variants increase cancer risks. It would be of interest to explore whether they could participate carcinogenesis through shaping gastric microbiota.

Conclusions

Gastric microbiota shows a distinct profile in gastric cancer. This is possibly resulted from reshaping microbiota by gastric acid, *H. pylori* infection and other factors during the carcinogenic process. Otherwise, inter-individual variations may account for the distinct microbiota profile in gastric cancer. At present, definite cancer-associated bacteria or properties of microbiota remain unclear. Resolving these issues would benefit our understanding of the carcinogenic mechanisms and exploring novel cancer preventing approaches.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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