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Gastric cancer and the microbiome

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PEER REVIEW

ABSTRACT

The human body harbours more than 10^{19} microbial species, a majority of which reside in the alimentary tract. Alterations in the organ specific microbiome have been associated with carcinogenesis, especially in colorectal cancer. *Helicobacter pylori* (*H. pylori*) has been known to be involved in gastric tumorigenesis for almost 2 decades. The ability of *H. pylori* to evade host immune responses and induce genomic aberrations via a gamut of virulence factors such as VacA and CagA probably contributes to its significant role in gastric carcinogenesis. However, recent studies have determined that *H. pylori* is not the sole microbe found in the gastric milieu. Moreover, observed differences in the microbial composition of the gastric milieu between healthy, gastritis afflicted and cancer patients point towards a probable role of these microbes in pathogenesis of gastric diseases. This review aims to summarize the current understanding of the gastric microbiome and its role in influencing gastric carcinogenesis.

1. Introduction

Each individual possesses a distinct set of genetic material that not only defines them as human, but also confers uniqueness upon them. With the exception of monozygotic twins, no two people possess the exact same DNA. In recent times, it has become clear that apart from the genetic material in our own cells, we also possess another entirely different set of genetic material which is just as much a part of us as our own cells – the microbiome. And just like our own DNA, it makes us similar to each other yet distinct at the same time. The term 'microbiome' was coined by Joshua Lederberg and refers to the ecological community of commensal, symbiotic and pathogenic microorganisms that share our body space. The human body harbours 10^{19} microbial species which is 10 times greater than all our somatic and germ cells combined. The gastrointestinal tract is home to the majority of our microbiome. In this review, we aim to outline what is currently known regarding the role of these microorganisms in gastric carcinogenesis.

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Gastric cancer (GC) is the fifth most common cancer type worldwide with 952 000 new cases diagnosed in 2012[1]. Asian countries bear the brunt of the disease with the rate of new cases being 4 times higher than in Africa. The primary treatment of GC continues to be surgical by, unless restricted by tumour size, recurrence or metastasis[2]. Clinical trials have demonstrated that perioperative chemotherapy significantly improves both overall survival and progression free survival compared to surgery alone[3]. Chemotherapy and supportive care are often the only available options for those who present late, have metastasis or recurrence. Targeted therapies have been explored with trastuzumab (monoclonal antibody against human epidermal growth factor receptor 2) and ramucirumab (monoclonal antibody against vascular endothelial growth factor receptor 2) being Food and Drug Administration approved for the treatment of GC[4]. Unfortunately, the prognosis of advanced or metastatic GC continues to remain abysmal with a median overall survival of less than one year[2].

2. Helicobacter pylori (H. pylori) and GC

The International Agency for Research on Cancer has classified *H. pylori* as a Group 1 human carcinogen. The link between *H. pylori* and GC has been known since the early 1990s[5]. Between the two histological subtypes of GC, the intestinal subtype has a statistically significant higher association with *H. pylori* than the diffuse subtype. The diffuse subtype is thought to have a more prominent genetic association. Besides these two, *H. pylori* has a critical role in the development and progression of gastric (mucosal-associated lymphoid tissue) lymphoma and the primary treatment of early stage tumours is eradication of the organism with a complete remission rate of 70%-80%[6,7].

H. pylori harbours a number of virulence factors that contribute to its pathogenicity in humans[8]. The CagA protein is a 120-140 kDa protein that is translocated into the host cell after bacterial attachment. Once inside, it is tyrosine phosphorylated and induces cell morphological changes associated with increased cellular migration, a prominent feature of malignant cells[9]. The presence of cag pathogenicity islands prevents efficient phagocytosis of *H. pylori* by macrophages[10,11]. VacA derives its name from its ability to induce intracellular vacuolization. Internalization of the VacA toxin is also known to elicit a number of cellular responses including disruption of the gastric epithelial mucosal barrier and modulation of the immune response[12]. VacA is instrumental in downregulation of host T cell proliferation by interfering with calcium signalling and eventual interleukin (IL)-2 inhibition in immune cells and thus aids in evasion of host immune defences[13-15]. Besides these two, a number of newly identified factors are also postulated to contribute to the ability of *H. pylori* to induce epithelial damage. These include duodenal ulcer promoting antigen which is associated with increased IL-8 production and increased risk of duodenal ulceration in some populations, blood group antigen binding adhesin which helps *H.*

pylori maintain persistent colonization, sialic acid-binding adhesin, outer inflammatory protein which mediates increased neutrophil infiltration, FlaA and peptidoglycan, to name a few[16].

3. Tracking changes in the gastric microbiome

For a long while it was believed that no microorganisms could survive in the acid milieu of the stomach. The characterisation of *H. pylori* laid that misconception to rest and paved the way for identification of other inhabitants of the gastric microenvironment. It is believed that the gastric mucus forms a pH gradient, thus protecting its guests from the acid attack. One of the initial studies used cloning, sequencing methods and restriction fragment length polymorphism of 16S rRNA to characterise the gastric microbiota. In this study by Dicksved *et al*[17], it was discovered that there were representatives from 5 bacterial phyla present in the gastric milieu, namely, Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria and Fusobacteria. Interestingly, species of the genera *Streptococcus*, *Lactobacillus*, *Veillonella* and *Prevotella* had a clear dominance. The same study concluded a lack of any statistically significant difference between the microbial compositions in GC patients versus controls. However, a more recent study conducted in China, by Eun and colleagues[18], reported a significant difference between the microbiota in GC, intestinal metaplasia (IM) and chronic gastritis using pyrosequencing methods. A possible reason for the discrepancy is the variation in prevalence of *H. pylori*, diet and lifestyle between the study groups as well as the molecular method used. A point of agreement in the two studies was the higher relative abundance of streptococci in GC compared to the control groups used. In another recent study, Aviles-Jiminez *et al.* concluded that there was a gradual shift in the microbiome composition from non-atrophic gastritis (NAG) to IM to GC[19]. Interestingly, this study also demonstrated a gradual decrease in bacterial diversity as the epithelium shifts from NAG to IM to GC, which they postulate is due to lack of healthy mucin production by the pre-neoplastic or neoplastic epithelium. The same study therefore concluded that a shift from MUC5AC and MUC6 produced by healthy gastric mucosa, to MUC2 produced by metaplastic intestinal mucosa could render the microenvironment less suitable for certain bacteria, while favouring the growth of others.

Their findings were in somewhat discordance with previous studies which postulated that as the epithelium moves to a neoplastic state, the pH of the gastric microenviroment rises and makes it more favourable for bacterial proliferation. It has also been demonstrated that treatment with acid suppressing drugs leads to an increase in the overall bacterial load in the stomach which normalizes once the treatment is discontinued[20]. The conclusion drawn was that with the increased bacterial load, there was an enhanced production of nitrite in the stomach. N-nitroso compounds are known carcinogens found in diet (especially smoked foods) and tobacco smoke and are classically linked to GC.

To summarize, all the studies had varied results when it came to the exact details of the progression of changes in microbiota as the normal epithelium shifts to cancer, with each suggesting a different mechanism. However, a simple conclusion that may be drawn is that as the pH of the stomach changes, the microbiota changes. Instead of just an increase in total bacterial burden or a decrease in diversity, a cross talk between certain types of bacteria and the host might exist, which tends to make the environment more favourable to some bacterial species while eliminating others. Future studies may potentially be able to identify these elusive bacteria.

4. The role of probiotics

The next question that presented itself was that if certain microorganisms contribute to carcinogenesis, could there be some that protect against it. This led to the investigation of the role of probiotics in gastric epithelial changes. *Lactobacillus* and *Bifidobacterium* are the most commonly used microbes in probiotics. Studies have shown that within the stomach, these "friendly" bacteria enhance the very immune response that *H. Pylori* attempts to evade[21]. These probiotic bacteria can bind to toll like receptors expressed on the surface of epithelial cells, and trigger a cascade of immunological defence mechanisms[21]. These interactions between probiotics and epithelial cells also result in modulation of inflammatory cytokine production, resulting in a reduction in gastric inflammation. Kabir *et al.* demonstrated that *Lactobacillus salivarius* inhibits *H. pylori*-stimulated secretion of IL-8 by gastric epithelial cells in a gnotobiotic murine model[22]. Clinical studies have validated the animal studies by providing evidence that use of probiotics either before or after standard triple therapy enhances the *H. pylori* eradication rate[23]. Besides the observed effect on *H. pylori*, there are *in vitro* studies which report that the extract from certain *Lactobacillus* species directly induce apoptosis of GC cell lines[24]. Another study showed that the supernatant from milk fermented by *Propionibacterium freudenreichii* was not only able to induce typical features of apoptosis on HGT1 human GC cells, but it also enhanced the cytotoxicity of camptothecin, a chemotherapeutic agent used for GC[24]. In a meta-analysis of five randomized controlled trials with 1 307 patients, another probiotic containing *Saccharomyces boulardii* and given along with triple therapy increased eradication rates (4 randomised controlled trials; relative risk: 1.13, 95% confidence interval: 1.05-1.21) and decreased the risk of therapy-related adverse effects (5 randomised controlled trials; relative risk: 0.46, 95% confidence interval: 0.3-0.7)[25].

5. *H. pylori* and genomic aberrations

Correa and Piazuelo described the histopathological changes as gastric epithelium progresses from normal to neoplastic. The mucosa passes through the stages of NAG, gastritis without IM, gastritis with IM, IM of the incomplete type, low and high grade dysplasia and finally invasive adenocarcinoma[26]. A number of genomic aberrations have been detected in gastric malignances, the most common being TP53, PIK3CA, ErbB2, ErbB3, ARID1A and KRAS[27,28]. It is believed that the chronic inflammation induced by *H. pylori* is a trigger of cellular and DNA damage, thus bringing in cytokines and growth factors that contribute to carcinogenesis. All these factors may act in synergy to lead to epigenetic changes, microRNA gene expression changes and alterations in cellular signalling pathways. Kim *et al.* demonstrated that the levels of MLH1, a DNA mismatch repair enzyme are lower in *H. pylori* infected cells compared to controls[29]. Deficiency of mismatch repair enzymes is known to cause microsatellite instability. The reported frequency of microsatellite instability in GC is 15%-30% and is known to affect oncogenes such as epidermal growth factor receptor, KRAS, PIK3CA and mixed-lineage protein kinase 3[30]. It has been shown that *H. pylori* is also capable of inducing hypermethylation, particularly of CpG islands, thereby inactivating tumor suppressor genes[31,32]. Another study demonstrated an increase in signal transducer and activator of transcription 3 (STAT3) signalling in *H. pylori* co-cultured GC cell lines, and an absolute increase in the levels of activated STAT3 in *H. pylori* positive gastritis tissue samples compared to controls[33].

However, *H. pylori* surely couldn't be the sole agent to blame for the initiation and progression of such an aggressive disease. Host factors are known to play a major role in the development of most cancers, and GC is no different. Just like only 10% of smokers develop lung cancer over their lifetime, only 2.9% of *H. pylori* infected individuals develop GC[34]. Cytokines are believed to play a major role in this regard. IL-1 is a known pro-inflammatory cytokine that is upregulated by *H. pylori*. Figueiredo *et al.* stratified *H. pylori* infected subjects on the basis of high IL-1 expression polymorphisms along with the virulence genotyped the *H. pylori* strains they carried and observed that the odds for development of GC was highest for those with both the high risk bacterial strain and the high IL-1 polymorphism[35]. Moreover, IL-1 transgenic mice overexpressing human IL-1 specifically in the gastric epithelium have been found to spontaneously develop gastritis and dysplasia and have increased susceptibility to dysplasia and carcinoma when infected by *Helicobacter felis*[36]. Besides IL-1, polymorphisms that increase tumor necrosis factor and toll like receptor 4 have also been associated with an increased risk of GC[37]. In all likelihood, it is a complex interaction between the host, the environment and the microbiome that continually tips the balance from normal epithelium to dysplasia to cancer.

6. Implications of *H. pylori* eradication

Although the association between *H. pylori* and GC has been extensively researched, and it is clear that eradicating the organism

reduces the eventual risk of development of cancer, investigating the point of no return for the damaged epithelium is of paramount importance. A recent meta-analysis of 10 randomized controlled trials with a total of 7955 patients attempted to answer that very question[38]. The authors concluded that for patients with normal epithelium, NAG, and atrophic gastritis without IM, the eradication of *H. pylori* significantly reduced the relative risk of development of GC. However, the same could not be said for patients who had already developed IM or dysplastic changes. Their results support the effect of *H. pylori* eradication as both primary (for normal epithelium) and secondary (for NAG and atrophic gastritis without IM) prevention of GC but they concluded that patients who had already developed IM or dysplastic changes would not benefit from eradication of *H. pylori*. These results may eventually affect the treatment guidelines of *H. pylori*. The current guidelines recommend treating only symptomatic patients or those with mucosal-associated lymphoid tissue lymphomas, but if eradication reduces carcinoma risk even in healthy people who simply harbour the bacteria, it may eventually lead to more widespread testing and treatment.

7. Conclusions

Needless to say, GC is a devastating disease that claims hundreds of thousands of lives worldwide each year. *H. pylori* is a major player in gastric carcinogenesis but certainly not the only one. A variety of host and environmental factors contribute to the development of this cancer. Current treatment modalities include surgeries, chemotherapy and targeted therapies. However, as for all diseases, prevention is better than cure. Identification of markers of early disease as well as further exploration of the molecular mechanisms of carcinogenesis are necessary to not only improve on current treatment strategies but also for prevention.

Conflict of interest statement

We declare that we have no conflict of interest.

Comments

Background

H. pylori have been associated with human gastric carcinogenesis and other gut microbes play an important role in modulating *H. pylori*'s effect on gastric carcinogenesis. A number of studies have also commented on the role of probiotics in prevention and treatment of GC.

Research frontiers

The review article summarizes the current understanding of gastric microbiome and its role in GC pathogenesis. It addresses the role of *H. pylori* and other gut microbes in mediating and influencing cancer initiation and progression.

Related reports

The review article incorporates a number of preclinical and clinical studies addressing the issues of tracking changes in gastric microbiome in patients afflicted with gastritis, precancerous lesions and GC as well as the role of *H. pylori* in affecting genomic aberrations in gastric epithelium.

Innovations & breakthroughs

The article is a review article which has summarized data from a number of articles on different aspects of gastric microbiome.

Applications

This article will provide a great summary of the relevant topics concerning the gastric microbiome and its role in disease and should be of use for clinicians and researchers who wish to review these topics.

Peer review

The review article nicely summarizes previous studies on the topic of gastric microbiome, its components, how it changes with disease and the role of probiotics in normalizing its aberrations. The articles mentioned in the paper are relevant and the authors have done a great job of addressing a number of relevant topics in their article.

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