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 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.

**Key words**

Gastric cancer, Microbiome, Microbiology, *Helicobacter pylori*
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, 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].

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 microbial compositions in GC patients versus controls. The same study concluded a lack of any statistically significant difference between the microbial compositions in GC patients versus controls. The 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 microenvironment 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.

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].
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 1307 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 malignancies, 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]. A further 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
and other gut microbes play an important role in modulating the microbiome and its role in GC pathogenesis. It addresses the role of the review article summarizes the current understanding 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.

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.

References

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