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Microbiota and Gastric Adenocarcinoma Carcinogenesis

Ana João Bezerra Cruz

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Orientador: Dr. Pedro Miguel Ribeiro Marcos

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Declaração de Integridade

Eu, Ana João Bezerra Cruz, que abaixo assino, estudante com o número de inscrição 41451 do curso de Medicina da Faculdade de Ciências da Saúde, declaro ter desenvolvido o presente trabalho e elaborado o presente texto em total consonância com o **Código de Integridades da Universidade da Beira Interior**.

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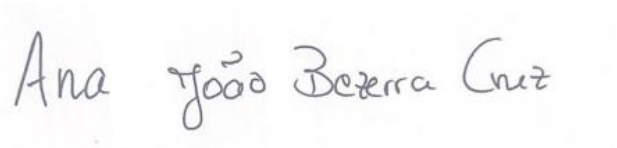

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Dedicatória

Esta tese é dedicada a todos os doentes oncológicos, cuja garra, determinação e resiliência são uma verdadeira inspiração. Que este trabalho contribua para o avanço do conhecimento na oncologia e proporcione novas esperanças e melhores resultados no combate ao cancro.

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Resumo

Introdução: O adenocarcinoma gástrico (AG) é um problema significativo de saúde global e a investigação tem demonstrado que a microbiota pode desempenhar um papel crucial no seu desenvolvimento.

Objetivo: Resumir o conhecimento atual sobre o papel da microbiota e as suas alterações no desenvolvimento do AG.

Métodos: Revisão da literatura disponível na PubMed. Revisões sistemáticas, ensaios clínicos, estudos de coorte e estudos de caso-controlo foram preferidos para análise.

Resultados: A composição da microbiota do estômago altera-se durante o desenvolvimento do AG. Um aumento de bactérias *Firmicutes* e uma diminuição de bactérias *Bacteroidetes* podem estar associados ao desenvolvimento desta neoplasia. A gastrite não atrófica é caracterizada por uma diversidade microbiana reduzida em comparação com estômagos saudáveis, provavelmente devido à inflamação. A presença de *Helicobacter pylori* exacerba esta redução, tornando-se a espécie dominante. A gastrite atrófica assemelha-se à gastrite não atrófica, mas apresenta um ligeiro aumento de outras bactérias, como *Streptococcus*, que podem promover a carcinogénese. À medida que a gastrite atrófica progride para a metaplasia intestinal e adenocarcinoma gástrico, há um aumento de bactérias potencialmente prejudiciais, particularmente do grupo *Firmicutes*, que desempenham um papel significativo na inflamação, mutagénese e progressão tumoral. Alterações nas bactérias também foram observadas na boca e nas fezes dos pacientes com AG.

Conclusões: A microbiota tem um impacto significativo no desenvolvimento do AG. A bactéria *H. pylori* é um carcinogéneo confirmado para AG e as alterações na microbiota durante a cascata de Correa levam à dominância do phylum *Firmicutes*. São necessárias mais investigações para entender estas alterações na microbiota e desenvolver ferramentas para a prevenção e diagnóstico precoce do AG.

Palavras-chave: Microbiota; Microbioma; Disbiose; Carcinogénese; Adenocarcinoma Gástrico; Cancro Gástrico.

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Abstract

Introduction: Gastric adenocarcinoma (GA) is a significant global health issue, and research has shown that the microbiota plays a crucial role in its development.

Objective: To summarize the current understanding of the microbiota's role and its changes in the development of GA.

Methods: Comprehensive review of relevant literature available on PubMed. We focused on systematic reviews, clinical trials, cohort studies, and case-control studies for analysis.

Results: The composition of stomach microbiota changes during the development of GA. An increase in *Firmicutes* bacteria and a decrease in *Bacteroidetes* bacteria may be associated with GA development. Non-atrophic gastritis is characterized by lower microbial diversity compared to healthy stomachs, likely due to inflammation. The presence of *H. pylori* exacerbates this reduction, becoming the dominant species. Atrophic gastritis shares similarities with non-atrophic gastritis but displays a slight increase in other bacteria, like *Streptococcus*, which can promote carcinogenesis. As the condition progresses to intestinal metaplasia and GA, there is an increase in potentially harmful bacteria, particularly from the *Firmicutes* group, which play a significant role in inflammation, mutagenesis, and tumor progression. Changes in bacteria have also been observed in the mouth and feces of patients with GA.

Conclusions: The microbiota has a significant impact on GA development. *H. pylori* is a confirmed carcinogen for GA, and changes in the microbiota during the Correa cascade lead to the dominance of the *Firmicutes* phylum. Further research is needed to understand these microbiota changes and to develop tools for the prevention and early diagnosis of GA.

Keywords

Microbiota; Microbiome; Dysbiosis; Carcinogenesis; Gastric Adenocarcinoma; Gastric Cancer.

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List of Acronyms

AG	Adenocarcinoma Gástrico
GA	Gastric Adenocarcinoma
GC	Gastric Cancer
IM	Intestinal Metaplasia
rRNA	Ribosomal ribonucleic acid
NO	Nitric oxide

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Introduction

Gastric cancer (GC) is the fifth most common cancer worldwide and the fifth leading cause of cancer-related death. It is more prevalent in East Asia, Eastern Europe, and some countries in Central and South America (1, 2). Gastric adenocarcinoma (GA) is the most common type of GC, accounting for 95% of cases. According to the Lauren classification, GA can be divided into two main subtypes: intestinal (the most common) and diffuse (3).

Most gastric adenocarcinomas develop through a series of changes known as the Correa Cascade. This process begins with chronic gastritis, followed by gastric atrophy, intestinal metaplasia (IM), dysplasia, and ultimately invasive neoplasia. Gastric atrophy involves the loss of pre-existing gastric glands, while gastric IM refers to the replacement of gastric epithelium with intestinal-type epithelium. Both gastric atrophy and gastric IM are considered premalignant conditions because they increase the risk of developing GA and create a background in which dysplasia and adenocarcinoma may occur (2). *Helicobacter pylori (H. pylori)* infection is recognized as the primary environmental factor driving this multistep carcinogenic process. The toxins produced by *H. pylori* can disrupt the normal gastric mucosal environment, initiate inflammation, and potentially damage nucleic acids (3).

The human microbiota comprises a diverse array of bacteria, fungi, viruses, and other living microorganisms, that reside predominantly in the gastrointestinal tract, skin, and oral cavity (3, 4). Although often confounded, microbiota and microbiome have different meanings, as the microbiome includes not only the community of living microorganisms, but also the microbial structural elements, metabolites, and environmental conditions in a single environment (5).

The human microbiota plays crucial roles in aiding digestion, regulating metabolism, enhancing immune responses, and protecting against harmful pathogens (6). Its composition can be influenced by genetic factors, dietary choices, lifestyle habits, and environmental exposures, resulting in variations observed between individuals and across different cultures (6, 7). Perturbations in the microbiota, often characterized by shifts in its composition, abundance, or diversity, are termed dysbiosis (5, 6). This imbalance commonly results in a decrease in beneficial microbes, an increase in potentially harmful ones, and a decrease in overall microbial diversity, frequently contributing to the development of disease (3).

For a long time, stomach was believed to be a sterile place, however advancements in bacterial genome sequencing methods, particularly 16S ribosomal ribonucleic acid (16S rRNA) sequencing, came to contradict this notion (8). The 16S rRNA gene, which is

consistent across bacterial species, makes it easy to identify the microbial taxa present in each bacterium (3). Hence, this method has become one of the most widely used approaches for analyzing the microbiota (3).

While the study of the gastric microbiome is relatively new compared to the gut microbiome, emerging research suggests that dysbiosis in this region can have significant implications for health, particularly in relation to GA (9). Therefore, we proposed to perform a literature review to summarize the current knowledge about the role of the microbiota and its changes in GA carcinogenesis, going beyond the well-established role of *H. pylori*, which is already recognized as a class I carcinogen. To conduct this review, the following query was used in PubMed:" (("gastric cancer" OR "stomach neoplasm" OR "gastric carcinoma" OR "gastric adenocarcinoma") AND (microbiota* OR "microbial communit*" OR microbiome* OR dysbiosis) NOT "review"). Original studies such as systematic reviews, clinical trials, cohort studies, and case-control studies were preferred for analysis. Studies using animal models were excluded, and only articles available in Portuguese or English were considered.

Gastric Microbiome Shifts in the Correa Cascade

Numerous studies investigating the microbiome's role in GA carcinogenesis consistently reveal an evolutionary connection with the Correa precancerous lesion cascade, aligning the microbiome composition with its distinct stages. These discoveries were made mainly through applying the 16S rRNA sequencing method (3).

Recent studies have shown that the normal gastric mucosa is predominantly composed of the following phyla in descending order of abundance (phyla: plural of phylum - the primary subdivision of a taxonomic kingdom, ranking above class and below kingdom, and grouping together all classes of organisms that have the same body plant): (i) *Firmicutes* (42%) - gram-positive, spore-forming, obligate anaerobes, including the *Enterococcaceae* and *Lactobacillaceae* families, as well as the Streptococcus genus; (ii) *Bacteroidetes* (24%) - gram-negative bacteria characterized by diverse shapes and predominantly composed by *Bacteroides* and *Prevotella* genera; (iii) *Proteobacteria* (17%) – a major phylum of gram-negative bacteria, encompassing a wide diversity of species with varied shapes, sizes, and ecological roles, including bacterial groups such as *Helicobacter pylori, Escherichia coli* and *Salmonella*; (iv) *Actinobacteria* (7%) – a group of unicellular filamentous bacteria that form a branching network of filaments and produce spores; (v) *Fusobacteria* (6%) - anaerobic, non-spore-forming, gram-negative bacteria that are frequently associated with periodontal disease (8, 10). Compared to chronic gastritis and stomachs with precancerous conditions or lesions, normal stomachs have the highest alpha diversity, meaning they contain the widest range of bacterial species (10, 11).

The findings associated with gastritis can vary significantly, considering whether it is associated with *H. pylori* infection or not and whether it is a non-atrophic or atrophic gastritis. In general, there is a decrease in the quantity and diversity of microbes in gastritis mucosal samples, particularly when *H. pylori* infection is present (12, 13). *H. pylori* can outcompete other bacteria for resources and space while also triggering immune responses that disrupt the microbial balance and compromise the protective mucus layer, namely by altering gastric pH levels (10). All these events result in a significant loss of microbiota diversity, characterized by the dominance of *H. pylori* (13-15). The microbiota in *H. pylori*-negative non-atrophic gastritis biopsies is similar to that of a normal stomach but with a slight decrease in overall community numbers (10). On the other hand, *H. pylori*-positive non-atrophic gastritis shows the poorest bacterial diversity, being completely dominated by the presence of *H. pylori* (10, 14).

It has been demonstrated that atrophic gastritis is associated with a more complex microbial co-occurrence, meaning more bacterial communities are present and interacting within the same habitat compared to non-atrophic gastritis (10). This was associated with an increase in pathogenic genera (a taxonomic category in the biological classification that ranks above species and below family) besides *Helicobacter* (10). In fact, a recent prospective cohort study developed in England showed that *H. pylori*-positive atrophic gastritis samples were primarily composed of *Helicobacteraceae* (62%). However, bacteria from the *Streptococcaceae* (5%), *Fusobacteriaceae* (2%), and *Prevotellaceae* (2%) families were increased compared to non-atrophic gastritis (14). Simultaneously, there were distinct negative correlations between *Helicobacter* and the other genera, such as *Streptococcus* (14). This indicates that as *H. pylori* levels increased, the presence of other species in the same environment decreased, highlighting the highly competitive and dysbiotic impact of this class I carcinogen (14).

Regarding *H. pylori*-negative atrophic gastritis, an Italian cohort study showed that it was associated with an increase in *Firmicutes* phyla, in particular *Streptococcus* genera, followed at lower percentages by *Proteobacteria* and *Actinobacteria* phyla, while *Bacteroidetes*, *Patescibacteria*, and *Spirochaetes* suffered a decrease (8). More specifically, the increase in *Firmicutes* and *Proteobacteria* included high levels of *Streptococcus mitis* and *Neisseria mucosa*, with some evidence suggesting their potential role in the development of GA (14).

Studies have shown that the presence of *H. pylori* decreases even further with the progression to IM (13, 16). Nevertheless, the bacterial diversity tends to increase, with IM representing a mid-point in quantitative diversity between gastritis and GA (17). These bacteria often exhibit pathogenic characteristics. The significant reduction of *H. pylori* and the decrease in acid secretion in these lesions may create an environment that promotes the colonization of other bacteria in the stomach, which could contribute to the development of GA (18).

Research indicates that *Firmicutes,* the predominant phylum observed to rise in both IM and GA instances, has negatively impacted gastric well-being (1, 19). For instance, *Filifactor alocis*, a species belonging to the *Firmicutes* phylum, was identified in a multicenter case-control study as being enriched in IM mucosa (18). It is known to be implicated in periodontal disease and to stimulate the secretion of proinflammatory cytokines from gingival epithelial cells (18). Other species from this phylum, like *Streptococcus bovis*, often linked to colorectal cancer, and *Lactobacillus*, are also augmented in IM (13, 16). Understanding the role of *Lactobacillus* is controversial, as some consider it to be protective, while others view it as pathogenic (20, 21). A multinational

cohort study conducted with GA patients indicated that it facilitates carcinogenesis (20). In contrast, a prospective cohort study conducted with Chinese subjects defended its beneficial role, showing that *Lactobacillus* and *Bifidobacteria* were depleted in both IM and GA (21). On the other hand, *Bacteroidetes* phylum was observed to be reduced in IM and GA (21). Interestingly, a clinical trial in patients with GC after gastrectomy revealed that *Bacteroidetes*, along with other probiotic compounds, helped in post-gastrectomy recovery by restoring a balanced gut microbiota, reducing inflammation, boosting immunity, and strengthening the intestinal mucosal barrier (22).

Additionally, ubiquinol biosynthesis pathways were found to be more prevalent in IM, while sugar degradation pathways were less represented (18). As most gram-negative bacteria produce ubiquinone, this may lead to the establishment of an inflammatory microbial environment (18). On the other hand, the utilization of sugar and glycolysis was found to be increased in GA compared to non-cancer samples, demonstrating a more generalized and diversified bacterial growth compared to IM (13). Besides the decrease in *Bacteroidetes* phylum, the GA microbiota has shown an increase in *Proteobacteria*, specifically the *Proteus* genus, reducing the functional characteristics for metabolizing galactose, sucrose, starch, and arginine (21). These findings suggest potential alterations in mucin production and the microbial composition that could potentially influence tumorigenesis (22).

In a cohort study conducted with a Chinese population, it was found that GA is associated with a higher degree of dysbiosis compared to cases of superficial gastritis. This suggests a dysbiosis progression of the microbiota across these stages (23). The data consistently indicate an enrichment of gastric mucosal bacteria throughout the cascade (24). These bacteria, including *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Fusobacteria*, are similar to those typically found in a healthy stomach (24). However, bacteria with potential cancer-promoting activities, mostly belonging to the *Firmicutes* phylum (such as *Streptococcus*), were associated with a more significant increase, supporting the potential tumorigenic role of this phylum (13, 20, 24, 25). A retrospective cohort study that analyzed one of the biggest GA patients cohort among all gastric microbiome studies described more deeply the roles of some of the augmented species in this stage, such as *H. pylori, Lactobacillus, Streptococcus*. It was shown that, by producing free radicals, and some important transcription and virulence factors, *H. pylori* can induce an intense inflammatory process, host cell mutation, infection, tumor aggressiveness and metastasis (20). This chronic inflammation can lead to a significant accumulation of nitric oxide (NO), stimulating the growth of precancerous lesions (20). Similarly, to *H. pylori*, *Lactobacillus* has been reported to produce high levels of N-nitroso compounds, which can

trigger mutagenesis and angiogenesis (20). Furthermore, it releases substances that are known to promote the growth of tumor cells (20). *Streptococcus* is believed to induce the formation of inflammatory cytokines in host epithelial cells through sulfate-reducing activity (20).

A retrospective analysis of two independent cohorts showed different results compared to previous studies. It suggested that an increase in *Bacteroidetes* in GA samples could promote the progression of the condition. The study proposed that *Bacteroidetes* might stimulate the production of pro-inflammatory cytokines and toxins, leading to inflammation of epithelial cells, premalignant changes, and proliferation. Additionally, the study mentioned that *Prevotella*, a species within the *Bacteroidetes* phylum, is believed to contribute to oxidative stress by producing the powerful redox protein thioredoxin (20).

Although most studies agree that there is an increase in bacterial diversity during the Correa cascade, a short cohort study performed in Mexico found it to be associated with a gradual decrease (26). However, species like *Lactobacillus coleohominis* and *Lachnospiraceae*, belonging to the *Firmicutes* phylum, were augmented and thought to favor the development of GA (26).

Regarding microbiota findings within each subtype of GA, some studies collected samples from GA without distinguishing results by subtype (9, 11, 13, 16), while others did differentiate (20, 23-25, 27). However, regardless of this distinction, all studies found no significant differences in the microbiota among GA subtypes, suggesting a consistent microbiome role across different GA types (20, 23-25, 27). Nonetheless, considering the interaction of the immune system with the microbiome and the varying immunogenicity among GA subtypes, additional comprehensive research is expected to reveal differences.

Gastric Adenocarcinoma and Microbiota Alterations: Beyond the Stomach

There is also data regarding changes in the oral mucosa and gut microbiota associated with GA carcinogenesis (18, 28-31). Regarding patients with IM, it was shown that their oral microbiota suffered an increase in *Firmicutes* and *Proteobacteria* phyla (18). Specifically, opportunistic pathogens such as *Peptostreptococcus stomatis*, *Johnsonella ignava*, *Neisseria elongata*, and *Neisseria flavescens* were enriched, whereas commensal bacteria like *Lactobacillus gasseri*, *Streptococcus mutans*, *S. parasanguinis* and *S. sanguinis* were diminished (18). These commensal bacteria are typically known to produce energy through a sugar degradation pathway (*Embden-Meyerhof-Parnas* pathway), recognized for its healthy function (18). This pathway was found to be underrepresented in IM, which suggests a decreased activity and quantity of these commensal bacteria in this stage (18). In addition, the oral microbiota of patients with GA was linked to a general reduction in the *Tenericutes* and *Actinobacteria* phyla (31). Conversely, an elevated risk of GA was connected to an increase in the *Proteobacteria* phylum, as well as *Prevotella pleuritidis* from the *Bacteroidetes* phylum (31).

In relation to the gut microbiota, it has been shown that *H. pylori* infection is connected to an imbalance in fecal microbiota (28-30). Research has revealed that individuals with *H. pylori* infection display a broader range of fecal microbial diversity, and higher levels of *H. pylori* stool antigens have been linked to a potentially harmful microbiota composition (28). A retrospective cohort study that compared the microbiome of individuals with GC to that of healthy individuals found that GC patients had higher levels of alpha and beta diversity compared to healthy controls (alpha diversity measures bacterial diversity within a single habitat, while beta diversity quantifies bacterial differences between multiple habitats). This indicates the presence of a dysbiosis progression of the microbiota (30). Genera from the *Firmicutes* and *Fusobacteria* phyla, such as *Lactobacillus*, *Streptococcus*, *Veillonella*, and *Fusobacterium*, were found to be more abundant in these samples (29, 30). These bacteria have also been identified in other types of cancers and have been associated with the production of reactive oxygen species, which are substances known to cause inflammation and stimulate tumor growth (29). As the relative abundance of these microorganisms might be a valuable diagnostic indicator, research in this field has suggested that changes in the fecal microbiome community could become a novel biomarker and target for preventing GA (29, 30).

Conclusion

The role of microbiota in the development of GA has garnered increasing interest. While numerous studies have explored this relationship, many had small sample sizes and did not account for other potential risk factors for GA. Additionally, inherent biases and variations in the populations studied have limited the findings. However, this review focuses on original articles of significant scientific relevance, gathering and summarizing relevant and updated data on microbiota and GA.

Studies consistently indicate that the gastric microbiota undergoes changes during the Correa cascade of carcinogenesis (13, 20). There is compelling evidence to suggest that an increase in the *Firmicutes* phylum may be associated with the development of GA, while a decrease in the *Bacteroidetes* phylum may contribute to the same (8, 13, 20, 21, 24-26).

Non-atrophic gastritis has been found to have lower microbial diversity and community compared to healthy stomachs, which is likely due to inflammation (10). The presence of *H. pylori* exacerbates this reduction, as it becomes the dominant species, leading to further declines in diversity (14). Atrophic gastritis shares similarities with non-atrophic gastritis in terms of gastric microbiota composition, although the former displays a slight increase in other bacteria, like *Streptococcus*. *Streptococcus* has been shown to be harmful and potentiate GA carcinogenesis, suggesting a gradual shift towards a more diverse and potentially tumorigenic microbiota as the condition advances (10).

As the condition progresses to IM, *H. pylori* significantly decreases, and different genera start to increase, especially those from the *Firmicutes* phylum (1, 13, 16-18). This phase represents an intermediate stage in microbial evolution, possibly attributed to the intense reduction of *H. pylori* and decreased acid secretion, creating conditions for the colonization of other potentially pathogenic bacteria in the stomach (18).

GA represents the final stage, with the highest number and diversity of bacteria. It is characterized by a general phylum increase, with no significant differences among GA intestinal or diffuse subtypes (16, 17, 26-28, 30). The *Firmicutes* phylum emerges as the dominant phylum, playing a significant role in inflammation, mutagenesis, angiogenesis, and tumor progression throughout the cascade (16, 17, 23, 27, 28).

Microbiota changes have been observed in patients with GA in both the oral mucosa and feces. In the oral microbiota, individuals with GA and IM were found to have higher levels of pathogenic bacteria from the *Firmicutes*, *Proteobacteria*, and *Bacteroidetes* phyla, while the commensal phyla (essential for maintaining oral mucosa health) showed a decrease (18, 31). Similarly, fecal samples also show an increase in microbial alpha and beta

diversity, primarily due to an increase in *Firmicutes* and *Fusobacteria*, which were significantly associated with GA (28-30).

To summarize, the gastric microbiota appears to significantly impact GA carcinogenesis. While it is widely known that *H. pylori*, a member of the *Proteobacteria* phylum, is a definitive carcinogen for GA, gastric microbiota changes significantly throughout the Correa cascade, ultimately resulting in *Firmicutes* phylum dominance. Further research is needed to understand how these changes in microbiota contribute to GA carcinogenesis and to develop new tools for preventing this cancer and enhancing patient care.

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