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The gastrointestinal microbiota and its role in oncogenesis

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ABSTRACT

Advances in research techniques have made it possible to map the microbial communities in the gastrointestinal (GI) tract, where the majority of bacteria in the human body reside. Disturbances in these communities are referred to as dysbiosis and have been associated with GI cancers. Although dysbiosis is observed in several GI malignancies, the specific role of these changes has not been understood to the extent of *Helicobacter pylori* (HP) in gastric cancer (GC). This review will address the bacterial communities along the GI tract, from the oral cavity to the anal canal, particularly focusing on bacterial dysbiosis and carcinogenesis. Just as non-HP bacteria in the stomach may interact with HP in gastric carcinogenesis, the same may hold true for other GI tract malignancies, where an interplay between microbes in carcinogenesis seems conceivable, especially in colorectal cancer (CRC). In the last part of this review we will discuss the potential mechanisms of bacterial dysbiosis in GI carcinogenesis.

1. Introduction

One of the first people to directly observe bacteria was Antoni van Leeuwenhoek, who in 1683 described the presence of single cell organisms living in the human oral cavity, which he called 'animalcules'. Nevertheless, it was not until the 19th century that the theory that bacteria could cause disease was commonly accepted. Today, we are starting to recognize the complex relationships between microbes and their hosts in health and disease. Historically, the identification and classification of microorganisms was based on microscopic and culture-based methods, and the advent of molecular technologies has contributed significantly to the emerging insight into the microbes that collectively reside in a given ecosystem (the microbiota) and their genomes (the microbiome) [1,2]. The human microbiome is mostly found at the interface between our body and the outside world – i.e. our skin, mucosa and in particular, the gastrointestinal (GI) tract, which can also be represented as one large complex microbial ecosystem. This tract, simplified as a hollow tube system from the oral cavity to the anal canal, is openly connected to the outside world and the epithelial layer of the GI tract therefore acts as an important barrier function to keep microorganisms from invading. Since a major part of the 10^{13} bacteria in the human body resides in the alimentary tract [3], continuous exposure to these microbiota is inevitable. Owing to the uneven distribution of the bacterial load, different parts of the GI tract are exposed to different amounts and types of micro-organisms. Bacterial counts drop from the oral cavity to the acidic stomach and then markedly increase from the small intestines to reach a maximum in the colon. As the latter harbors a quantity that exceeds other organs by at least two orders of magnitude, the bacterial content in the colon has been a preferred focus for study [3,4]. Nevertheless, there has been an exponential interest in unraveling the microbial content of the entire GI tract. Disturbances in this highly complex system (termed dysbiosis) can affect human health [5–7] and have been associated with different GI diseases, including infectious diarrhea, inflammatory bowel disease (IBD) and cancer.

Given the extensive research and data availability, this review will focus mainly on bacteria as a major component of the GI tract microbiota. The healthy human microbiome, sampled across different body parts, is mainly (>90%) represented by the phyla

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Actinobacteria, Firmicutes, Proteobacteria and *Bacteroidetes* [8]. Since each body habitat harbors its own characteristic microbiota, the relative abundances of these phyla and their sublevels (class, order, family, genus and species) are likely to vary across different GI sites in healthy and diseased states (Fig. 1). In this review, we will focus on the interaction between the microbiome and GI carcinogenesis, focusing on site-specific microbial changes and summarizing what is known about the molecular pathways involved.

2. The microbiome of the upper GI tract

Much effort has been directed towards the identification of causative microbial agents in carcinogenesis. The global burden of new cancer cases attributed to infectious agents was estimated to be 15.4% (2.2 million cases) in 2012 [9]. The leading contributor to this list was *Helicobacter pylori* (HP), with a total of 770,000 cancer cases that year. HP was involved in 29% of the gastric carcinomas of the cardia, and was accountable for 89% of the non-cardia gastric carcinomas and 74% of the non-Hodgkin lymphomas of the stomach. The involvement of HP in gastric cancer (GC) is the most notorious example of microbial infection related cancer within the human GI tract. Already in 1994, this Gram negative flagellated bacterium was classified as a group I carcinogen by the International Agency of Research on Cancer (IARC) [10]. In contrast, the microbial contribution to other GI tract malignancies has not been fully understood. Since the compartments of the alimentary tract are all virtually interconnected to one another, it is important to uncover site specific microbes involved in carcinogenesis.

2.1. Oral cavity

The oral cavity is the starting point of the GI tract and is lined by mucosa that covers the lips and the mouth. Despite the possibility of direct visualization, cancer affecting this site is often recognized at late stages [11]. Oral squamous cell carcinoma (OSCC) is the most common type and a major cause of morbidity and mortality [11,12]. Tobacco smoking remains an important contributor besides other factors such as alcohol and betel nut exposure. Despite these, the etiological picture is still not fully elucidated, and recently the attention has been shifted to the identification of potential microbial agents [13]. A minor role (4.3%) for *human papillomavirus* (HPV) infection in oral cancer was acknowledged earlier [9], but studies have not agreed on the role of specific bacteria, individually or collectively, in OSCC [14].

Two consecutive papers addressed the presence of living bacteria in OSCC tissue, and showed an increased presence of saccharolytic and aciduric bacteria in OSCC as compared to normal tissue of the same patient. This suggests the presence of specialized microbes attracted by the acidic and hypoxic tumour environment [15,16], and could point to a consequence of tumourigenesis rather than a driving factor for these bacteria. Differences in bacterial composition of tumour versus normal adjacent tissue included an increased abundance of phylum *Firmicutes* (85%), and a relative shift of Gram-negative to Gram-positive microbiota, including saccharolytic *Streptococcus* [17]. Using next-generation sequencing (NGS), a later study managed to classify almost all reads (99.6%) from three OSCC biopsies to species level (228 species). Thirty-five species were shared among the OSCC subjects studied, including potential pathogens *Fusobacterium* spp, *Aggregatibacter segnis* and *Prevotella oris (P. oris)*. Interestingly, a small group of non-oral taxa (5%), including *Bacteroides fragilis (B. fragilis)*, was found [18].

Meanwhile, non-invasive microbial profiling of saliva and oral swabs has been of great interest for the purpose of OSCC diagnostics. One study explored forty common oral microorganisms in saliva and suggested *Prevotella melaninogenica (P. melaninogenica), Capnocytophaga gingivalis (C. gingivalis)* and *Streptococcus mitis (S. mitis)* as potential biomarkers, with a promising sensitivity and specificity of $\geq$80% [19]. To pursue a more complete picture of bacterial saliva, Pulshalkar et al. conducted a 454-sequencing study which revealed *Firmicutes* as the most prevalent of 8 phyla in OSCC subjects. In total, 15 phylotypes were found to be unique for OSCC which included the above-named *P. melaninogenica* and also *Capnocytophaga* spp [20]. Similarly, salivary changes were also noticed in a high throughput study investigating oral leukoplakia (OLK), OSCC and controls. While *Firmicutes* was again the dominant phylum, unlike biopsies, it showed a lower prevalence in saliva from OSCC patients [21]. A significant decrease of *Firmicutes* (genus *Streptococcus*), as well as *Actinobacteria* (genus *Rothia*), was also found in oral swabs from both cancerous and precursor oral lesions when compared to the non-affected contralateral sites, although not in comparison to healthy controls [22]. However, a recent 16S rDNA sequencing study did show significant differences between the oral microbiota from swabs taken from patients presenting with different stages of malignancy and normal controls. Interestingly, the microbial community of potentially malignant disorders overlapped and was positioned between those of oral cancer and healthy controls, suggesting a gradual shift of microbiome during carcinogenesis. One of the microbes isolated from precancerous lesions was *Megasphaera micronuciformis*, which, together with other bacteria that were in higher abundance in these lesions compared to either the normal (*P. melaninogenica*, *Prevotella veroralis*) or the cancerous sites *(Rothia mucilaginosa),* might serve as potential biomarker [23].

Taken together, while relatively few studies have examined the microbiome associated with oral cancer, they have indicated shifts in microbial diversity. However, methodological differences between these studies make it difficult to draw definite conclusions about the bacterial association in OSCC [14,22], as the sampling sites (i.e. mucosa vs fluid) can affect outcomes. While *Firmicutes* is undoubtedly one of the most abundant phyla present in the mouth, both up- and down-regulation of this phylum in OSCC samples have been observed. It remains undetermined whether the shift within the bacterial community contributes to OSCC carcinogenesis or reflects the changed micro-environment.

2.2. Oropharyngeal cavity and oesophagus

The next section of the GI tract is the oropharyngeal cavity, which includes the tonsils. As for other head and neck cancers, tobacco and alcohol are important risk factors in oropharyngeal cancers. Nevertheless, HPV infections are highly associated with this malignancy [24,25] and have been estimated to contribute to 30% of the oropharyngeal cancer cases worldwide [9]. On the other hand, bacterial associations with oropharyngeal malignancies are so far not evident. HP has shown the ability to colonize the oropharyngeal tissue, but its role in carcinogenesis could not be confirmed [26].

Another understudied area is the microbial structure of the oesophagus, especially the bacterial community [27]. While HPV has been linked to Barrett's oesophagus (BE) and oesophageal cancer [28,29], bacterial involvement in oesophageal disease has not been fully determined. Nevertheless, interesting findings have been observed in a limited number of non-culture based studies that have been performed [29,30]. The normal oesophagus harbors a complex bacterial community and has shown to be predominated by the phylum *Firmicutes*, followed by *Bacteroidetes, Actinobacteria*, *Proteobacteria* and *Fusobacteria* which appeared in decreasing order [31]. Supervised and phenotype directed analyses have demonstrated two types of microbiomes present in the distal oesophagus [32]. Type I was more closely associated with normal oesophagus

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