

FOCUS ON PERIODONTAL DISEASE AND COLORECTAL CARCINOMA

D. LAURITANO¹, L. SBORDONE², M. NARDONE³, A. IAPICHINO⁴, L. SCAPOLI⁴, F. CARINCI⁵

¹ Department of Medicine and Surgery, University of Milan-Bicocca, Milan, Italy

² Department of Medicine, Surgery and Dentistry, University of Salerno, Baronissi (Salerno), Italy

³ Ministry of Public Health, Rome, Italy

⁴ Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy

⁵ Department of Morphology, Surgery and Experimental Medicine, University of Ferrara, Ferrara, Italy

SUMMARY

Diagnosis of focal disease, the theory that the human oral microbial (HOM) could affect the onset and development of systemic diseases, was very popular in the past, but the lack of scientific evidence has led to the abandonment of this idea. Interestingly, increasing evidence over the past 3 or so decades suggests that HOM can indeed serve as a reservoir for systemic dissemination of pathogenic bacteria and their toxins in distant body sites, favouring the developments of malignant tumours.

Malignant tumours are complex communities of oncogenically transformed cells with aberrant genomes, associated non-neoplastic cells including immune and stromal cells, and sometimes HOM, including bacteria and viruses. Recent data suggest that HOM and periodontal disease play an active role in the pathogenesis of colorectal cancer, in fact HOM has been found within the colorectal cancer microenvironment, and the composition of the HOM was different from that of adjacent non-neoplastic tissue. An association of *Fusobacterium nucleatum* with the colonic mucosa of colorectal cancer has been proven.

Several questions thus arise. Is periodontal disease a risk factor for colorectal carcinoma? Given the connectivity of the digestive tract, could *Fusobacterium nucleatum* or other HOM be involved in additional gastrointestinal disorders? Furthermore, based on the “mobility” of *Fusobacterium nucleatum* and the omnipresence of cadherins, could this organism be involved in cancers beyond the gastrointestinal tract? Answers to these questions will shed new lights on the role of the HOM in onset of diseases.

Key words: colorectal carcinoma, oral microbioma, *Fusobacterium nucleatum*, periodontal disease.

Introduction

Diagnosis of local disease, the theory that the human oral microbial (HOM) could affect the onset and development of systemic diseases, was very popular in the past, but the lack of scientific evidence has led to the abandonment of this idea. Interestingly, increasing evidence over the past decades suggests that HOM can indeed serve as a reservoir for systemic dissemination of pathogenic bacteria and their toxins in distant body sites. In fact a different group of HOM

species has been found to be directly involved in infections at extra-oral sites. Advances in the HOM research have also facilitated a shift of focus from oral diseases to oral bacteria in systemic infections.

Human microbioma and cancer

Human microbioma (HM) has been unequivocally linked to cancer. Agents of infections such

as Human Papilloma Virus, Hepatitis B and C virus, and *Helicobacter pylori*, alone are responsible for an estimated 15% of the global cancer burden, based on strength of the association and prevalence of infection (1). Metagenomics methods developed over the past decade provide a useful approach to identifying HM sequence signatures in diseases that have a possible or suspected infectious etiology (2, 3). For example, the detection of a new polyomavirus in Merkel Cell carcinoma with metagenomics approach highlights the link between HM and cancer (4).

Malignant tumours (MTs) are complex communities of oncogenically transformed cells with aberrant genomes, associated non-neoplastic cells including immune and stromal cells, and sometimes HM, including bacteria and viruses. Several viruses that can integrate into the human genome directly cause MTs, such as human papillomavirus in cervical cancer and herpes virus in Kaposi's sarcoma (5). In other cases, HM lead indirectly to MTs through chronic inflammatory responses, a mechanism by which *Helicobacter pylori* contributes to both gastric cancer and MALT lymphoma (6).

Human microbioma and colorectal carcinoma

Colorectal carcinoma (CC) is the fourth cause of MTs deaths, responsible for approximately 610,000 deaths per year worldwide (7). CC is one of the first and best genetically characterized MTs, and specific somatic mutations in oncogenes and tumour suppressor genes have been found that are associated with progression from adenomatous lesions (polyps) to invasive MTs. The pathogenic mechanism of CC is unknown, but inflammation is a well-recognized risk factor (8). Since the link between *H. pylori*-mediated inflammation and gastric cancer has been demonstrated, we asked if HM is associated with other gastrointestinal MTs. Recent data suggests that HM plays an active role in the

pathogenesis of CC, in fact HM has been found within the MTs microenvironment, and the composition of the HM of MTs was different from that of adjacent non-neoplastic tissue. An association of *Fusobacterium nucleatum* (FN) with the colonic mucosa of colorectal CC has been proven (9, 10).

Recent publications have provided mechanistic evidence for the involvement of gut HOM in the development of CC. In fact HOM would comprise: production of DNA damaging superoxide radicals, production of genotoxins, T helper cell-dependent induction of cell proliferation, and Toll-like receptor mediated induction of pro-carcinogenic pathways (11, 12). However, no evidence have been demonstrated the direct relationship between HM colonization of intestinal lumen and CC. In fact, the link between HM colonization of intestinal lumen and CC was undemonstrated till Eckburg et al. revealed the presence of 400 bacterial species by sequencing prokaryotic ribosomal RNA gene sequences from multiple colonic mucosal sites and feces of healthy subjects (13). Further researches highlighted intra-individual variation of intestinal HM, whereas the microbial colonization of the mucosa within adult individuals is relatively stable through the colon.

Human oral microbioma and colorectal carcinoma

The advancements in HOM studies have changed our opinion about the microorganisms associated with disease, including MTs. A certain number of studies consistently identified FN, a Gram-negative anaerobe, to be highly enriched in CC (14). A subsequent study reported that FN was not only enriched in CC but also in benign precancerous polyps (15).

FN is found in high bacterial loading in periodontal disease (16, 17). Increasing evidence suggests that HOM is not confined to the oral cavity and can migrate to extra oral sites, causing infection and inflammation (12). FN has

been isolated from a wide range of organ abscesses and infections, although it is never or rarely detected in those floras under normal conditions. In particular, FN is capable of crossing the placental barrier, causing pregnancy complications such as preterm birth, stillbirth, and neonatal sepsis (12).

Detection of FN in the colorectal adenomas and carcinomas does not prove causality. The oral cavity is at the beginning of the digestive tract; it is not surprising that HOM species find their way down this path. Recent studies by the Author's group (10) and others (9) aimed to address this issue and demonstrate that FN is indeed a driver of CC. The Author's group showed that the unique FadA (Fusobacterium adhesin A) adhesion of FN stimulated human CC cell growth (10). FadA mediates FN binding to endothelial and epithelial cells, and, perhaps most importantly to this discussion, it is only present in limited spaces including FN and is absent in non-oral *Fusobacterium* species (12). FadA binds vascular endothelial-cadherin on endothelial cells, causing increased endothelial cell permeability thus allowing bacteria to penetrate, a likely mechanism used by FN for systemic dissemination. Rubinstein et al. (10) demonstrated that *F. nucleatum* binds to and invades both normal and MTs epithelial cells via FadA binding to epithelial (E)-cadherin. This binding leads to growth stimulation of human CC cells but not the non-cancerous cells. FadA binding to E-cadherin on MTs cells activates b-catenin-regulated transcription, resulting in increased expression of oncogenes cyclin D1 and c-Myc, Wnt (wingless-related integration site) signalling genes Wnt7a, Wnt7b, and Wnt9a, and inflammatory genes nuclear factor- κ B, interleukin-6 (IL-6), IL-8, and IL-18, all of which are hallmarks of carcinogenesis. The FadA binding site on E-cadherin has been mapped to an 11-amino acid domain. A synthetic peptide corresponding to this domain prevents FN from binding and invasion of MTs cells, thus blocking the oncogenic, Wnt, and inflammatory responses/gene expression. It also inhibits FN-driven MTs growth both *in vitro* and in xenograft mice (10).

Other evidences indicate that FN has increased fadA expression in the MTs that correlate with increased tumorigenesis responses (10). The fadA gene levels are significantly increased in the adenoma and carcinoma tissues compared with the normal controls (10). The increase is stepwise, from normal controls to the precancerous lesions (including benign polyps and tissues surrounding the benign and malignant polyps), and from the precancerous state to MTs, with an average 10-fold increase between each step. FadA transcription in FN in the MTs tissues is significantly increased compared with that in the normal controls and the precancerous tissues, indicating an increased virulence activity of FN in MTs.

In another study FN was shown to induce tumour multiplicity and selectively recruit tumour-infiltrating myeloid cells to promote tumorigenesis in APC \pm mice. Unlike other HOM, FN does not induce colitis, enteritis, or inflammation-associated MTs; instead, it induces sporadic colorectal tumorigenesis, which is the most common form of CC in humans. FN was found to be enriched in the stools from patients with adenoma and carcinoma (9).

Together, these studies demonstrate that FN stimulates MTs. The discovery of a microbial driver of MTs provides new perspective on the aetiology, mechanism, diagnosis, treatment, and prevention of this debilitating disease. The mechanistic studies identified new diagnostic and therapeutic targets. Because it is unique to FN, FadA may be an ideal diagnostic marker for early detection of CC. Diagnostic criteria may be developed to define healthy, precancerous, and cancerous states according to the FadA gene levels. The inhibitory peptide and/or its derivatives may be used in precision medicine to specifically eradicate FN to treat CC or reduce CC risk, similar to eradicating *Helicobacter pylori* to reduce gastric cancer risk (18). Compared with antibiotic therapies, the precision elimination avoids disturbance of the flora. The potential use of FadA in disease diagnosis, treatment, and prevention warrants additional testing.

Periodontal disease and colorectal cancer

Periodontal tissues include four defined structures that constitute the support of teeth: gingiva, cementum, alveolar bone, and the periodontal ligament. Periodontal diseases are extremely prevalent worldwide, affecting roughly half of the adult population. Gingivitis, the mildest form of periodontal disease, is rapidly inducible and reversible inflammatory affection of gingiva mainly caused by accumulation of bacterial biofilm. The combination of bacterial infection and persistent inflammatory response can eventually induce the progressive destruction of the deeper periodontal tissues, a worst form of periodontal disease called periodontitis. Additional risk factors include genetic susceptibility, tobacco smoke, alcohol use, and systemic conditions such as diabetes, osteoporosis, malnutrition and stress. Effective treatment of periodontal infections is important to reduce local inflammation and bacteraemia. In addition, poor periodontal health appears to increase the risk of cardiovascular disease, pulmonary disease, and preterm and low birth weight (19-29).

The oral cavity is populated by a large number of bacteria species that form polymicrobial communities called biofilm. The biofilm formed by the oral microbiota includes both symbiotic and potentially pathogenic species. The advent of periodontal diseases and peri-implantitis appear associated with a microbial shift, more commonly known as dysbiosis, that could be considered either a decrease in the number of beneficial symbionts and/or an increase in the number of pathogens. While the classical infection disease are caused by a single exogenous species, periodontal diseases are considered effects of biofilm community changes involving a set of species that could either be endogenous and/or exogenous. In this complex scenario the identification of the pathogenic species become a hard task.

It will be challenging to prove causation by periodontal disease, especially because it would re-

quire impractical numbers of patients to do a periodontitis intervention study to assess the prevention of CC. Despite these challenges, the work by the Author's group (10) and others (9) has started to draw direct causal links between a specific sub gingival HOM found in high numbers in periodontitis and peri-implantitis and the cancerous changes that lead to CC. Discovering of the role of FN in CC has revealed the link between oral and systemic diseases. FN is abundantly present in the oral cavity and increases in the presence of periodontal disease. Both periodontal disease and CC are frequent in older with their risks increasing with age. Several questions thus arise. Is periodontal disease a risk factor for CC? Given the connectivity of the digestive tract, could FN or other OHM is involved in additional gastrointestinal disorders? Furthermore, based on the "mobility" of FN and the omnipresence of cadherins, could this organism be involved in cancers beyond the gastrointestinal tract? Answers to these questions will shed new lights on the role of the HOM in onset of diseases.

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Correspondence to:

Dorina Lauritano, DDS
 Department of Medicine and Surgery
 University of Milan Bicocca
 Via Cadore 48
 20052 Monza, Italy
 Phone: +39.0392332301
 Fax: +39. 03923329892
 E-mail: dorina.lauritano@unimib.it