Trends in Molecular Medicine

CellPress

Opinion

Bacterial Biofilms in Colorectal Cancer Initiation and Progression

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Intestinal microbiota have emerged as an important factor in colorectal cancer (CRC) initiation and progression. The currently prominent view on bacterial tumorigenesis is that CRC initiation is triggered by local mucosal colonization with specific pathogens (drivers), and that subsequent changes in the peritumoral environment allow colonization by opportunistic (passenger) microbes, further facilitating disease progression. Screening for CRC 'driver-passenger' microorganisms might thus allow early CRC diagnosis or preventive intervention. Such efforts are now being revolutionized by the notion that CRC initiation and progression require organization of bacterial communities into higher-order structures termed biofilms. We explore here the concept that a polymicrobial biofilm promotes pro-carcinogenic activities that may partially underlie progression along the adenoma–CRC axis.

Trends

The organization of bacterial communities into biofilms (higher-order spatial structures of bacterial species) may be necessary for bacteria-induced CRC initiation.

The interaction of the intestinal epithelium with the microbiota is highly dependent on the nature of the spatial organization of bacterial communities.

Bacterial biofilms might act as direct triggering factors contributing to colorectal cancer.

The biofilm confers highly-invasive properties to opportunistic bacteria, and a putative tumor-promoting potential.

In experimental models, biofilm microbial populations can significantly impair the intestinal epithelial barrier function, alter polyamine metabolism affecting cellular proliferation, enhance proinflammatory/pro-oncogenic responses, and exacerbate intestinal dysbiosis.

The invasive and co-aggregation capacity of microbiota may be essential for biofilm-promoted colon tumorigenesis.

The Intestinal Microbiome: A New Window to Studying Colon Carcinogenesis

Nowhere in the human body are interactions between the **microbiome** (see Glossary) and host physiology as pronounced as in the gastrointestinal (GI) tract. It hosts an estimated 40 trillion microbes composed of at least 1000 species of which the vast majority reside in the colon [1]. It is thus to be expected that, if the microbiome and human pathophysiology are interlinked, this should be especially pronounced in the colon. A principal role for the microbiome in the pathophysiology of acute and chronic inflammatory diseases of the colon is indeed well established [2,3]. Intriguingly, despite the relatively negligible bacterial colonization of the stomach, the link between gastric cancer and *Helicobacter pylori* infection is beyond discussion. Emerging evidence suggests that other microbiota colonizing the stomach might also be involved in human gastric cancer progression [4,5]. However, a causal link between colorectal cancer (CRC) and the microbiome has been less evident. Recently, however, this field has moved forward by linking colonic intestinal microbiota to CRC progression [6–13]. Such studies have generated high expectations that screening for microbiological constituents might provide early diagnosis of CRC, or that disease might be potentially prevented through dietary or other interventions that could modulate colonic microbiome composition. In view of the substantial challenge that CRC poses to society, such efforts are considered to be of high importance [14,15].

It is now becoming clear that CRC is not attributable to a single pathogenic microorganism, and instead that it requires a complex intestinal bacterial community. Studies comparing fecal matter from patients with CRC relative to healthy controls have demonstrated substantial differences in human gut microbiome composition [16,17]. These studies also show that CRC is characterized by **microbial dysbiosis** [12,18]. Furthermore, recent work has shown that mice deficient in the

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immune sensor **absent in melanoma 2 (Aim2)**, and which were colonized with dysbiotic gut microbiota, are highly susceptible to tumorigenesis in the colon compared to the same mice colonized by healthy microbiota [19]. Important questions in the field involve the extent to which these changes are a cause or a consequence of CRC, in addition to identifying the mechanisms mediating such changes. Currently, various models for bacteria-induced carcinogenesis have been postulated, suggesting how intestinal microbiota as well as microbe–microbe and microbe–host interactions contribute to CRC [20–22]. Nevertheless, the mechanisms by which the intestinal microbiota interact with themselves and the human host to induce CRC initiation and progression remain largely obscure. It is becoming clear that these CRC-eliciting interactions are highly dependent on the nature and spatial organization of multispecies bacterial communities in higher-order structures (termed **biofilms**) [11,13]. In this opinion article we argue that polymicrobial biofilms promote pro-carcinogenic activities, and that invasive biofilm appears to be indispensable for CRC initiation.

From Single Pathogenic Microorganism to Polymicrobial Infections and Cancer

As shown in the quintessential example of *H. pylori* in gastric cancer, specific microorganisms *per se* are capable of driving carcinogenic and other cancerous processes in the human GI tract [23–26]. In addition, other types of human cancer can also be provoked by infection with a specific pathogen, examples being liver cancer (chronic hepatitis B or C virus), cervical cancer (human papilloma virus), Burkitt's lymphoma (Epstein–Barr virus), and bladder cancer (induced by *Schistosoma haematobium*) [20,21,27,28].

Regarding human CRC, two North American studies in 2012 showed over-representation of *Fusobacterium nucleatum* in CRC tumors compared to the surrounding normal tissue [7,9]. This bacterium was linked to CRC development, as evidenced by its capacity to invade the colonic mucosa, induce local inflammation and increased expression of cytokines, such as interleukin 6 (IL-6), IL-8, IL-12, transforming growth factor β (TGF-β), and tumor necrosis factor ∝ (TNF-∝), thereby potentially exacerbating CRC [10,29,30]. *F. nucleatum* bacterial infection directly contributes to colorectal carcinogenesis, as evidenced by two recent reports using the ***Apc*$^{Min/+}$ mouse** model and human CRC cell lines; the data show that *F. nucleatum* invasion results in the recruitment of tumor-infiltrating CD11b^{+} (integrin subunit ∝M, ITGAM) immune cells in the colon and establishing an oncogenic/proinflammatory microenvironment [10,31]. Moreover, this recruitment process seemed to depend on bacterial FadA (adhesin fatty acid degradation A)-mediated adhesion [10,31]. FadA has been previously shown to bind to **epithelial cadherin** (**E-cadherin**; also known as cadherin 1, CDH1), leading to activation of β-catenin signaling [31]. With regard to *F. nucleatum*, a recent human study reported that a higher abundance of *F. nucleatum* was correlated with fewer CD3^{+} T cells in CRC biopsies, suggesting a putative immunosuppressive effect linked to CRC progression [32]. Thus, it is reasonable to suppose that *F. nucleatum* may play a relevant role in CRC initiation/progression.

Of note, several other pathogenic bacteria of relatively low abundance in the colonic microbiota may exhibit pro-oncogenic activity in CRC development via the action of unique virulence factors [33]. As an example, enterotoxigenic *Bacteroides fragilis* (ETBF) has been proposed to be a **keystone pathogen** in CRC initiation; although ETBF typically comprises a small proportion (∼1 to 2%) of the human fecal bacterial community it causes significant pro-carcinogenic effects linked to its abundance [20,21]. The *B. fragilis* toxin, a metalloprotease toxin secreted by ETBF, can lead to the recruitment of **type 17 T helper (Th17) cells**, which in turn elicit rapid and robust inflammatory responses characterized by the production of **genotoxic** oxygen radicals, while concomitantly depressing T cell-mediated **tumor immune surveillance** through the selective activation of the signal transducer activator of transcription 3 (STAT3)-dependent pathway in the human colon [20,21,34,35]. It is thus possible that some bacteria exhibiting invasive behavior

Glossary

Absent in melanoma 2 (Aim2): an innate immune sensor encoded by the *AIM2* gene that is frequently mutated in patients with CRC. The mouse homolog is *Aim2*.
Adenoma: a benign tumor originating in epithelial tissue within glandular structures; it may affect various organs such as stomach, colon, and lung.
Adenoma–carcinoma sequence model: an experimental model described as a stepwise progression from normal colorectal epithelium to adenoma, and eventually to invasive carcinoma as a result of the accumulation of genetic and epigenetic mutations.
Amyloid fibrils: highly-ordered peptide or protein aggregates formed in human organs and tissues under abnormal conditions that are associated with various human diseases, such as peptide aggregates in brain tissue of Alzheimer patients. Bacteria can also assemble amyloid fibrils to contribute to bacterial biofilm formation.
***Apc*$^{Min/+}$ mice:** a well-established murine model for studying human colon cancer. Mice carry a truncating mutation of the mouse homolog of the *APC* gene and develop multiple intestinal neoplasia (Min).
Bacterial driver-passenger: bacteria-induced CRC carcinogenesis model; it defines driver bacteria as inducers of DNA damage to epithelial cells, initiating mutagenesis, allowing colonization of the epithelium by opportunistic bacteria (passenger pathogens) which outcompete driver bacteria, and ultimately resulting in cell proliferation/neoplasia.
Biofilm: a higher-order structure generated by an assembly of microbial pathogens that may display enhanced pathogenicity to the host. When microbes attach and reproduce on a living or non-living substrate surface, bacterial communities will be influenced by so-called extracellular quorum-sensing signals. These signals can modify the microbiota into a specific structure encased in a polymeric matrix including polysaccharides, proteins, and other components.
Bone marrow-derived dendritic cells (BMDCs): potent antigen-presenting cells that induce antitumor immunity; they can be exploited to assess immunomodulatory and