

Review

Linking Gut Microbiota to Colorectal Cancer

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Abstract

Pre-clinical and clinical data produce mounting evidence that the microbiota is strongly associated with colorectal carcinogenesis. Dysbiosis may change the course of carcinogenesis as microbial actions seem to impact genetic and epigenetic alterations leading to dysplasia, clonal expansion and malignant transformation. Initiation and promotion of colorectal cancer may result from direct bacterial actions, bacterial metabolites and inflammatory pathways. Newer aspects of microbiota and colorectal cancer include quorum sensing, biofilm formation, sidedness and effects/countereffects of microbiota and probiotics on chemotherapy. In the future, targeting the microbiota will probably be a powerful weapon in the battle against CRC as gut microbiology, genomics and metabolomics promise to uncover important linkages between microbiota and intestinal health.

Key words: microbiota, dysbiosis, mucosal defense mechanisms, inflammation, carcinogenesis, colorectal cancer.

Introduction

The general understanding of CRC: colorectal cancer; is that it is basically a genetic disease resulting from a sequence of mutations following well-established molecular pathways and accumulating over many years. Sporadic CRC develops through the CIS: chromosomal instability pathway; while HNPCC: hereditary non-polyposis colon cancer; and CRC in FAP: familial adenomatous polyposis; develop along the MIS: microsatellite instability; pathway and from a germline mutation in the APC: adenomatous polyposis coli; gene respectively – both leading to invasive cancer at younger ages.^{1,2} More recently an additional pathway – the serrated pathway – displaying both MIS and non-MIS tumors has been defined.^{3,4}

The molecular biology of colorectal carcinogenesis is mapped in great detail with the majority of CRC demonstrating accumulation of mutations and epigenetic changes according to the adenoma-carcinoma sequence with loss of function of tumour suppressor genes and gain of function in

oncogenes.⁵

However, pathways used by pathogens to establish infections (quorum sensing, invasion, biofilm formation) are able to derail mechanisms controlling cellular proliferation and pre-clinical and clinical research are producing mounting evidence that the gut microbiota is strongly associated with CRC carcinogenesis. Perturbations of the microbiota may effectively change the course of carcinogenesis directly as well as indirectly as microbial actions seem to impact both genetic and epigenetic alterations promoting dysplasia, clonal expansion, tumour growth and invasive cancer.^{6,7}

Intestinal microbial eubiosis and host-microbiota interactions are extremely important for human health. Dysbiosis and chronic, subclinical, low-grade inflammation in the bowel wall contribute to and may even initiate development of gastrointestinal diseases, such as CRC, IBD: inflammatory bowel disease; and IBS: irritable bowel syndrome. Dysbiosis may also contribute to extra-intestinal, systemic conditions

such as atherosclerosis, obesity, type 2 diabetes, metabolic syndrome and mental perturbations.

Only recently the microbiota was integrated in the framework of translational CRC research and it seems clear that dysbiosis and subsequent immunological responses facilitate CRC carcinogenesis and is linked to prognosis as well.⁸⁻¹⁵ Although the microbiota is stable over long periods of time, a variety of factors such as aging, obesity, western diet, lack of exercise, diseases and antibiotics shifts the microbiota towards a less diverse and pro-inflammatory profile.¹⁶

Potential bacterial drivers and carcinogenic metabolites, intraluminal events and inflammatory pathways are subject to intense research^{15, 17-19} as manipulation of the microbiota (e.g. use of pro-, pre- or antibiotics) changes the course of tumorigenesis, may alter or arrest tumorigenesis.

16S rRNA sequencing and whole genome shotgun sequencing metagenomics studies show lower bacterial diversity and a higher rate of certain pro-tumorigenic bacteria (e.g. Fusobacteria and Porphyromonas) associated with CRC.^{6, 13, 14, 17, 18, 20-27}

Here, we review recent research linking gut microbiota to CRC and discuss future implications. We also present the latest findings regarding possible implications of gut microbiota on sidedness of CRC and effects on chemotherapy.

Colorectal Cancer

CRC is a common malignancy in Western Europe, North America, Australia and New Zealand affecting 5-7% of the populations. Despite population screening, advances in surgery and oncology, CRC is still among the leading causes of cancer deaths. Due to Western influences on lifestyle and diet, CRC rates are on the rise in many Eastern nations as well and the global burden of CRC is estimated increase by 60% with 2.2 million new cases and 1.1 million deaths annually by 2030.²⁸ Although the 5-year survival rate has improved in the US (from 50 % to 65 % over the last 40 years), CRC remains a heavy burden on health care economics. More than 1.18 million US residents were living with CRC in 2013 and the prevalence is increasing.^{29, 30}

Recently attention has been drawn to the impact of primary tumor sidedness in terms of reduced progression-free and overall survival for patient with right-sided CRC.³¹ The CRYSTAL and FIRE-3 studies looked at 764 KRAS wild-type patients with mCRC: metastatic CRC; receiving conventional chemotherapy combined with bevacuzimab or cetuximab. Patients with right-sided primary tumors had significantly reduced progression-free survival

and overall survival compared to patients with a left-sided primary.³²

These findings were confirmed in a prospective study (CALGB/SWOG 80405) of 1137 patients with mCRC from right- or left-sided primary tumors and RAS-wild-type, both receiving conventional chemotherapy with bevacuzimab or cetuximab. Patients with left-sided primary CRC had a median overall survival 33.3 months compared to 19.4 months for right-sided primary CRC. The overall conclusion from retrospective analyses of these studies was that patients with right-sided CRC did not benefit from EGFR: epidermal growth factor receptor; antibodies, regardless of their RAS-status.³³

Interestingly, in a recent study substantial biofilm formation was found in the right colon from virtually all right-sided colon adenomas and cancers, while left-sided cancers infrequently were biofilm positive (89% vs. 12%). Importantly, histologically normal colon mucosa, collected from the surgical resection margin, was also biofilm positive or negative, demonstrating 100% concordance with their paired cancer.³⁴

Microbial biofilm (figure 1) may be regarded as an independent driver at an early stage of CRC carcinogenesis, before the malignant transformation from adenoma to carcinoma occurs. Various processes mediating the action of biofilms in driving the CRC process have been proposed, including reduced levels of E-cadherin in colonic crypts, increased intestinal permeability, the production of polyamine metabolites and their subsequent acetylation, and elevated induction of IL-6: Interleukin-6;/STAT3: Signal Transducer and Activator of Transcription 3; signaling.³⁵

CRC carcinogenesis- molecular pathways

The two major pathways involved in most cases of CRC are the CIS pathway developing through mutations in KRAS, APC, MYC, Wnt, MAPK and P53 along the adenoma - carcinoma sequence (figure 2) and the MSI pathway through mutations in MMR genes. More than 90 % of CRC are sporadic with only a small number caused by germline mutations.

CRC in FAP is a rare malignancy (<1% of CRC) caused by a frameshift mutation in the APC gene on chromosome 5q22. The mutation simulates a stop codon causing a truncation of the APC protein inhibiting β -catenin from binding to the cytoplasmic part of the e-cadherin complex. The free intra-cytoplasmic β -catenin translocates to the cell nucleus resulting in upregulation of Wnt-signalling. FAP results in multiple adenomas and inevitably CRC before the age of 40 years.²

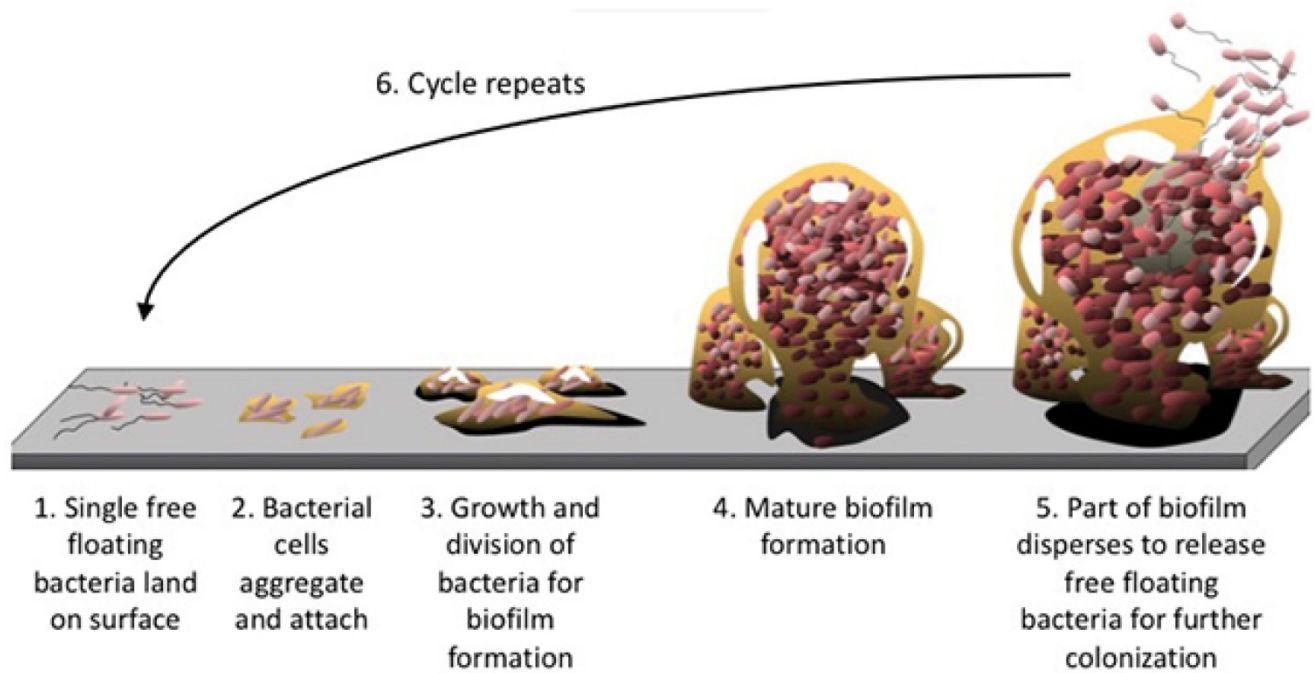
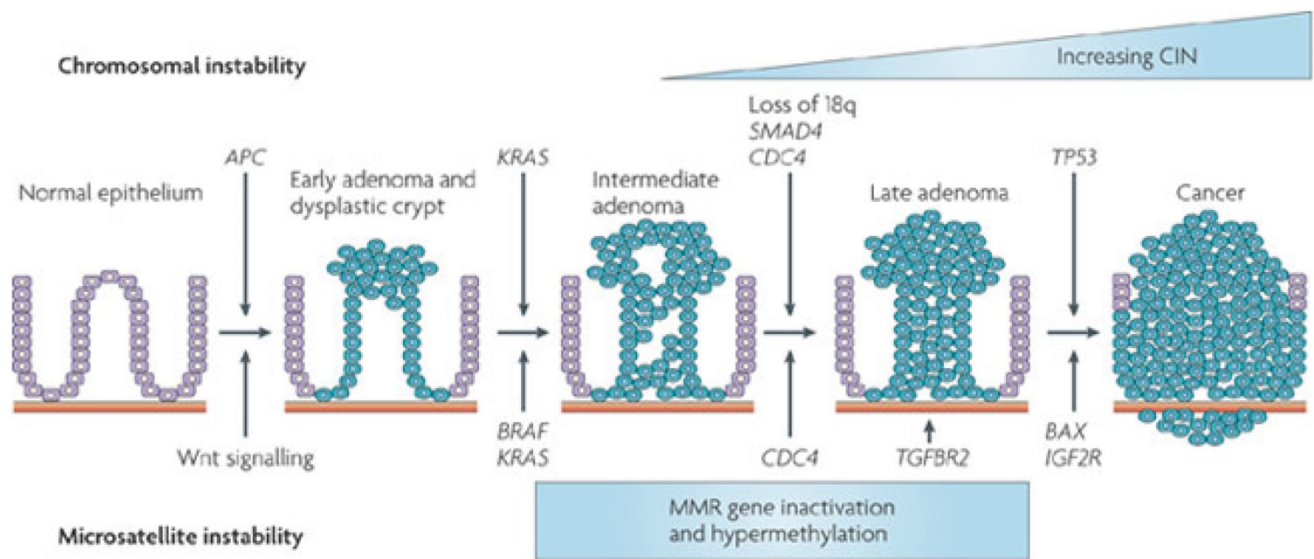


Figure 1. Biofilm formation. The structural components of the extracellular matrix is a highly hydrated, robust structure with high tensile strength and represents up to 90% of the biofilm mass keeping bacteria in close proximity, enabling intimate cell-to-cell interactions and DNA exchange while at the same time protecting the biomass from damaging agents. No copyright.



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Figure 2. Adenoma-carcinoma sequence. CIS involves loss of APC function and KRAS, followed by loss of chromosome 18q with SMAD4 and mutation in TP53. MSI CRC is characterized by a deficiency of the MMR leading to slippage in microsatellites. CIN: chromosomal instability. From Walther & al. Nat Rev Cancer 2009;9:489-99. Permission from Macmillan Publishers Limited.

Chronic inflammation is linked to carcinogenesis as 20 % of patients with ulcerative colitis develop CRC within 30 years of onset.³⁶ IBD (ulcerative colitis and Crohns disease) is widely accepted as one of the important risk factors for CRC. A meta-analysis of 116 studies found the mean age of IBD-CRC diagnosis to be 43 years, which is 10-15 years younger than sporadic CRC but with a similar 5-year survival rate.³⁷

Furthermore, it is well established that long term use of NSAIDs reduce lifetime risk of CRC, again pointing towards a link between inflammation and carcinogenesis.³⁸

The finding that long-term antibiotic use in early adulthood seems to be associated with increased risk of colorectal adenomas later in life also points towards an association between microbiota and neoplasia. ³⁹

In sporadic CRC, the accumulation of multiple mutations in growth promoting oncogenes and growth limiting tumor suppressor genes occurs with no apparent genetic predisposition although familial clustering is seen.

A constant proliferation rate in the normal colorectal mucosa is a result of a delicate equilibrium between proto-oncogenes and tumor-suppressor genes. Proliferation studies show that the rapid turnover and immense number of mitoses in the colon crypts lead to tens of thousands of mutations every day.⁴⁰⁻⁴²

Efficient DNA repair mechanisms, among others the MMR, the base excision repair system, the nucleotide excision repair system and the double strand break repair systems continuously scan the genome for replication errors. DNA defects are repaired immediately, the base excision repair system alone accounting for more than 10,000 repairs in the colon per day.⁴³ If time is needed for DNA repair, the G1 cell cycle phase is slowed down or put on stand-by by tumor-suppressor genes, among which the p53 is one of the most important and well known.^{44, 45} If mutations are too many or too extensive to repair, apoptosis is initiated through a complex signal pathway shutting down mitochondrial function resulting in immediate cell death.⁴⁶

Although the DNA repair systems are extremely effective, mutations do slip through from time to time explaining both evolution and genetic diseases. Apart from germline/hereditary cancers, the development of CRC usually requires decades to accumulate mutations in key oncogenes and tumor suppressor genes powerful enough to initiate colorectal carcinogenesis.⁴⁷

Although it is not yet proven that any specific strains of bacteria are able to provide the first hit to initiate carcinogenesis, the current understanding of the adenoma-carcinoma pathway has expanded to include environmental factors and luminal events including perturbations in the microbiota and dysbiosis.⁴⁸

Colon and the microbiota

Microorganisms have evolved over more than a billion years and the human body is host to trillions ($3.5-4 \times 10^{13}$) of microbes residing in the GI tract. The number of microbes is slightly higher than the number of cells in our body (approx. $3 \times 10^{13}/70\text{kg}$) and they harbor 150 times as many genes (3.3 mill.) compared to the human genome. It is postulated that the human colon is one of the most densely populated microbial ecosystems on the planet.⁴⁹⁻⁵²

In the colon, the concentration of bacteria reaches 10^{11} bacteria pr. gram content, which is a

million times higher than in the small bowel. The small bowel contains a very different microbiota much more dynamic and dependent on diet. Despite the harsh chemical environment in the small bowel, the risk of small bowel cancer is 12 times less than the risk of CRC, suggesting a potential role of the microbiota in carcinogenesis.^{13, 53-55}

The gut microbiota is an ecologic system of diverse commensal microorganisms metabolizing food remnants, intestinal secretions, digestive juices and exfoliated colonocytes. The colon produces vitamins (vitamin K, B12, thiamin, riboflavin), absorbs water and electrolytes and transports waste products (feces) to the rectum for defecation. Proteolytic fermentation increases with high protein intake and results in production of phenolic compounds, amines, ammonia, NOC: N-nitroso compounds; and indoles, all considered to have a carcinogenic impact on epithelial cell differentiation and proliferation.⁵⁶

Colonic metabolism involves primarily extraction of nutrients and energy from otherwise indigestible carbohydrates such as starch and proteins by fermentation. Providing 10% of our daily energy requirements, the colon is a bioreactor also producing heat, which is important for maintaining a stable intraluminal environment for microbes as well as a stable body temperature. Microorganisms produce more heat than any other organism per weight unit, and it is estimated that 70% of the body heat at rest results from bacterial metabolism in the gut.^{14, 53, 57, 58}

The human tissues are separated from the microbiota by a single layer of epithelial cells representing the mucosal surface. Epithelial cells are protected from potential pathogens by a mucus layer and by the immune system. A breach in this otherwise robust barrier may result in a state of chronic inflammation.⁵⁹

In the human colon, the total proliferation rate is 3-10 billion colonocytes per day, being the highest proliferation rate in any mammalian organ. This constant proliferation requires readily available nutrients and energy, making the process very responsive to dietary changes. In the bottom of each colonic crypt, 4-6 stemcells proliferate into an enormous amount of colonocytes while accumulating genetic and epigenetic changes.⁶⁰ As a result of the continuous proliferation, new colonocytes move from the bottom of the crypt towards the surface by approximately 1 cell position per hour. Colonocytes reaching the luminal surface are exfoliated. A crypt, and thus the entire colonic mucosa, is fully renewed in only a few days.⁶¹⁻⁶³

The microbiota has a great influence on the expression of a broad array of human genes, e.g. particular strains of Bifidobacteria, Lactobacilli and E.

coli influence gene expression of mucins, TLR: Toll-like receptors; on macrophages and dendritic cells as well as caspase expression and thus modulate immunological activity and apoptosis. However, during eubiosis the interaction between commensal bacteria and the immune cells constitute a delicate balance of pro-inflammatory genes and proto-oncogenes on one side and anti-inflammatory genes and tumor-suppressor genes on the other side.^{64, 65} Although identification of the gut microbiota, consisting of a great variety of microbes with a majority of anaerobic bacteria, has been ongoing for some years, the majority of the microbiota is still not characterized⁶⁶. Multidisciplinary approaches for characterizing the microbiota show major discrepancies between morphological and molecular studies. It is therefore necessary to perform multiple techniques to assess the overall bacterial diversity in the gut.⁶⁷⁻⁶⁹ Currently, it is estimated that 500-1000 different bacterial species and more than 7000 individual bacterial strains create the delicate community of commensal organisms.^{50, 70}

Besides bacteria the microbiota includes vast numbers of viruses, protozoa, archae and fungi. 99.9 % of colonic bacteria are strict anaerobes (bacteroidetes, bifido-, fuso-, peptostreptococcus, atopobium and eubacterium).⁷¹

Table 1. The gut microbiota is dominated by five bacterial phyla

Firmicutes (50-60% of the microbiota) including more than 180 species of Lactobacillus, Clostridiales and Streptococcaceae.
Bacteroidetes (25-40% of the microbiota) with Bacteroides Fragilis and Porphyromonadaceae both reported upregulated in CRC
Actinobacteria (2-5%) including the Bifidobacteriae
Proteobacteria (2-5%) E. coli, salmonella, yersinia, shigella, vibrio, haemophilus,
Fusobacteria (2-5%) Fusobacterium Nucleatum, faecalibacterium prausnitzii

Although a state of symbiosis exists between the microbiota and the human body, most microbes are pathogenic if translocated from the gut lumen into surrounding tissues. Hospital infections are most often Gram-negative with E. coli as the most common etiologic organism, followed by Pseudomonas aeruginosa, Klebsiella species and Enterobacter species. Bacteroides are found in most anaerobic infections and carry a high mortality.⁷²⁻⁷⁴

In the colon, the fecal (luminal) microbiota differs substantially from the microbiota within the mucine layer of the bowel wall (the juxta- or mucosal microbiota). The fecal microbiota is much richer in diversity than the mucosal microbiota and stool samples are thus poor mirror images of the mucosal microbiota as many microbes in the stool are simply passing through. Therefore, the adherent microbes in the deeper mucus layer with access to the epithelial

cells (directly or indirectly) are more likely key players in carcinogenesis.⁷⁵ In fact, most of the mucosal microbes are residing in the outer layer of the mucus membrane while the innermost part of the mucus layer contains only small amounts of bacteria.⁷⁶

The microbiota is in part determined by the genetic profile of the host and the microbiota constantly evolves during the lifespan of the individual. The microbiota is generally stable for long periods of time and in younger adults the microbiota is substantially different from the elderly, especially with regard to the proportion of the firmicutes phylum decreasing with age.^{77, 78}

A core microbiota comprising 66 taxonomic units is shared by approximately 40% of all humans but still we have no clear understanding of what constitutes a healthy microbiota. Conversely, 60% of the microbiota is variable and determined by a combination of host factors.^{50, 79, 80} Although each individual has a unique microbiota (microbiological fingerprint), the abundance and distribution between the bacterial phylotypes is almost similar among healthy persons.

With increasing age and age-related loss of CD4 T cells, the microbiota shifts towards a more pro-inflammatory and less diverse profile linked to adverse health issues and tumorigenesis. Loss of T cell differentiation diminishes the ability of the immune systems to suppress tumor-promoting inflammation in the colon.^{16, 79} Also among the age-related changes in the microbiota is a reduction in butyrate producing bacteria (especially the Firmicutes phyla) resulting in a decrease of the primary energy source for colonocytes as well as an increase in intra-colonic pH values. The higher pH values, inflammation and dysbiosis create a hostile environment for colonocytes and contribute to tumorigenesis.⁴⁸

When looking at individual bacterial strains in relation to colorectal carcinogenesis, there appears to be opposing roles for protective, butyrate-producing Gram-negative populations and pro-inflammatory, mucin-degrading Gram-positive populations. Several papers have looked at the levels of different strains in relation to CRC carcinogenesis in animal settings and in CRC patients. Methods, materials and results are inconsistent but a reproducible pattern in most studies show a reduction in lactic acid bacteria and an increase in fusobacteria, staphylococcaceae and porphyromonadaceae. Fusobacteria and porphyromonadaceae are known to synergistically promote oral cancer.⁸¹

The microbiota located in close relation to tumor tissue and polyps did not differ significantly from the normal mucosa within the same individual,

suggesting that the microbiota in CRC patients had not changed secondary to the neoplasia per se, suggesting that a CRC distinctive microbiota was already present in early stages of carcinogenesis.^{14,36}

In general, studies were based on small sample numbers and controls were often not comparable and at this point in time, it is not possible to characterize a specific cancer associated gut microbiome. Rather than a single carcinogenic bacterial strain, it seems more likely that certain bacterial compositions and their metabolites create an environment involved in carcinogenesis mediated through continuous/chronic inflammatory processes and changes in the immune system.⁷

Mucosal defense mechanisms and inflammation

In healthy individuals, the structure and function of the colorectal epithelium together with the immune system preserve a mutually beneficial relationship between the microbiota and the host. The preservation of eubiosis is important for maintaining the integrity of the intestinal gut barrier as well as the participation in the uptake of nutrients and production of vitamins. The healthy microbiota is able to sort food ingredients and other intraluminal substances into useful, useless and pathogenic and to handle them accordingly.⁸²

The single-layer of epithelial cells between the microbiota and the host is not only a physical and chemical barrier counteracting microbial invasion. It also balances the communication between the host, the microbiota and the intraluminal environment. During eubiosis, a physiologic and controlled state of low-grade inflammation is present in the bowel wall, supervised by the innate immune system. The healthy microbiota does not cause significant inflammatory responses in the host mucosa as the immune system is trained to recognize and accept the normal and naturally occurring microorganisms.

Predominant anti-inflammatory cytokines such as TGF- β : transforming growth factor- β ; and IL-10 are produced by epithelial cells, T regs: regulatory T cells; and stromal cells in order to keep colonic inflammation in check. Even commensal bacteria induce IL to maintain a basal level of Th17: T helper cells; protecting against mucosal pathogens in the bowel wall.⁸³⁻⁸⁵

The immune system contains an immunologic archive, based on PRR: pattern recognition receptors; able to distinguish potentially pathogenic microorganisms from harmless commensals. PPR primarily recognizes surface molecules derived from microbes, especially bacterial lipopolysaccharides, lipoproteins, prokaryotic DNA and foreign nucleic

acids, so-called MAMP: microorganism-associated molecular patterns; or PAMP: pathogen-associated molecular patterns.

TLR belong to a major class of PRR expressed on membranes of macrophages and dendritic cells (fig. 1). TLR signaling trigger immune defense mechanisms, primarily through production of proinflammatory cytokines, and enhances barrier function by reinforcing tight junctions and zonula occludens thereby obstructing paracellular microbial invasion.⁸⁶ CpG (cytidine-phosphate-guanosine) motifs of damaged human DNA or apoptotic debris (damage-associated molecular pattern - DAMP) may activate TLR9, an intracellular DNA sensor, and trigger a self-destructive, chronic B-cell immune response. Chronic TLR9 triggering may accelerate growth and dissemination of tumors in the GI tract.^{87,88}

Another group of PRR is NLR: NOD-like receptors; a big family of closely related intracellular sensors belonging to a super-family of glycoprotein receptors, among those the NOD1 - 14, Ipaf and Naip detecting PAMPs entering cells via phagocytosis or pores in the cell membrane. NLR are located in the cytoplasm of both immune cells and non-immune cells such as colonocytes. NLR not only recognize bacterial wall fragments being the typical NLR activators, but also react to damaged host cell membranes exposing glycans from the outer leaflets of membrane to the cytoplasmic NLR receptors. NLR activation triggers structural rearrangement of the receptor to conduct signal spread activating multiple signal pathways to induce production of pro-inflammatory cytokines and autophagy activity.⁸⁹

Disruption or mutations in PPR result is a dramatic thinning of the mucus layer and a sustained inflammatory reaction. Especially NOD2 mutations are associated with Crohns disease and increased risk of CRC as well as worsened prognosis in CRC.⁹⁰⁻⁹⁵

Dysbiosis favors invasion and growth of pathogenic species and disrupt homeostasis of the immune system and mucosal barrier. The subsequent inflammatory process results in increased permeability allowing gut microbes to drive a continuous state of inflammation.^{66, 86, 96-98}

How inflammation is linked to microbiota and carcinogenesis

When pathogens invade the well-calibrated functions of microbiota and innate immune system, the normal, anti-inflammatory environment driving anti-tumor immunity is transformed by the prompt activation of immune cells releasing cytokines and growth factors such as interleukins, TGF- β , TNF: tumor necrosis factor and VEGF: vascular endothelial

growth factor.⁹⁹

While PPR try to maintain epithelial barrier function by reinforcement of cellular junctions, an opposite effect is produced by the pro-inflammatory cytokines causing increased mucosal permeability because of cytoskeletal contraction of the peri-junctional ring providing paracellular passageways for microbes. Both cytokines and growth factors drive the inflammatory process negatively influencing cell differentiation and thereby supporting the growth and survival of dysplastic cells. In a self-sustaining process, tumor cells and even bacteria may produce increasing amounts cytokines themselves.^{100, 101}

If a state of persistent inflammation develops, the ongoing cascades of inflammatory signals result in proliferation, angiogenesis, inhibition of apoptosis and increased release of growth factors - all paving the way for cancer.¹⁹

Several cytokines may be used as biomarkers and treatment targets. A classification system (Immunoscore®) may improve prognostication of CRC patients. A multivariate analysis revealed that Immunoscore was superior to testing of microsatellite instability in predicting recurrence-free survival and overall survival.¹⁰²⁻¹⁰⁵

When eubiosis is impaired TGF- β , IL-6 and TNF produced by macrophages and T cells trigger the differentiation of naive CD4 T cells into pro-inflammatory Th17 cells leading the adaptive immune response against pathogens. In case of sustained mucosal inflammation, the prolonged presence of Th17 has been linked to development of CRC. Infiltration of Th17 cells in CRC tissue and elevated levels of Th17-mitogenic cytokines, especially IL-17 and IL-22, are associated to poor prognosis and reduced survival.^{75, 99}

Interestingly, certain types of commensal bacteria, especially Clostridia species, as well as dysplasia promote the accumulation of Th17 in the intestinal mucosa and drive IL-17 production in epithelial cells and Th17 has been shown to be a key driver of CRC in Min-mouse models exposing the animals to ETBF: Enterotoxigenic Bacteroides Fragilis. In experimental settings, limiting Th17 by depletion of Tregs reduces formation of microadenoma.^{75, 91, 106-109} On the other hand, tumours with marked infiltration of CD3+ (and maybe of CD8+) are reported to be associated with good clinical outcome in CRC in terms of longer disease-free survival and overall survival.¹¹⁰

IL-6 is a powerful pro-inflammatory cytokine clearly associated to CRC development and important for angiogenesis in cancer tissue. IL-6 produced by macrophages, T cells and fibroblasts of the

tumor-supporting stroma leads to activation of STAT3 and tumor progression. An elevated level of serum IL6 is an independent prognostic marker for tumor size, liver metastases and poor survival in CRC patients.^{111, 112}

Another multifunctional cytokine, TGF- β , produced by inflammatory cells and macrophages, plays an important role in cell growth, differentiation and apoptosis. Under normal circumstances TGF- β activation in epithelial cells inhibits abnormal cell growth by inducing cell-cycle checkpoints and growth arrest, but inactivating mutations causing loss of TGF- β signaling molecules and alterations in TGF- β receptors are frequent in CRC.^{113, 114} TGF- β produced by tumor stromal fibroblasts has the opposite effect and drives proliferation and metastatic potential of CRC cells. Tumors characterized by elevated TGF- β production are associated with a high-risk of recurrence upon treatment because of a survival advantage to metastatic cells.¹¹⁵

Macrophages also produce TNF, a powerful pro-inflammatory cytokine, recruiting inflammatory cells, increasing vascular permeability and mitogenic signaling.

TNF activates oncogenic signaling pathways in epithelial cells, including the Wnt and NF- κ B: Nuclear Factor κ -B; a transcription factor, thereby regulating growth and survival. Exposure of epithelial cells to TNF increases CIS and mutation rates, suggesting a direct mechanism by TNF on cancer development.¹¹⁶⁻¹¹⁹ TNF serum levels positively correlate with disease progression and poor survival in CRC patients.^{120, 121}

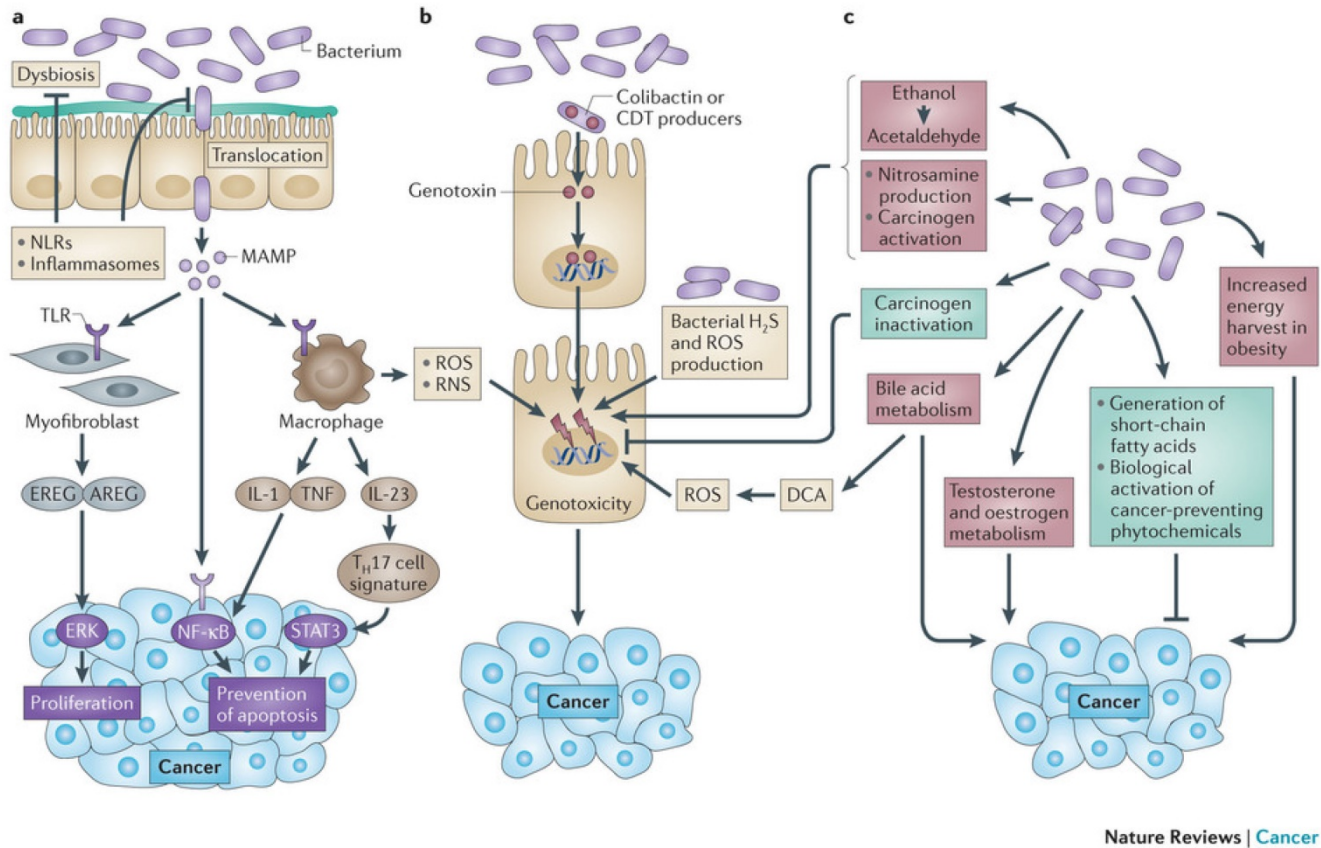
Possible pathophysiological mechanisms of inflammation also include the oxidative stress caused by an imbalance between the production of ROS: Reactive Oxygen Species; and antioxidant defenses resulting in DNA damage, dysplasia and thus a tumor-supporting environment. Oxidative stress induces NF κ B linking chronic inflammation to cancer through the ability of up-regulation of pro-inflammatory and tumor promoting cytokines, such as TNF α , IL-1 and -6 as well as mitogenic and anti-apoptotic signaling. NF- κ B directly regulates a complex of genes (including bcl-x and survivin) increasing proliferation and protecting tumor cells from apoptosis.^{100, 122} Interestingly, obesity increases NF κ B expression in most tissues also increasing the risk of type-2 diabetes. NF κ B could be a factor linking obesity, type 2 diabetes, cardio-vascular disease, IBD and cancer.^{13, 123}

Evading host immune system

Gut bacteria are able to invade host tissues, evade host immune system and maintain

inflammation. Bacteria communicate internally using chemical molecules in a process called QS: quorum sensing; (figure 4) and by nanotubes, both of great importance for bacterial virulence. Ways to interrupt QS (quorum quenching) and the formation of biofilms

are believed to be next generation of antibiotics and may impact tumorigenesis and is therefore focus intense research. QS involves production, release, detection and response to chemical signalling by small hormone-like molecules called autoinducers.



Quorum Sensing

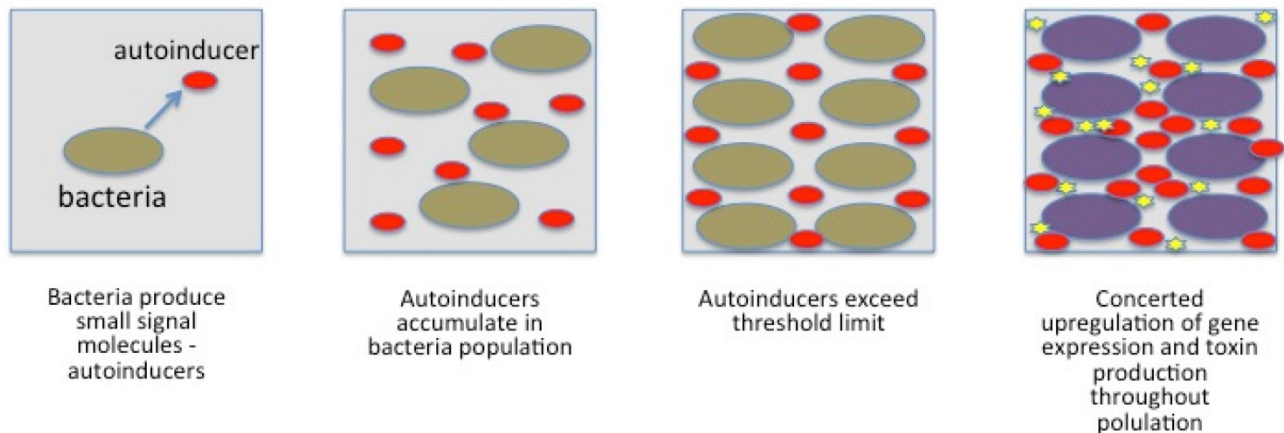


Figure 4. Schematic presentation of bacterial gene regulation by Quorum sensing. No copyright.

Bacteria also use nanotubes (small bridging tunnels of 30-130 nm in diameter), through which cargo can be exchanged in the form of cytoplasmic components (e.g. resistance). Furthermore, nanotubes are not restricted to interaction between the same species.⁹ For bacteria to launch a successful attack on the host, timing and communication is the key. If not executed precisely, bacteria are immediately wiped out by the host's immune system. By the use of quorum sensing, a finely tuned and powerful attack may overcome the defense mechanisms and even kill the host. Bacteria use quorum sensing to count themselves, synchronize their behavior and to express specific genes at the same time. They wait until their numbers exceed a critical mass (threshold for signal molecules) for a successful invasion (swarming) and secrete their toxins simultaneously. Once gene expression is upregulated, a number of bacteria produce exopolysaccharides to glue themselves together in a sticky, protective layer (biofilm) having significant importance for virulence and resistance.¹²⁴

Bacteria have evolved ways to perceive environmental changes and gauge whether it is beneficial to remain as planktonic (free-flowing) organisms or whether it is time to resume a safer life within a biofilm. When bacteria switch from planktonic to community mode, they undergo a shift

in behavior that involves alterations in the activity of numerous genes. Once colonization of the biofilm has begun, it grows through a combination of cell division and recruitment.¹²⁵

Biofilm formation enables bacterial pathogens to colonize a wide variety of host niches and to persist in harsh environments making their eradication by the innate immune system particularly difficult. Because biofilms often develop their own metabolism, they are sometimes compared to tissues of higher organisms, in which closely packed cells benefit from the collective gene-pool for the greater good and safety of the community.¹²⁶

Biofilm formation is not a random process but have distinct architectures developing into 3-dimensional structures housing millions or billions of individual bacteria. Attached to the surface by pili and fimbriae and with the help of the self-produced intercellular polymeric matrix the bacteria glue themselves together and form colonies resembling small cities with towers, roads, bridges and channels for the flow of nutrients and at the same time allowing residents to enter and exit the biofilm. Living inside biofilms, bacteria increase their chance of survival by increased persistence and resistance to hostile environmental factors.^{124, 127}

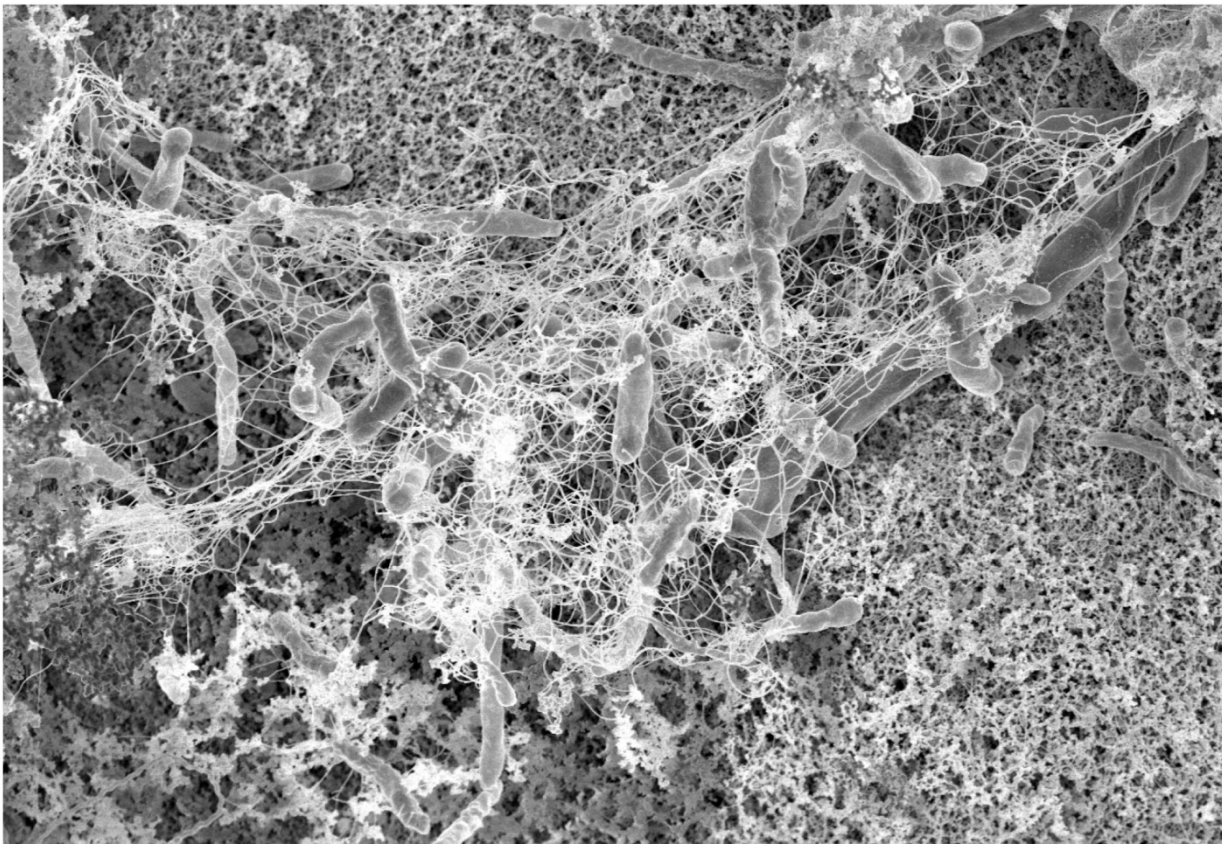


Figure 5. Early stage of bacterial biofilm with visible intercellular polymeric matrix.

If overcrowding in the biofilm occurs, communication molecules prompt bacteria to leave the biofilm (figure 1). In the colon, biofilms may be found in the inner protective mucus layer allowing for direct interaction between bacteria and colonocytes thereby triggering inflammation. Approximately 15% of healthy individuals harbour thin bacterial biofilms predominantly in the right side of the colon whereas nearly 100% of right-sided CRC were found to have substantial biofilms. In addition, biofilms proximal to the hepatic flexure constitutes a higher frequency of epithelial KRAS and BRAF mutations and are linked to a worse prognosis for right-sided cancers.^{9, 12, 34, 75, 128-130}

Once an attack is launched, bacteria have developed highly effective mechanisms to evade the immune system. Wearing coats of carbohydrates camouflaging their surface antigens (lipopolysaccharides and peptidoglycans) bacteria are able to disguise themselves and evade immune surveillance and PPR recognition. They can invade their new territory without detection and may even circulate systematically and unnoticed within the body.¹²⁹

Inside the host, bacteria disguise themselves by changing expression of surface molecules by switching simple structural molecules. Some bacteria even possess a genetic switch to turn gene expression on and off. As the acquired immunity of the host relies on memory of previous exposure to antigens, variation or covering of surface antigens is very effective in circumventing both humoral and cellular responses.^{129, 131}

Bacteria may alter host immune response or even avoid it by affecting downstream inflammatory cytokines and by secretion of enzymes such as IgA proteases degrading immunoglobulins. Bacteria anchor to host cell surfaces by adhesins or receptor ligands and by the use of invasins or fusion proteins they force their way into host cells. Inside infected cells, bacteria may block normal apoptosis mechanisms, and using the cell like a Trojan horse, bacteria multiply within the infected cell.¹³²

If discovered by the immune system, several bacteria are able to defend themselves by injecting cytotoxic agents directly into immune cells through pili and nanotubes. Due to their size, bacteria are perfect phagocytic targets but many bacteria have developed ways of avoiding phagocytosis by injection of effector mediators neutralizing phagocytic activity. The ability to avoid internalization and killing plays a central role in bacterial virulence.¹³³

When caught and trapped inside a macrophage, bacteria can use several strategies to avoid killing. Escaping the phagosomes, most commonly by

blockage of phagosome-lysosome fusion or by utilization of mechanisms to allow survival in phagolysosomes. Species of *Shigella* and *Listeria* and some *Rickettsia* species secrete lysins that are highly effective at lysing the vacuolar membrane that engulfs internalized organisms.¹²⁹

Drivers, passengers and keystone pathogens

The mechanisms by which the microbiota potentiate carcinogenesis range from production of carcinogenic toxins to manipulation of inflammatory pathways. The driver-passenger model describes the existence of certain bacteria in the microbiota possessing special virulence traits (e.g. ETBF, *Fusobacteria* spp. and *Porphyromonadaceae*) creating DNA damage and driving genomic instability. Drivers are subsequently replaced by commensals (passengers) with tumor-promoting or tumor-suppressing properties such as the pro-inflammatory *Streptococcaceae*, *Klebsiella* and *Proteus*. During bacterial invasion, passengers or "back-seat drivers" do not play any active role. They are excluded from the communication network and are not impacted by quorum sensing but can be activated at a later stage. By the use of nanotubes, passengers may bypass any diffusion barrier or inhibitory systems put up by the immune system.⁹

According to the keystone pathogen (or α -bug) hypothesis, the effects of some bacterial species are disproportionately large relative to their abundance. Thus, small-abundance pathogens like ETBF, *Fusobacteria* and colibactin-producing *E. coli* can cause dysbiosis, increase permeability, translocate through the mucosal barrier and with their toxins cause chronic inflammation, inflammatory diseases and contribute to carcinogenesis.^{18, 49, 134}

Bacteria of special interest

Although dysbiosis may take many forms and no consistent cancer-related dysbiotic signature is likely to be discovered, carcinogenesis may depend on structure and metabolic activities of communities of bacteria (polymicrobial synergy), i.e. no single virulence factor, disruption of mucosal barrier or long lasting inflammation, but rather all these components in concert.

Although conflicting data exist, there are a number of reports of significant differences between the mucosal and fecal microbiota from CRC patients and controls.^{75, 135}

Overall, the common findings in CRC patients are loss of bacterial diversity and dysbiosis. Particularly there is often a marked decrease in firmicutes, bacteroides and lactic acid bacteria

together with an increase in fusobacterium, and porphyromonas.

E. faecalis, *E. coli* and *Fusobacterium nucleatum* are capable of triggering Wnt mitogenic signalling, DNA damage, interference with DNA repair processes and to increase mucosal permeability by rendering adhesion molecules (e-cadherins) dysfunctional - all of which are critical to CRC carcinogenesis.^{49, 55, 75}

In experimental settings, proteobacteria, especially a relative abundance of *Lactobacillus*, was inversely proportional to tumour burden, whereas Bacteroidetes and proteobacteria consistently were among the phylae increasing tumour counts and tumour burden. Indeed, the balance between the predominant Bacteroidetes and Firmicutes phyla seems to be critical in relation to disease progression.^{7, 8, 75}

In 2014, European Molecular Biology Laboratory performed metagenomic sequencing of stool samples from 156 individuals with CRC and from controls. Metagenomics predicted the presence or absence of CRC using the relative abundance of 22 bacterial species, including *Porphyromonas* and *Fusobacterium*. It was possible to predict CRC with about the same accuracy as fecal occult blood testing commonly used as a screening tool. Fifty percent of cancers were correctly identified with less than 10 percent of false positives. When metagenomics and fecal occult blood testing were combined, they found more than 70 percent of cancers. The accuracy of metagenomic CRC detection did not differ between early- and late-stage cancers. The method was later validated in independent patient and control populations from several countries.¹³⁶

Fusobacterium Nucleatum

FN: *Fusobacterium Nucleatum*; is an anaerobic Gram-negative bacteria linked to CRC. 16S rRNA and whole genome sequencing has shown enrichment of FN in CRC associated mucosa and in the mucosa of adenoma patients when compared to healthy individuals. FISH: fluorescence *in situ* hybridization; technique also show excess FN in tumor tissue as well as invasiveness of the bacteria has been demonstrated.^{137, 138}

FN is able to directly promote carcinogenesis when the secreted anchoring adhesin FadA binds to the extracellular domain of E-cadherin on epithelial cells rendering the adhesive complex dysfunctional. The result is openings of paracellular passageways for pathogens to the submucous tissues. The malfunctioning intercellular part of the adhesion complexes cannot bind free cytoplasmic beta-catenin, which instead translocates to the cell nucleus. Here

β-catenin upregulates mitogenic signaling through the Wnt pathway, leading to increased expression of transcription factors, Wnt genes, inflammatory genes and growth stimulation of CRC cells.¹³⁹ FadA may even compromise DNA repair as high-level FN colonization has been significantly associated with microsatellite instability.⁵⁵ A 3-fold increase in risk of adenomas among individuals with the highest tertile of FN counts has been demonstrated. Presence and counts increased with malignant transformation from adenomatous polyp to CRC.⁷ FadA levels in the colon tissue from patients with adenomas and adenocarcinomas is >10–100 times higher compared to normal individuals and the potential as a diagnostic marker is being investigated.¹³⁹ Examination of adenomatous and carcinomatous tissues demonstrated an elevated number of FadA gene copies and high levels of FN-DNA in tumor tissue was associated with increased lymph node metastases and poorer outcome in terms of shorter survival for CRC patients.^{140, 141}

FN is often found in tumor microenvironment, and it seems that the function of infiltrating natural killer cells is inhibited in the presence of FN. The cytotoxicity of NK: natural killer; cells appear to be inhibited through a direct interaction between the Fap2 protein produced by FN and the inhibitory immune receptor TIGIT: T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibition motifs; present on all human NK cells and various T cells. A recent study identifies how the tumors exploit the Fap2 protein of FN to evade the immune system and inhibit immune cell activity via TIGIT.

FN potentiates CRC tumorigenesis in APC^{min} mice and represents a typical driver bacterium. FN cannot colonize the colon on its own but needs the interaction of several other species to establish colonies, which in turn support the growth of peptostreptococcus and porphyromonas. Furthermore, FN is implicated in other diseases like rheumatoid arthritis and liver cirrhosis.^{81, 138, 142}

Enterotoxigenic Bacteroides fragilis

BF: *Bacteroides Fragilis*; is a common anaerobic commensal in most humans representing approx. 1 percent of the microbiota. A subgroup of BF is the ETBF, a common cause of diarrhea in children. ETBF has only one recognized virulence factor, the ETBF-toxin or fragilysin being a 20kDa zinc-dependant metalloproteinase toxin binding to epithelial receptors on colonocytes.⁷

Fragilysin rapidly alters structure and function of epithelial cells including cleavage of E-cadherin thereby increasing mucosal permeability and cytokine secretion, upregulation of Wnt signaling, NF-κB

activation, cell proliferation and DNA damage. *In vivo* and *in vitro* experimental models together with early human data support that ETBF may promote colon carcinogenesis.^{6, 49, 143} Small amounts of Fragilysin can be demonstrated in up to 40% of healthy adults. Persistent colonization with ETBF may result in a subclinical IL-17 dominant colitis with concomitant STAT3 activation. Increased amounts of ETBF and Fragilysin have been demonstrated in 100% of advanced CRC cases^{144, 145}. In Min mice (Apc min/+), colonization with ETBF rapidly resulted in adenoma formation and visible colon tumors already after only one month. Thus, ETBF may represent the optimal bacterial driver for CRC.⁴⁹

Escherichia coli

E. coli is a facultative anaerobic commensal bacteria belonging to the proteobacteria, not extremely abundant in the colon but easily cultivable. There are several phylogroups of which the B2 strain is pathogenic and often involved in IBD and CRC. The B2 strain harbors a genomic island called “pks” codings for the production of a polyketide-peptide genotoxin, Colibactin.¹⁴⁶

Colibactin is able to penetrate the cell membrane of colonocytes and translocate to the cell nucleus and function as a DNase causing double strand DNA breaks, cell cycle arrest and incomplete DNA repair, all of which will lead to chromosome aberrations. Micronuclei, aneuploidy, ring chromosomes, and anaphase bridges persisted in dividing cells up to 21 days after infection, indicating occurrence of DNA breaks and chromosomal instability. The infected cells showed significant mutation frequency demonstrating the mutagenic and transforming potential of *E. coli* type B2.^{147, 148}

Germfree mice have very few IgA producing cells in their intestinal mucosa. IgA is important for the exclusion of pathogens and neutralization of toxins. If these mice are colonized with either *E. coli* type B2 or *E. faecalis*, they both develop inflammation, but only those colonized with *E. coli* type B2 developed colon tumors characterizing colibactin as a carcinogenetic bacterial metabolite.¹⁴³

E. coli phylotype B2 harboring the pks island represents another perfect driver bacteria and colonization with the B2 strain may contribute to the development of sporadic CRC. The B2 strain and colibactin represents the overall current strongest candidate for bacterial contribution to CRC carcinogenesis.^{49, 149}

Porphyromonas

PM: Porphyromonas; compromises the integrity of the epithelial cell layer, allowing for invasion and

colonization. Once inside the host cell, PM inhibit cell apoptosis by inhibiting cytochrome C release from mitochondria and downregulating of caspase 3 activity. Also, there is upregulation of anti-apoptosis genes encoding Bcl-2 and survivin as well as changes in the level and phosphorylation status of cyclins, p53 and PI3K controlling the cell cycle. Furthermore, it appears that PM may increase TNF α and nitric oxide synthetase expression. Taken together, the actions of PM lead to a pro-tumorigenic inflammatory environment.^{150, 151}

Bacterial metabolites: Short chain fatty acids and secondary bile acids

Probiotics produce SCFA such as butyrate, acetate, propionate and valerate. SCFA provide colonocytes with fuel and suppress cancer and inflammation through several pathways: depressing anti-apoptotic Bcl-2, converting procaspase to active caspase, irreversible binding to mutagens such as nitrosamines and hydrogen peroxide, cell cycle regulation and induction of autophagy. Inflammation is primarily suppressed by inhibiting IL-2,-6,-10,-12, TNF α and NF κ B. Probiotics contribute to the barrier function of the mucosa by maintenance and assembly of tight junctions and increasing mucin production.¹⁵²

Other microbial metabolites, e.g. SBA promote carcinogenesis. Butyrate and SCFA are both thoroughly investigated in relation to inflammation and CRC carcinogenesis. Beneficial effects of SCFAs are listed in table 2. From bacterial breakdown of dietary fiber SCFA has the capability of influencing host gene expression by a number of different routes and also modulate host inflammatory response, among others by histone-deacetylase inhibitor affecting gene expression. The Bacteroidetes and Firmicutes phylae produce large amounts of acetate and propionate, whereas Bifidobacteriae are the main source of butyrate production.¹⁵³

Table 2. Beneficial effects of SCFA produced by probiotics

Predominant energy source for colonocytes
Induction of mucin synthesis
Augmentation of tight junction assembly
Mediation of cross-talk between commensals and host immune system for maintenance of gut homeostasis
Conditioning gut epithelial cells to mount protective immunity through MAP kinase signaling
Inhibiting pro-inflammatory cytokines, NF κ B and TNF α
Inactivation of mutagens

Besides liberating beneficial SCFA, fermentation of amino acids produces several harmful compounds potentially playing roles in CRC and IBD. Compounds like ammonia, phenols, p-cresol, certain amines and H₂S, may take part in the initiation or

progression of cancer through chronic inflammation and DNA damage.⁵³ Butyrate possesses anti-tumorigenic properties; among others the induction of IL-8 in the colonic epithelium, driving T cell differentiation of naive T-naive cells to regulatory T cells and reduction of tumorigenic ILs and metalloproteinase activity.^{36, 152, 154}

A reduction of butyrate producing bacteria results in an increase in intra-colonic pH values creating a hostile environment for colonocytes. From the cecum to the rectum the pH increases contributing to increased susceptibility for tumorigenesis in the left colon and rectum.⁴⁸

The PBA: primary bile acids; cholic acid and chenodeoxycholic acid, produced from cholesterol in hepatocytes, are conjugated with taurine or glycine to increase solubility and impermeability critical for the formation of micelles. SBA: secondary bile acids; primarily lithocholic acid and DOC: deoxycholic acid; are a very potent bacterial metabolites produced by commensal bacteria by deconjugation (dehydroxylation) of PBA.^{155, 156}

High-fat diets increase formation of SBA and may induce significant changes in the composition and function of the microbiota together with changes in the gut immune system. Repeatedly exposure of colonocytes to increased levels of DOC induced resistance to apoptosis.¹⁵⁷ Exposure of colonocytes to high concentrations of DOC induces formation of ROS and RNS and the primary carcinogenic actions of SBA are through ROS, NF-kappa-B activation and direct DNA damage.¹⁵⁸

Ridlon *et al.* propose mechanisms by which microbial metabolism of taurocholate (whose formation is favored by diets high in animal protein) may promote CRC through sulfide formation from taurine and via the tumor-promoting activity of DOC.¹⁵⁹

Animal studies

Based on the initial composition of the microbiota in an animal study using the inflammation based model (AOM/DSS) and a pattern recognition algorithm, it was possible to predict subsequent tumor development and even time antibiotic treatment to arrest/revert carcinogenesis. Eight different bacterial community structures were generated by different antibiotic treatments prior to AOM injection and despite a non-significant reduction in the bacterial load due to the antibiotics they varied in the ability to drive tumorigenesis.^{6, 36}

When gnotobiotic mice are colonized with microbiota from mice with CRC of chronic inflammation the number of tumors increases.³⁶ However, IL-10 and p53 knockout mice do not

develop CRC under germfree conditions. If colonized with feces of healthy mice tumorigenesis is significantly reduced.⁶ In the AOM model using DSS as inflammatory agent, mice do not develop tumors if they receive antibiotics as well as antibody blockade of IL-17.¹⁰⁶

In conclusion, animal studies have shown that perturbing the microbiota may halt carcinogenesis and that the microbiota interacts with the host to impact tumor susceptibility. A tumor-associated microbiota transferred to germ-free mice resulted in increased tumor development when compared to healthy mice.

Chemotherapy

Microbiota may also influence tumorigenesis in a positive manner providing a critical boost to antitumoral T cell responses. Some chemotherapeutics (e.g. Oxaliplatin) even rely on the differentiation gene MyD88: Myeloid Differentiation Primary Response 88 gene; signalling triggered by microbes. The MyD88 encodes a cytosolic adapter protein playing a central role in the innate and adaptive immune response. The gene product functions as a signal transducer in the interleukin-1 and Toll-like receptor signaling pathways. Both pathways regulate the activation of numerous proinflammatory genes. The protein consists of an N-terminal death domain and a C-terminal Toll-interleukin1 receptor domain.

Efficacy of immunotherapy is found to rely on commensal bacteria such as bifidobacteria and bacteroides providing augmented dendritic cell functions, enhanced CD8+ T cell priming and accumulation in the tumour microenvironment.⁷⁵

Clinical trials have demonstrated lack of effect of EGFR antibodies in patients with mCRC from right-sided primaries irrespective of their RAS-status and one could speculate these results are related to the biofilm formation found in these patients.^{32, 34}

Animal trials have shown that a normal functioning microbiota is required for optimal effect of both oxaliplatin and cyclophosphamide^{160, 161} and *in vitro* studies showed prolonged and increased response to 5-fluorouracil, a fluoropyrimidine, in the presence of a healthy microbiota composition. Capecitabine and TAS-102 (tipiracil hydrochloride) are both fluoropyrimidines used for systemic treatment in colorectal cancer patients.¹⁶²

In theory, this could in part explain some cases of lack of effect of chemotherapy (e.g. Oxaliplatin in rectal cancer) due to preoperative radiotherapy followed by a quite extensive surgical trauma and frequent use of antibiotics postoperatively. Consequently, the use of antibiotics in cancer patients

should be administered with care as several chemotherapeutics may depend on a functional microbiota. Increasing production of SCFA and modulating the microbiota towards a health-promoting profile by increasing lactic acid bacteria may prove beneficial during recovery from surgery and chemotherapy.^{75, 163, 164}

Targeting the microbiota may also improve the effect on certain chemotherapeutics, e.g. inhibiting β -glucuronidase produced by gut bacteria prevents the metabolism of the topoisomerase-inhibitor Irinotecan and the administration of Bifidobacteria enhances anti-tumor immunity partly by increasing anti-PD-L1 (check-point blockade) efficacy.^{164, 165}

Obesity, inflammation and cancer

Obesity rates are on the rise in most parts of the world and increase with age and lower educational attainment. In Europe, 16% of adults are obese and 11% of CRC cases have been attributed to obesity. In the US, obesity constitutes the fifth leading risk for overall mortality in the US and is associated with a number of diseases.^{164, 166}

Obesity (BMI > 30) is often combined with widespread low-grade inflammation in many organs and tissues, especially the adipose tissue. Obese individuals are known to harbor different types of microbiota in terms of phylum-level changes, reduced bacterial diversity and altered representation of bacterial genes and metabolic pathways. Although an obesity-related decrease in the ratio of Bacteroidetes to Firmicutes has been demonstrated, it remains controversial if this is a general obesity trait. Epidemiological data associate obesity to a 30-70 percent increased risk of CRC.¹⁶⁰

Obesity may also be associated with worse outcome, e.g. recurrence and mortality. Several factors, including reduced sensitivity to antiangiogenic-therapeutic regimens, might explain these differences. Except for wound infection, obesity has no significant impact on surgical procedures. The underlying mechanisms linking obesity to CRC are still a matter of debate, but metabolic syndrome, insulin resistance and modifications in levels of adipocytokines seem to be of great importance. NF κ B could be a factor linking obesity, type-2 diabetes and cancer.^{53, 167, 168}

Conclusion

Dysbiosis generates a proinflammatory response and a self-reinforcing hostile environment in the colonic mucosa. It is unlikely that dysplasia and malignant transformation is related to only a few microbes but rather to a polymicrobial change in microbiota. Convincing data show that microbiota is

deeply involved in colorectal carcinogenesis and the understanding of the microbial processes, especially with regard to interpreting the crosstalk between the microbes and the host, will help us explore the manipulation of the microbiota in the prevention and treatment of CRC through diets, pre-, pro- and antibiotics including next-generation antibiotics of quorum sensing inhibitors and biofilm inhibitors.

Biofilms are advanced microbial arrangements or higher-order structures that may enhance or accelerate dysplasia and malignant tissue transformation. A biofilm invading the colonic mucus layer indicates a pathologic state. The recent discovery of colonic biofilms in patients with right-sided colon cancer adds to the concept that colorectal cancer may arise as a result of the actions of microorganisms and therefore may, at least in theory, be regarded as an infectious disease. Data on biofilm formation in patients with CRC may change screening practice to also include biopsies from the right colon and examination (FISH, electron microscopy) for biofilm if results are confirmed in larger trials. Biofilm-positive individuals maybe should be offered the same attention and follow-up as adenoma patients. New biofilm inhibitors may change the course for CRC the same way antibiotics did for *Helicobacter Pylori* and gastric cancer.

Probiotics and their SCFA seem to be a safe tool in conjunction with conventional treatment of CRC and already one clinical trial has demonstrated beneficial effects of probiotics for CRC patients including shorter hospital stay and lower infection rate.⁸⁹

A fecal or microbiota transplant (MT) to restore a healthy microbiota is an interesting concept in CRC but has still not been elucidated in CRC. MT is a simple, inexpensive and presumably safe way to manipulate the microbiota. MT is effective in recurrent or chronic clostridial infections.¹⁶⁹ Effective components of MT still need to be identified and there is no consensus as to the optimal route of MT (nasoduodenal or rectal), composition or amount of the optimal MT. An optimal donor profile also has to be defined and the donor screened for contagious diseases. To our knowledge, there are no reported adverse effects of MT, but until we know more about benefits and potential harms of MT authorities are reluctant to approve it. With the current speed of pharmacological and microbiological research, these issues are likely to be solved in the near future. An ongoing study aims to detect a bacterial profile that predicts if patients with mCRC will respond to systemic treatment and/or experience chemotoxicity. If a favorable microbiota composition can be identified, MT is a better alternative to halt/reduce or

switch chemotherapy.¹⁶²

In the future, targeting the microbiota will probably be a powerful weapon in the battle against CRC. The combination of gut microbiology with new genomic and metabolomics data promises to uncover more important linkages between microbial metabolites and health in the future.

In affluent and modern societies, the microbiota and the relation between microbiota and host is dramatically changing as a consequence of lifestyle, environment and diet, especially processed food, increase in carbohydrate intake, lack of exercise and obesity.

Research and increasing knowledge will provide better understanding of the impacts of diet, obesity and inter-individual variation and should reveal new avenues for disease prevention. In addition, prebiotics, probiotics and microbiota transplantation may restore microbial homeostasis, reduce the toxic effects of certain microbes, inflammation, mitogenic and antiapoptotic pathways and even antibiotics may contribute to the efficacy of chemotherapeutics in cancer patients.

Lastly, new classes of antibiotics like quorum sensing inhibitors and biofilm inhibitors may also become prominent tools in terms of changing the virulence and resistance of certain bacterial drivers and the course of bacterial driven carcinogenesis.

Abbreviations

AOM: azoxymethane; APC: adenomatous polyposis coli; AREG: amphiregulin; BA: bile acids; BF: bacteroides fragilis; CDT: cytolethal distending toxin; CIS: chromosomal instability; CRC: colorectal cancer; DOC: deoxycholic acid; DSS: dextran sodium sulphate; EREG: epiregulin; ETBF: Enterotoxin bacteroides fragilis; ETBFT: Enterotoxin bacteroides fragilis toxin; FAP: familial adenomatous polyposis; FISH: fluorescence in situ hybridization; FOBT: fecal occult blood test; FN: fusobacterium nucleatum; HNPCC: Hereditary non-polyposis colorectal cancer; IBD: inflammatory bowel disease; IBS: irritable bowel syndrome; IL: interleukin; MAMP: microorganism-associated molecular pattern; MAPK: mitogen-activated protein kinase; MIS: microsatellite instability; MMR: mismatch repair; NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; NLR: NOD-like receptor; NOC: N-nitroso compounds; NOD: nucleotide-binding oligomerization domain-like receptor; NSAID: non-steroidal anti-inflammatory drugs; PAMP: pathogen-associated molecular pattern; PBA: primary bile acids; PM: porphyromonas; PRR: pattern recognition receptor; RNS: reactive nitrogen species; ROS: reactive oxygen species; SBA: secondary bile

acids; SCFA: short chain fatty acids; STAT3: signal transducer and activator of transcription 3; TGF: transforming growth factor; Th cells: T helper cells; TIGIT: T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibition motifs; TLR: toll-like receptor; TNF: tumor necrosis factor; UC: ulcerative colitis; VEGF: vascular endothelial growth factor; Wnt: wingless-related integration site.

Competing Interests

The authors have declared that no competing interest exists.

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