The gut microbiota, bacterial metabolites and colorectal cancer

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Abstract | Accumulating evidence suggests that the human intestinal microbiota contributes to the aetiology of colorectal cancer (CRC), not only via the pro-carcinogenic activities of specific pathogens but also via the influence of the wider microbial community, particularly its metabolome. Recent data have shown that the short-chain fatty acids acetate, propionate and butyrate function in the suppression of inflammation and cancer, whereas other microbial metabolites, such as secondary bile acids, promote carcinogenesis. In this Review, we discuss the relationship between diet, microbial metabolism and CRC and argue that the cumulative effects of microbial metabolites should be considered in order to better predict and prevent cancer progression.

Recent advances in our understanding of the composition and metabolism of the human microbiota have established that it exerts an important influence on human health. Importantly, accumulating data suggest that the microbiota has a role in the aetiology of several types of cancer by influencing inflammation, DNA damage and apoptosis. As our greatest exposure to microorganisms occurs in the gut, particularly the large intestine, the involvement of the gut microbiota in colorectal cancer (CRC) is an active area of research.

CRC is the third most common cause of cancer mortality in the world. The disease typically develops over many years via a sequence of genetic changes, which is known as the adenoma–carcinoma sequence (BOX 1). Tumours are more frequent in the distal large intestine (which includes the descending colon and rectum) compared with the more proximal regions of the large intestine, which might reflect differences in the luminal environment of these gut compartments. Although some forms of CRC tend to be heritable, most CRC cases show an association with diet and lifestyle, and dietary factors are among the most strongly established risk factors. Diet and the composition of the gut microbiota

The large intestine contains the most dense and metabolically active microbial community (>10^{11} cells per g contents) in healthy adults, which is dominated by anaerobic bacteria that belong to two phyla — the Firmicutes and Bacteroidetes — in addition to Actinobacteria, Proteobacteria and Verrucomicrobia. Despite substantial inter-individual variation in the composition of the microbial community, human studies have shown that dietary composition has an important effect on the gut microbiota, such that changes in the faecal microbiota are detectable as early as a few days after switching between carefully controlled diets. Many of the species that respond to changes in carbohydrate intake seem to belong to the Firmicutes and Actinobacteria, which are nutritionally-specialized. Among the Firmicutes, Ruminococcaceae (particularly
**Non-digestible carbohydrates**

Dietary carbohydrates that are not digested by mammalian enzymes in the small intestine and reach the colon, where they may be used as substrates for the resident microbiota.

**Mucin**

A high molecular weight glycoprotein that is produced by the gut epithelium and forms the mucus layer that lines the gut wall; it can be used as an energy source by some gut bacteria.

**Non-starch polysaccharides**

Non-digestible carbohydrates other than resistant starch, including cellulose, arabinoxylans, xylanoglucons, pectins and gums.

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**Box 1 | Genetic basis of CRC development: the adenoma–carcinoma sequence**

More than 1.2 million new cases of colorectal cancer (CRC) are reported each year, most of which occur sporadically as a result of the accumulation of mutations and epigenetic modifications in several genes. The sequential accumulation of genetic alterations that is thought to drive malignant progression involves the transition from normal mucosa to pre-malignant lesions, with progression to colorectal adenomas and fulminant CRC occurring over several years. This pathogenetic framework is known as the adenoma–carcinoma sequence. The initial mutations most often occur in the adenomatous polyposis coli (APC) tumour-suppressor gene, which encodes a multifunctional protein that has important roles in the WNT signalling pathway, intercellular adhesion, cytoskeleton stabilization, cell cycle regulation and apoptosis. Mutations in APC confer a selective growth advantage and thereby potentiate the growth of the mutated cell. Further mutations in another gene, such as KRAS, which is a gene that is usually involved in G protein signal transduction and the modulation of cellular proliferation and differentiation, promotes rapid clonal growth and an increase in cell numbers. This process of mutation followed by clonal expansion continues, and mutations in genes such as PIK3CA, SMAD4, TP53, CTNNB1 and BRAF eventually result in malignancy. Not all adenomas progress to invasive cancer, although all adenomas have the capacity for malignant transformation. The pathological features of adenomas (such as size, type, histological grade and presence of dysplastic foci) are all predictive of their malignant potential; however, it is still unclear why some adenomas progress to malignancy, whereas others stabilize or even regress. Notably, adenomas harbour increased numbers of inflammatory cells, which are much higher than those expected in healthy colonic tissue.

Substantial changes in the composition of faecal microbiota, such as a decrease in butyrate-producing Firmicutes (mainly Roseburia spp. and Eubacterium rectale) and Actinobacteria (such as Bifidobacterium spp. and Collinsella aerofaciens), have also been observed in response to low-carbohydrate, weight-loss diets. More wide-ranging compositional changes were recently reported in response to a switch between extreme plant-based diets (that contained high levels of fibre and low levels of fat and protein, comprising 32% and 10% of caloric intake, respectively) and animal-based diets (that contained no fibre and had high levels of fat and protein, comprising 70% and 30% of caloric intake, respectively). In response to the animal-based diet, the abundance of Bacteroidetes (such as Bacteroides spp. and Arthrobacter spp.) and Bilophila wadsworthia increased, whereas the number of several members of the Firmicutes decreased. There is also evidence that variations in habitual dietary intake are responsible for differences in gut microbiota profiles; for example, individuals with a high proportion of Prevotella spp. in their faecal microbiota tend to consume more fibre, whereas Bacteroides spp. are enriched in individuals who consume high levels of protein and fat. This suggests an ecological division within the Bacteroidetes, in which Prevotella spp. are better equipped than Bacteroides spp. for the degradation of fibre. Interestingly, metagenomic analysis has recently revealed that the faecal microbiota has a bimodal distribution in the general population, such that some individuals show less diversity (known as low gene count (LGC)) than others (known as high gene count (HGC)). The LGC communities tend to be dominated by Bacteroides spp. and show a decrease in butyrate-producing Firmicutes, and individuals who have this profile have a higher incidence of obesity and metabolic syndrome. Furthermore, obese volunteers with an LGC microbiota who switched to a controlled weight-loss diet showed an increase in the diversity of the microbiota, which approached that of a HGC community. Thus, diet clearly has a major impact on the composition of the gut microbiota, so dietary interventions are likely to influence susceptibility to diseases that have a microbial component, such as CRC.

Although the link between fibre intake and cancer risk has been debated, recent meta-analysis studies indicate that a high-fibre intake, particularly of cereals and whole grains, is associated with a decreased risk of CRC, and patients with advanced colorectal adenomas (which are CRC precursor lesions) are reported to have lower dietary fibre intake compared with healthy controls. By contrast, diets that are rich in red and processed meat, fat and alcohol are associated with an increased risk of CRC. The lower incidence of CRC in rural native Africans compared with African Americans corresponds to higher dietary intake of non-digestible carbohydrates relative to protein and fat, as well as major differences in the fermentation capacity of the gut microbiota.

**Microbial metabolism in the gut**

Undigested dietary components that reach the large intestine and host products (mainly mucin) are fermented by the anaerobic microbial community to produce an extraordinarily wide range of metabolites, which reflects both the chemical diversity of the available substrates and the remarkable biochemical capacity of the microbiota. The major fermentation products in healthy adults are gases and organic acids, particularly the three short-chain fatty acids (SCFAs) acetate, propionate and butyrate (typically in a 3/1/1 ratio), which have a combined concentration of 50–150 mM in the colon. Non-digestible carbohydrates are usually the primary substrates for microbial fermentation and include the structural polysaccharides of plant cell walls (non-starch polysaccharides), resistant starch and certain soluble oligosaccharides (for example, fructo-oligosaccharides). The availability of non-digestible carbohydrates in the colon varies with diet and with meal times, in contrast to the almost constant supply of endogenously derived products, such as mucin.

Bacterial metabolism in the colon is not solely fermentative but can also include anaerobic respiration, in...
which nitrate, sulphate and various organic compounds function as electron acceptors\(^ {30}\). Facultative anaerobes, including Proteobacteria, are able to use available oxygen as an electron acceptor, which increases their energy recovery from substrates, compared with most obligate anaerobes, except *Bacteroides* spp.\(^ {31}\) and *Faecalibacterium prausnitzii*\(^ {32}\), which can also use oxygen. *Bacteroides* spp. have cytochromes, whereas *F. prausnitzii* seems to depend on extracellular electron transfer by flavins and thiols. Importantly, owing to the oxygen sensitivity of these anaerobes, this form of metabolism can occur only at low oxygen concentrations, and its consequences for the microbial ecology of the gut mucosa require further investigation\(^ {33}\). Microorganisms that use hydrogen and formate (FIG. 1), including methanogenic archaea (such as *Methanobrevibacter smithii*), acetogenic bacteria (such as *Blautia hydrogenotropha*) and sulphate-reducing bacteria (such as *Desulfovibrio* spp.)\(^ {34}\), have a particularly important role in anaerobic metabolism via interspecies cross-feeding interactions\(^ {35}\). The abundance of methanogenic archaea in the adult gut varies, and variations in gut transit (which is influenced by diet) might have an important role in determining the relative contributions of methanogenesis, acetogenesis and sulphate reduction\(^ {36}\).

**Protective metabolites**

For simplicity, bacterial metabolites and enzymatic activities are divided in the following sections according to whether they are predicted to have mostly protective or mostly detrimental effects on gut health and carcinogenesis. However, it should be emphasized...
Wood–Ljungdahl pathway
The collection of sequential biochemical reactions that lead to the formation of acetate from carbon dioxide and hydrogen. Several bacteria use this pathway for the generation of energy, and it is also used by certain bacteria and archaea for the assimilation of carbon dioxide into biomass.

Acrylate pathway
The collection of sequential biochemical reactions that lead to the conversion of lactate to propionate by certain Firmicutes bacteria.

Propanediol pathway
The collection of sequential biochemical reactions that lead to the conversion of deoxy-sugars (such as rhamnose and fucose) to propionate by certain gut bacteria.

Histone deacetylases (HDACs).
Enzymes that remove acetyl groups from histones, which are structural proteins that package DNA into structural units. Deacetylation leads to more condensed chromatin, which alters gene expression.

Colonic regulatory T cells (cTreg cells).
A subset of T lymphocytes that are found in the colon and are crucial for the maintenance of immune tolerance.

Microbial production of SCFAs
The production of microbial SCFAs is influenced by diet (BOX 2). Acetate, which is the most abundant SCFA, is produced by most enteric bacteria as a fermentation product, but it is also formed by acetylogenic bacteria, such as *B. hydrogenothermophilica*, from H₂ and CO₂, or from formate via the Wood–Ljungdahl pathway (FIG. 1). Acetogenic bacteria can produce three molecules of acetate from one molecule of glucose, but non-acetylogenic anaerobes, which comprise most of the microbiota, must dispose of reducing equivalents by forming other products in addition to (or instead of) acetate, including succinate, propionate, butyrate, formate, d-lactate, L-lactate and ethanol (FIG. 1). The relative synthesis of the different fermentation products varies according to the composition of the microbiota and environmental conditions, including pH, hydrogen partial pressure and available substrates. Recent work on cultured isolates and metagenomics data indicate that propionate and butyrate are mainly produced by the action of different groups of enteric bacteria on carbohydrates (FIG. 1). Propionate is mostly formed via the succinate pathway by Bacteroidetes and by some Firmicutes (such as *Roseburia inulinivorans* and *Ruminococcus obeum*). The proportion of propionate that is present in total faecal SCFA correlates with the relative abundance of Bacteroidetes, which confirms that the succinate pathway is the dominant source of propionate.

Butyrate is produced by some Firmicutes using either the butyryl-CoA:acetate CoA-transferase enzyme or, less commonly, phosphotransbutyrylase and butyrate kinase to catalyse the final steps of the pathway (FIG. 1). Species that use the butyryl-CoA:acetate CoA-transferase route include several of the most abundant species in the healthy gut microbiota (including *Faecalibacterium prausnitzii*, *Roseburia spp.*, *Eubacterium rectale*, *Eubacterium hallii* and *Anaerostipes spp.*), which are generally net users of acetate, such that the concentration of acetate in the gut lumen is determined by the balance of production, use and mucosal uptake. A subset of Lachnospiraceae, including *E. hallii* and *Anaerostipes spp.*, can use lactate and acetate to produce butyrate. Thus, these organisms may have an important role in stabilizing the microbial ecosystem by preventing the accumulation of lactate. Only a few anaerobes are known to produce both propionate and butyrate, and they do so from different substrates: *R. inulinivorans* produces butyrate from glucose and produces propionate from fucose; whereas *Coprococcus catus* produces butyrate from fructose and produces propionate from lactate (via the acrylate pathway).

Impact of SCFAs on host cells.
Acetate, propionate and butyrate are rapidly absorbed from the gut lumen, but their subsequent distribution, fate and effects on host cell metabolism differ. Butyrate is preferentially used as an energy source by gut epithelial cells, and its concentration in the systemic circulation is low. Propionate is mostly metabolized in the liver, and only acetate achieves relatively high concentrations (0.10–0.15 mM) in peripheral blood.

Intracellular butyrate and propionate (but not acetate) inhibit the activity of histone deacetylases (HDACs) in colonocytes and immune cells, which promotes the hyperacetylation of histones, in addition to some transcription factors and proteins that are involved in signal transduction. This has multiple consequences for gene expression and cellular differentiation, including the downregulation of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and IL-12, in colonic macrophages. SCFAs exert potentially important anti-inflammatory effects and have been shown to regulate colonic regulatory T cells (cTreg cells) in mice. Recent evidence shows that butyrate and propionate induce the differentiation of regulatory T cells that express the transcription factor FOXP3, which have a crucial role in controlling intestinal inflammation. It is proposed that butyrate causes increased acetylation of histone H3 in the promoter and enhancer regions of the FOXP3 locus, which results in increased expression.

**Box 2 | Impact of diet on the production of SCFAs**

In general, ingestion of non-digestible carbohydrates (such as fibre and resistant starch) leads to increased colonic fermentation, increased gut transit and stool output and a decrease in the pH of the intestinal lumen. Gut transit has an important impact on substrate fermentation and the absorption of short-chain fatty acids (SCFAs) and is partly regulated by SCFA concentrations. In overweight human volunteers, low-carbohydrate weight-loss diets result in a decrease in the concentrations of all three faecal SCFAs (that is, acetate, butyrate and propionate; the greatest decrease is in butyrate levels) compared with volunteers on a control diet with normal carbohydrate intake. It was suggested that this effect is mainly caused by a decrease in the abundance of one major group of butyrate-producing Firmicutes in the colon (*Eubacterium rectale* and *Roseburia spp.*), which reflects a diet-mediated change in the composition of the gut microbiota. In another recent study in healthy human adults, supplementation with resistant starch and non-starch polysaccharides led to an increase in the faecal concentrations of acetate and butyrate, and a significant positive correlation was observed between butyrate levels and ammonia excretion. This could be caused by the decrease in colonic pH that results from more active fermentation. An inverse relationship between faecal pH and butyrate formation has been shown in vivo and also in an in vitro continuous culture model, which involved the replacement of a microbial community that contained abundant butyrate-producing Firmicutes at pH 5.5 by a Bacteroides-dominated community that produced more propionate and acetate at pH 6.5. More acidic conditions also favour the excretion of ammonia as a result of protonation, which produces the poorly absorbed ammonium ion. In conclusion, the actual concentrations of SCFAs in the gut, as well as total SCFA production, are influenced by diet composition and intake.
A pathway that regulates gene expression via the transcription factor activator protein 1 (AP-1).

Figure 2 | Anti-inflammatory and anti-apoptotic effects of colonic bacteria and their metabolites that are thought to mitigate colorectal carcinogenesis. The major bacterial fermentation products, which are the short-chain fatty acids (SCFAs) butyrate, propionate and acetate, can be recognized by receptors (such as the G protein-coupled receptors (GPCRs) GPR41, GPR43 and GPR109A) on the surface of colonocytes (as shown) and immune cells (not shown). SCFAs are also transported into host cells, which results in the subsequent inhibition of histone deacetylase (HDAC) activity by butyrate and propionate, causing hyperacetylation of histones. Several studies have shown that the interactions between SCFAs and GPCRs, as well as SCFA inhibition of HDACs, also occurs in cell types other than colonocytes, including macrophages and T cells. HDAC inhibition and GPCR signalling result in an increase in total colonic regulatory T cell (cTreg) numbers and the production of the anti-inflammatory cytokines interleukin-10 (IL-10) and transforming growth factor-β (TGFβ). HDAC inhibition is also thought to promote apoptosis of colorectal cancer (CRC) cells. Other potential anti-inflammatory molecules, for which mechanisms remain to be established, are also indicated with dashed lines. For example, several phytochemicals that are formed by microbial transformation have been shown to have anti-inflammatory effects, and in vitro and in vivo models indicate that they inhibit pro-inflammatory mediators (including tumour necrosis factor (TNF), IL-6 and prostanoids). Finally, microorganism-associated molecular patterns (MAMPS) of some commensal bacteria are thought to contribute to anti-inflammatory signalling.

of FOXP3 (Ref. 51). It is possible that propionate functions by the same mechanism, but this requires further study.46,51-57. Lactate has also been reported to inhibit HDACs, although the high concentrations that are required are probably not physiological.46 Interestingly, the transporter SLC5A8, which is a tumour suppressor protein, is involved in the transport of propionate, butyrate and lactate.46,60 Although SLC5A8−/− mice do not show an increase in carcinogen-induced tumour formation,46,70,71 alternative transport mechanisms for SCFAs (which include passive diffusion) might occur when their concentrations are sufficiently high.46

Extracellular SCFAs are involved in several potentially important interactions with surface-exposed receptors of host cells (FIG. 2). G protein-coupled receptor 41 (GPR41; also known as FFA3), GPR43 (also known as FFA2) and GPR109A are expressed on various host cells, including colonocytes. GPR43 recognizes all three major SCFAs (acetate, propionate and butyrate) on the order propionate > butyrate >> acetate, whereas GPR109A interacts only with butyrate.46 Butyrate-driven signalling interactions that involve GPR109A might be involved in the anti-inflammatory action of butyrate by promoting the differentiation of regulatory T cells (Treg cells) and IL-10-producing T cells, as well as by blocking activation of nuclear factor-kB (NF-kB) and induction of apoptosis by a mechanism that is independent of HDAC inhibition.47 Interactions of acetate and propionate with GPR43 are proposed to have an important role in inducing their anti-inflammatory effects via the modulation of Treg cells.48,49 GPR4 and GPR109A are tumour-suppressor genes and might mediate some of the cancer-protective effects of propionate and butyrate that are associated with high fibre intake.50 Other important antigutmyogenic effects of butyrate include inhibiting proliferation and selectively inducing apoptosis of CRC cells.46,52,53. The mechanisms that are involved remain to be fully elucidated, but the promotion of apoptosis involves changes in transcriptional regulation owing to HDAC inhibition and possibly G protein-coupled receptor interactions, as discussed above.46 As a likely consequence of HDAC inhibition, butyrate and, to a lesser extent, propionate have been shown to activate the AP-1 signalling pathway in epithelial cell lines, which has an important role in the control of cell proliferation and apoptosis.54 By contrast, a recent study using a mouse line that is genetically susceptible to CRC, suggests that low concentrations of butyrate might promote CRC by stimulating the proliferation of colonic epithelial cells. However, the influence of concurrent changes in the composition of the microbiota could not be excluded.55

The anti-inflammatory effects of SCFAs (FIG. 2) are not only important because of their influence on host cells but it is also possible that they contribute to homeostasis of the gut microbiota. An intriguing hypothesis proposes that the anti-inflammatory effect of high butyrate levels tends to limit immune responses towards the gut microbiota, whereas low butyrate concentrations trigger a pro-inflammatory state that results in remodelling of the gut microbiota via the suppression of potential pathogens and restoration of butyrate-producing species.56

Biotransformation of phytochemicals and xenobiotics. Many different dietary compounds that are present in fruit, vegetables, cereals, seeds, nuts, spices and beverages have been suggested to protect against different types of cancer.57 Most studies have investigated polyphenols, but non-phenolic compounds, such as glucosinolates (which are derived from brassica vegetables), are also protective.58 Phytochemicals have several effects, including antioxidant
effects, modulation of xenobiotic detoxification pathways and modulation of cell proliferation, apoptosis and inflammation\(^6\) (FIG. 2). Antioxidants neutralize reactive oxygen species (ROS), which are by-products of energy metabolism and can damage cellular structures, including DNA, which results in the generation of mutations that predispose the cell to the development of cancer. It is currently unclear whether direct antioxidant effects have an important role in cancer prevention in vivo, as it has been questioned whether sufficient systemic concentrations can be achieved\(^7\). Furthermore, some studies have reported an increase in the occurrence of certain cancers in response to supplementation with some antioxidants\(^8\).

Dietary phytochemicals are usually present as glycosides or are bound to fibre, and only limited uptake takes place in the small intestine. Up to 95% of dietary phytochemicals reach the large intestine and are released and transformed to other metabolites by the gut microbiota\(^9\)-\(^12\). The conversions that occur include hydrogenation, dehydroxylation and demethylation, which can change metabolite bioactivity. Several phenolic metabolites that are formed by microbial transformation have been shown to inhibit pro-inflammatory mediators (including tumour necrosis factor (TNF), NF-κB and prostanoids)\(^13\)-\(^17\) (FIG. 2). Many phenolic compounds have been shown to exert antimicrobial effects that can alter the composition of the gut microbiota, as different bacterial species show varying levels of sensitivity\(^18\). The resultant changes in microbial composition could have knock-on effects — for example, via changes in SCFA profiles or the suppression of pathogenic microorganisms.

After absorption in the gut, phytochemicals are conjugated to methyl, sulphate and glucuronic acid groups in the liver, which facilitates their excretion into the gut via bile. In the gut, bacterial β-glucuronidases convert glucuronides back to the respective aglycones, which can then be re-absorbed. Thus, bacterial β-glucuronidase activity and enterohepatic circulation extend the detention time of phytochemicals in the body. However, microbial β-glucuronidase activity also interferes with the detoxification and excretion of toxic xenobiotics, such as drugs and environmental pollutants, and has been reported that high β-glucuronidase activity is associated with an increased risk of cancer\(^19\). Furthermore, protein-rich diets deliver toxic compounds, such as nitrosamines and polycyclic aromatic hydrocarbons, which can be formed endogenously via acid-driven nitrosation in the stomach and by the nitrosation of amines that are derived from the microbial fermentation of protein in the large intestine\(^20\). An increase in faecal NOCs has been found in individuals on high-protein diets in controlled dietary intervention studies\(^21\). Nitroreductases and nitrate reductases that are encoded by Proteobacteria probably contribute to nitrosation reactions. Ammonia, which is another product of protein fermentation, is also a potential carcinogenic agent at relatively low concentrations, as shown by the increase in mucosal damage and colonic adenocarcinoma in a rat model\(^22\).

Polymamines are involved in a range of essential physiological functions, such as the maintenance of the structural integrity of membranes and nucleic acids, gene regulation and translation\(^23\). \(^24\). The major polymamines putrescine, spermidine and spermine are produced from arginine in host tissues, but polyamine synthesis also occurs in gut bacteria\(^25\). High levels of polymamines are toxic and are associated with various diseases, including cancer, and oxidative stress that results from polyamine catabolism is thought to be the underlying mechanism of toxicity\(^26\). In addition to contributing directly to the polyamine pool by synthesizing these compounds, certain gut bacteria (such as enterotoxigenic Bacteroides fragilis) upregulate polyamine production by host cells\(^27\). Indeed, several pathogens, including Shigella flexneri, Streptococcus pneumoniae, Salmonella enterica subsp. enterica serovar Typhimurium and Helicobacter pylori, exploit polymamines to increase their virulence\(^28\). There is evidence from both humans and animal models that dietary supplementation with non-digestible carbohydrates can decrease protein fermentation in the large intestine, which coincides with a decrease in the genotoxicity of faecal water\(^29\). Diets that include resistant starch lead to a reduction in DNA damage and tumour formation in a rat model\(^30\) and may attenuate the detrimental effects of high levels of dietary protein\(^31\). However, the effects of a high-protein diet on the risk of CRC in humans remains a complex question, as it is strongly influenced by the specific source of protein.
Several bacterial metabolites, including hydrogen sulphide, secondary bile acids, polyamines and reactive oxygen species (ROS) have the potential to cause direct DNA damage or to provoke inflammation (via interleukin 6 (IL-6) and tumour necrosis factor (TNF) production), which thus promotes carcinogenesis. N-nitroso compounds (NOCs) can promote cancer by generating mutations owing to DNA alkylation. Pathogenic bacteria, in particular, also exert pro-inflammatory effects via the recognition of microorganism-associated molecular patterns (MAMPs) by Toll-like receptors (TLRs), which leads to detection by dendritic cells and the activation of T helper 17 (T\textsubscript{H}17) cells, T\textsubscript{H}17 cells promote the expression of the pro-inflammatory mediator IL-23 and block expression of the anti-inflammatory mediator IL-10. Tumour-associated loss of barrier function, which is mediated by MAMPs, can also result in increased bacterial translocation, and this further drives pro-inflammatory pathways, thereby increasing tumorigenesis.

**Figure 3 | Pro-inflammatory and DNA-damaging effects of colonic bacteria and their metabolites that are thought to contribute to colorectal carcinogenesis.**

Secondary bacterial metabolites, including hydrogen sulphide, secondary bile acids, polyamines and reactive oxygen species (ROS) have the potential to cause direct DNA damage or to provoke inflammation (via interleukin 6 (IL-6) and tumour necrosis factor (TNF) production), which thus promotes carcinogenesis. N-nitroso compounds (NOCs) can promote cancer by generating mutations owing to DNA alkylation. Pathogenic bacteria, in particular, also exert pro-inflammatory effects via the recognition of microorganism-associated molecular patterns (MAMPs) by Toll-like receptors (TLRs), which leads to detection by dendritic cells and the activation of T helper 17 (T\textsubscript{H}17) cells, T\textsubscript{H}17 cells promote the expression of the pro-inflammatory mediator IL-23 and block expression of the anti-inflammatory mediator IL-10. Tumour-associated loss of barrier function, which is mediated by MAMPs, can also result in increased bacterial translocation, and this further drives pro-inflammatory pathways, thereby increasing tumorigenesis.

**Bile acid metabolism.** The interplay between diet, bile acids and the gut microbiota is complex. High-fat diets, which are positively correlated with the incidence of CRC, lead to an increase in bile secretion, and increased faecal bile acid concentrations have been reported in patients with CRC\textsuperscript{99,100}. In addition, higher fat intake correlates with higher faecal concentrations of secondary bile acids in African Americans compared with rural Africans\textsuperscript{101}, and recent evidence suggests that the secondary bile acid deoxycholic acid promotes liver cancer\textsuperscript{102}. Bile acids exert strong antimicrobial activities, as they damage bacterial cell membranes owing to their amphipathic properties and are therefore likely to modify the composition of the gut microbiota. Rats that are fed a diet that is supplemented with deoxycholic acid show a decrease in the production of SCFAs and major changes in the composition of the microbiota, with a relative increase in Gammaproteobacteria and certain Firmicutes at the expense of Bacteroidetes, which is similar to what is observed in response to high-fat diets in mice\textsuperscript{103}.

The primary bile acids cholic acid and chenodeoxycholic acid are produced in the liver from cholesterol, are conjugated to glycine or taurine (which render the bile acids more hydrophilic and facilitate their action as emulsifiers) and are excreted into the duodenum to absorb in the small intestine before they are re-absorbed in the distal ileum to facilitate fat digestion. Limited biotransformation of primary bile acids by the gut microbiota occurs in the small intestine before they are re-absorbed in the distal ileum for enterohepatic circulation\textsuperscript{104}. However, the fraction of bile acid that escapes re-absorption in the small intestine (approximately 5% of the total pool) undergoes extensive transformation by the microbiota in the large intestine\textsuperscript{98}. Bile salt hydrolases (which are found in all the major bacterial divisions and the methanogenic archaea) cleave glycine and taurine residues from the primary bile acids\textsuperscript{104,105}, which converts them into several different secondary bile acids by dehydrogenation and dehydroxylation reactions. The main secondary bile acids that are produced are deoxycholic acid and lithocholic acid, both of which are produced by 7α-dehydroxylation\textsuperscript{104}. The genes that are responsible for this conversion (which are encoded in the bai operon) have been investigated in detail in *Clostridium scindens*\textsuperscript{106}, but they seem to be less widespread than bile salt hydrolases in the human gut microbiota.

Bile acids have been implicated in carcinogenesis in different regions of the intestinal tract and associated organs, owing to the generation of ROS and reactive nitrogen species (RNS), both of which cause DNA damage\textsuperscript{106}. In addition, animal studies have shown that the...
administration of bile acids results in a higher incidence of tumours in the gut. The molecular mechanisms that mediate the cytotoxic effects of bile acids are complex. Secondary bile acids are more hydrophobic and thus more potent at disrupting cell membranes, which is likely to lead to the generation of ROS via the activation of membrane-associated proteins such as NAD(P)H oxidases and phospholipase A2, but other mechanisms may also be involved. For example, bile acids function as hormones that interact with nuclear receptors and activate cellular signalling pathways that promote apoptosis; however, constant exposure to high levels of bile acids can lead to resistance to apoptosis. Furthermore, some bile acids seem to counteract the cytotoxic effects of others; for example, urosodeoxycholic acid, which is produced by Ruminococcus gravis, seems to inhibit the production of ROS and to protect cells from the cytotoxic effects of deoxycholic acid. Taurine conjugation increases in individuals who are on meat-rich diets, and the sulphonic acid moiety of taurine is reduced to hydrogen sulphide after deconjugation of the bile acid. Diets that promote high levels of taurine conjugation lead to a bloom in the sulphite-reducing bacterium Bilophila wadsworthia, which is associated with pathological gut conditions.

Ethanol. Excessive consumption of ethanol is considered to be an important risk factor for several cancers, and microbial metabolism may contribute to its toxicity, particularly in the upper gastrointestinal tract. Ethanol is produced by many anaerobic bacteria that inhabit the colon when they are grown in pure culture, but the level of endogenous ethanol production by the colonic microbiota in vivo is unknown. Although ethanol itself is not regarded as a carcinogen, its immediate oxidation product acetaldehyde is highly toxic and carcinogenic, and causes effects that range from DNA damage to the degradation of the vitamin folate. Interestingly, studies of the oral microbiota have shown that microorganisms contribute to the production of acetaldehyde from ethanol, which suggests that the gut microbiota might also contribute to this process.

The microbiota and inflammation

It has become increasingly clear that the microbiota has a major influence on immune responses, and chronic inflammation is a well-established risk factor for CRC. As the colonic mucosa is constantly exposed to the gut microbiota and its metabolites, bacterial stimulation of immune responses has the potential to cause continuous low-grade inflammation. The tumour microenvironment contains several different immune cell types, including tissue-associated macrophages and other innate immune cells, as well as T cells and B cells, which communicate with each other and the other cells in the tumour microenvironment via direct contact or via cytokine and/or chemokine signalling to control tumour growth. TAMs primarily promote tumour growth, and high numbers of TAMs generally correlate with cancer progression. After TAMs, T cells are the most numerically abundant immune cells in the tumour microenvironment and can exert both tumour-promoting and tumour-suppressive effects. Increased numbers of CD4+ T helper 1 (Th1) cells and CD8+ cytotoxic T cells are associated with the direct lysis of cancer cells and the production of cytotoxic cytokines that limit the progression of CRC. However, other T cell subsets, such as interferon-γ (IFNγ)-producing Th1 cells, promote tumorigenesis via cytokine production and cytotoxic mechanisms. Interestingly, inflammation in the absence of the gut microbiota or microbial products is insufficient to induce CRC. There is also clear evidence that the gut microbiota of mice influences adenoma formation. Adenomatous polyposis coli (ApcΔm) mice that have a mutation in one copy of the tumour suppressor gene Apc spontaneously form many benign adenomas in the small intestine, and the number of colon tumours that are formed in ApcΔm mice is greater than that in wild-type C57BL/6 mice. However, germ-free ApcΔm mice exhibit a twofold reduction in small intestinal adenomas compared with ApcΔm mice that have a conventional microbiota. In addition, disruption of microbial sensing by innate immune receptor signalling also results in reduced tumorigenesis. Host recognition of the microbiota occurs via various pattern recognition receptors (PRRs; such as Toll-like receptors (TLRs)) that control the inflammatory response to microorganism-associated molecular patterns (MAMPs; such as lipopolysaccharide (LPS), flagellin and nucleic acids). PRRs have a key role in maintaining mucosal homeostasis and controlling inflammation in the colonic environment. In particular, alterations in signalling of TLR4, which is the major receptor for LPS, have been linked to the progression of CRC.

The role of specific pathogens in CRC. In the previous sections, we have described the role of the microbiota (as an integrated community) and its metabolites in the aetiology of CRC. That said, several bacterial pathogens seem to be directly and specifically involved in promoting CRC; for example, enterotoxigenic B. fragilis and adherent-invasive E. coli strain NC101 have been shown to promote CAC in genetically susceptible mice. The pro-inflammatory effects of enterotoxigenic B. fragilis result in the induction of spermine oxidase in colonocytes, which leads to the production of ROS and consequent DNA damage. However, most cases of CRC do not arise from a background of colitis, which suggests that other microbial species are associated with the pathogenesis of CRC. Recent studies indicate that Fusobacterium spp. and Campylobacter spp. are over-represented in CRC tissue, thus, these genera may form part of a metagenomic CRC signature. Invasive strains of Fusobacterium nucleatum accelerate the onset of colonic tumours and drive the transition to a pro-inflammatory microenvironment that is conducive to colorectal tumorigenesis. Binding of the F. nucleatum adhesin FadA to E-cadherin induces its tumour-suppressor activity and activates β-catenin, which further promotes the growth of tumour cells. However, despite these associations, it remains difficult to establish whether there is a causal link to specific pathogens (BOX 3). However, it is clear that the
Box 3 | Microbiota changes associated with CRC

There has been a recent surge in studies that compare the composition of the microbial community in patients with colorectal cancer (CRC) with that of healthy subjects, in an effort to determine whether changes in the gut microbiota are a cause of the disease. However, as CRC develops over many years, it is a challenge to determine whether the associated changes in the gut microbiota are a consequence of diet alterations or physiology or whether they are causative. In one article, a ‘driver–passenger’ model for CRC was proposed in an attempt to distinguish between causative organisms and those that respond to disease progression. The analysis of faecal samples from patients with CRC using 16S ribosomal RNA gene sequencing has shown that Bacteroides fragilis and several enterobacterial operational taxonomic units (OTUs) are enriched compared with healthy controls, whereas levels of five OTUs that correspond to butyrate-producing Lachnospiraceae were reduced. Changes also occur in the tumour-associated microbiota, in which an increase in Fusobacterium spp. seems to be consistent between studies. Deep metatranscriptomic sequencing has more recently shown that Leptotrichia spp. and Campylobacter spp. co-occur with Fusobacterium spp. Clear differences in the composition of the gut microbiota have been reported in mice following tumour induction using carcinogenic agents; compared with untreated mice, Bacteroides spp., Akkermansia spp. and Odoribacter spp. increase, whereas Prevotella spp. and Porphyromonas spp. decrease. The increasing list of potentially carcinogenic bacteria provides support for the hypothesis that tumorigenesis is driven by mechanisms and/or pathways that are common to many bacterial groups rather than a single organism.

Dietary and environmental compounds

<table>
<thead>
<tr>
<th>Non-digestible carbohydrates</th>
<th>SCFAs</th>
<th>Microbiota modulation; Cellular differentiation; apoptosis; Inflammation</th>
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<tr>
<td>Phenolic acids; isothiocyanates</td>
<td>NOCs; ammonia</td>
<td>ROS production; genotoxicity</td>
</tr>
<tr>
<td>Polyamines</td>
<td>Hydrogen sulphide</td>
<td>Inflammation; ROS production; genotoxicity</td>
</tr>
<tr>
<td>Taurine</td>
<td>Secondary bile acids</td>
<td>Microbiota modulation; Cellular differentiation; apoptosis; ROS production; genotoxicity</td>
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<tr>
<td>Carcinogens</td>
<td>Acetaldehyde</td>
<td>ROS production; genotoxicity</td>
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E. coli. Inflammation creates an opportunity for certain bacteria, such as E. coli, to adhere to the colonic mucosa by decreasing the production of protective mucins and antimicrobial peptides. The reduced barrier function enables bacteria to more readily interact with the epithelium, resulting in increased delivery of mutagenic metabolites, including colibactin, which is a putative hybrid peptide–polyketide that is produced by the Enterobacteriaceae and causes DNA damage. Colibactin-producing E. coli strains can induce DNA double-strand breaks in the host cell and thereby activate DNA damage signalling cascades, which leads to chronic mitotic and chromosomal aberrations as well as an increased frequency of gene mutation and anchor-age-independent growth. Increased levels of Proteobacteria, particularly the Enterobacteriaceae, are found in the gut microbiota of patients with IBD, which is a known risk factor for CRC. Early genetic events in the pathogenesis of CRC, such as the activation of β-catenin and mutation of the APC gene, seem to result in a loss of barrier function in the colonic epithelium, which leads to the translocation of microbial products into the tumour microenvironment. This process results in the downstream production of tumour-promoting cytokines via the activation of IL-23-producing myeloid cells, which promote tumour growth. Although defective barrier function enables the translocation of microbial products, it also enables the colonization of invasive-adherent bacteria at neoplastic sites.

Outlook

The studies that are discussed in this Review highlight that progression to CRC is influenced not only by the presence of specific pathogens but also by the metabolic output of the entire microbiota. In addition to high microbial diversity and low pathogen abundance, microbial SCFAs have a major role in maintaining intestinal homeostasis. They suppress the growth of Gram-negative pathogens, function as energy sources and anti-inflammatory agents and promote apoptosis of cancer cells. Thus, prominent butyrate-producing species are not only indicators of a diverse, healthy
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microbiota — as suggested by recent studies — but also seem to be actively involved in maintaining a stable and healthy gut community. By contrast, dysbiosis is characterized by a reduction in microbial diversity and an increase in pro-inflammatory, pathogenic species, which can be caused by an unhealthy diet, antimicrobial therapy or genetic predisposition (which is exemplified by Crohn’s disease).

There is increasing evidence that diets that are low in fibre and high in fat and sugar result in a less diverse gut microbiota, which, combined with the detrimental effects of these diets that are mediated by dietary components and microbial metabolites, is likely to increase the risk of CRC. Metabolomics is yielding new information on microbial metabolite profiles and responsiveness to controlled dietary manipulations in patient groups that show differing risks of CRC. However, we need a better fundamental understanding of dietary factors and an ability to predict, the effects of diet on the microbial microbiome — as suggested by recent studies — but also seem to be actively involved in maintaining a stable and healthy gut community. By contrast, dysbiosis is characterized by a reduction in microbial diversity and an increase in pro-inflammatory, pathogenic species, which can be caused by an unhealthy diet, antimicrobial therapy or genetic predisposition (which is exemplified by Crohn’s disease).

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80. Islam, K. B. M. S. et al. Bile acid is a host factor that regulates the composition of the cecal microbiota in rats. Gastroenterology 143, 1775–1781 (2012). This study shows that bile acids have a strong modulatory effect on the gut microbiota, which suggests that they can contribute to the dysbiosis in the microbiota in response to high-fat diet.


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131. Grivennikov, S. I. et al. Adenoma-linked barrier defects and microbial products drive IL-25/IL-17-mediated tumour growth. *Nature* **491**, 254–258 (2012). This study demonstrates that defective intestinal barrier function at tumour sites facilitates bacterial translocation, which leads to the activation of myeloid cell-derived cytokine networks (involving IL-17 and IL-23) and the promotion of tumour growth.


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Competing interests statement

The authors declare no competing interests.