Microbial Metabolites as Molecular Mediators of Host-Microbe Symbiosis in Colorectal Cancer

Keane JM[1,2,3], Joyce SA[1,4], Gahan CGM[1,2], Hyland NP[*#1,5], Houston A[*1,3]

1. APC Microbiome Ireland, University College Cork, Ireland
2. School of Microbiology, University College Cork, Ireland
3. Department of Medicine, University College Cork, Ireland
4. School of Biochemistry and Cell Biology, University College Cork, Ireland
5. Department of Physiology, University College Cork, Ireland

Corresponding Author
Department of Physiology
3.81 Western Gateway Building
University College Cork
Cork,
Ireland
Email: n.hyland@ucc.ie

*Equal contributions

Key words: butyrate, bile, gut microbiota, colon, tumorigenesis

Abstract

The symbiosis between the gut microbiota and the host has been identified as an integral part of normal human physiology and physiological development. Research in germ-free or gnotobiotic animals has demonstrated the importance of this symbiosis in immune, vascular, hepatic, respiratory and metabolic systems. Disruption of the microbiota can also contribute to disease, and the microbiota has been implicated in numerous intestinal and extra-intestinal pathologies including colorectal cancer. Interactions between host and microbiota can occur either directly or indirectly, via microbial-derived metabolites. In this chapter, we focus on two major products of microbial metabolism, short-chain fatty acids and bile acids, and their role in colorectal cancer. Short-chain fatty acids are the products of microbial fermentation of complex carbohydrates and confer protection against cancer risk, while bile acids are compounds which are endogenous to the host, but undergo microbial modification in the large intestine leading to alterations in their bioactivity. Lastly, we discuss the ability of microbial modulation to mediate cancer risk, and the potential to harness this ability as a prophylactic or therapeutic treatment in colorectal cancer.

1 The Gut Microbiota

The human microbiota is a community of bacteria, archaea, protists, fungi, and viruses that live in and on the human body (1). The term gut “microbiome” is sometimes used synonymously with the gut “microbiota” but can also refer to the full collection of genes present in the microbiota of a community. The cells of our microbiota are estimated to outnumber our nucleated human cells by a ratio of about 13:1, about 70% of which occupy our gastrointestinal (GI) tract (2). A symbiotic relationship exists between the microbiota and host, and this relationship plays a vital role in host immune modulation, metabolism,

inhibition of pathogens and structural development (3, 4). Members of the microbiota may be classified by the nature of their symbiotic relationship with the host, ranging from harmful pathogens to beneficial probiotics. These probiotic bacteria are characterised as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host", while prebiotics are "selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (5-7). Some relationships are more complex however, with species displaying both harmful and beneficial behaviour. *Helicobacter pylori*, for example, is recognised as a major risk factor for stomach cancer, but the elimination of this species has been associated with increased rates of inflammatory diseases including inflammatory bowel disease (IBD), asthma and eczema, suggesting a role for *H. pylori* in immune modulation (8, 9).

The gut microbiota comprises over 5000 bacterial species and 3 million genes in a typical individual, with possibly over 35,000 species in the collective human microbiome (10, 11). It is dominated by the phyla Firmicutes and Bacteroidetes, featuring smaller proportions of Proteobacteria, Verrucomicrobia, Actinobacteria, Fusobacteria, and Cyanobacteria (3, 12). This consistency of phyla, combined with significant inter-individual variation within the phyla, suggests a selective pressure to maintain the higher taxonomic structure with a functional redundancy at lower levels (12, 13). The upper GI tract contains relatively few microbial inhabitants. The stomach and duodenum contain approximately 10^2 organisms per gram of contents. This rises to 10^4-10^7 in the jejunum, finally reaching ~10^9 colony forming units (CFUs)/mL in the terminal ileum and ~10^{12} CFU/mL of primarily anaerobic bacteria in the colon (3, 14). The composition also changes along the length of the GI tract, with *Bacillus* and Actinobacteria enriched in the small intestine, while Bacteroidetes and *Lachnospiraceae* are enriched in the large intestine (11).

The intestinal tract is generally considered sterile at birth, with colonisation beginning immediately through contact with the mother and environmental bacteria. Recent research, however, has suggested colonisation of the placenta by *Streptococcus agalactiae* in approximately 5% of pregnancies. However, the possibility remains that this is a result of sample contamination (15, 16). The newborn microbiota is reflective of the mode of delivery, with babies delivered by Caesarean section having a microbiota characterised by fewer *Bifidobacterium* species compared to vaginal births (17). The shift towards an adult microbial composition begins during weaning before the microbiota stabilises at approximately 1-2.5 years of age (18). The microbiota then remains largely stable until old age, in the absence of disruptions such as long-term dietary changes or migration (19, 20). Further changes to the microbiota are observed later in life, such as a reduction in diversity and in the number of symbiotic species, and an increase in enteric bacteria, which may be associated with the age-related physiological decline observed in these populations (21-23).

1.1 Host-microbe symbiosis and physiological development

The ancient association and co-evolution between host and microbe have lead to the deep integration of the microbiota into normal physiological processes and development. This is illustrated by germ-free (GF) animals, which, in the absence of normal gut microbiota, display several developmental abnormalities including an immature immune system (24). Potential mechanisms by which the neonatal microbiota mediate the development of the immune system differ between bacterial species, and likely involve the interacting influences of many different taxa. GF mice have a suppressed T Helper Type 1 (T_h1) cell response that can be restored by monocolonisation with *Listeria monocytogenes*, which stimulates interleukin

(IL)-12 production in macrophages. Likewise, the reduction in T_h17 cells observed in these animals can be normalised by colonisation by segmented filamentous bacteria (SFB) leading to the release of serum amyloid A from intestinal epithelial cells (25). Colonisation with SFB also upregulated the production of immunoglobulin A, which is crucial for a tolerance of commensal microbiota by the mucosal immune system (26, 27).

The host immune response also modulates the composition of the gut microbiota, and the ability of the mucosal immune system to differentiate between commensal and pathogenic bacteria is a topic of ongoing research (28). Members of the gut microbiota interact with the host directly by signaling through pathogen recognition receptors, such as Toll-like Receptors (61). The gut microbiota also produces a wide array of bioactive bacteria-derived metabolites, both from compounds endogenous to the host, e.g. bile acids, or exogenous compounds such as those found in the diet or environment, which allow them to interact indirectly with the host. These metabolites can also play an important role in host health and disease, including colorectal cancer (CRC) (discussed in Section 3).

2 Host-Microbiota Interactions in Colorectal Cancer

There is precedence for the involvement of bacteria in GI cancer, as *H. pylori* is the strongest known risk factor for gastric cancer (8). Given the close apposition between the gut microbiome and colonic epithelium, in particular, research efforts have focussed on the role of the microbiota in colon cancer (Table 1) (29). The proposed mechanisms by which the microbiota may impact CRC include its effects on the immune system and proto-oncogenic pathways such as proliferation and apoptosis, while microbial metabolites can also have pro- and anti-tumorigenic associations (30). The strongest links between the microbiota and potentially cancer-promoting inflammation involve pathogenic species such as *Fusobacterium nucleatum* or enterotoxigenic *Bacteroides fragilis*, both of which have been positively correlated with CRC (31, 32). The role of the microbiota in proliferation is evident in GF mice which display smaller intestinal crypts with a lower mitotic index (33), while the microbiota can mediate apoptosis via a number of mechanisms including the production of butyrate (Section 3.1). Moreover, tumour formation is reduced in GF animals (68), with faecal microbial transfer from CRC patients to GF mice increasing tumorigenesis in these animals (69-71). This capacity to regulate both intestinal proliferation and apoptosis highlights the importance of this delicate symbiotic relationship, which could contribute to cell cycle disruption if dysregulated.

[Table 1]

Substantial evidence exists in animal models for the role of gut bacteria in promoting CRC. These studies primarily utilise mouse models either genetically predisposed to CRC such as the APC^{MIN} mouse, or use genotoxic compounds such as azoxymethane (AOM) or its precursor dimethyhydrazine (DMH), to chemically induce CRC. AOM can also be combined with dextran sodium sulphate (DSS) to model colitis-associated CRC. Using this AOM/DSS model of CRC, manipulation of the microbiota with antibiotics was shown to result in reduced tumorigenesis, but antibiotic treatment had conflicting effects in APC^{MIN} mice (63, 64). Antibiotic treatment was protective, however, in APC^{MIN} mice when compound mutations in DNA repair or interleukin receptor genes were present (64-66). Furthermore, Onoue *et al*. observed decreased numbers of aberrant crypt foci (ACF) in DMH-treated GF rats compared to conventional rats (67). Conversely, the administration of bacteria associated with cancer risk, for example, *Streptococcus bovis* or *F. nucleatum*, to susceptible animals was shown to

increase proliferation, inflammation, and tumorigenesis (68, 69). Tumour multiplicity was also increased in gnotobiotic (GB) rats colonised by enterococci compared to GB rats without enterococci, with the tumour numbers in the former group significantly decreased by the inclusion of probiotic strain *Bifidobacterium breve* (67). A similar result was achieved by Horie *et al*. concerning adenomas, with the lowest incidence of adenoma development observed in rats mono-associated with probiotic *Lactobacillus acidophilus* (70).

In contrast, human studies present only associative evidence for the role of the microbiota in CRC. The microbiota is altered in the colon of CRC patients and in the tumour tissue compared to healthy controls, with adenomatous polyps representing an intermediate step between the two states (71). The colonic mucosa is the symbiotic interface between host and microbiota, and studies have shown colonisation of this interface by adherent and invasive *Escherichia coli* in carcinoma patients (58, 72). Moreover, CRC patients had increased carcinogenic microbial metabolites in their faeces compared to healthy individuals despite both groups having similar diets, with the difference ascribed to their different levels of enzymatically-active anaerobic bacteria (73). Similarly, *Lactobacillus* species have been shown to reduce faecal and urinary mutagenicity induced by fried meat consumption and to reduce faecal β-glucuronidase, β-glucosidase, nitroreductase and glycocholic acid hydrolase activity (74-76). The gut microbiota can also modulate the production of mucus in the intestinal lumen, which in itself can play an important role in CRC by regulating the interaction of the gut bacteria and luminal contents with the colonic epithelium (77).

The composition of the microbiota has also been investigated as a potential predictive biomarker for human CRC. Two meta-analyses of human faecal shotgun sequencing studies identified microbial taxonomic signatures with sensitivity to, and specificity for, CRC, which was comparable to common non-invasive clinical screening tests (78, 79). Models based on the functional gene content of the faecal microbiome were also generated, and enrichment of the bile acid-inducible operon, which is involved in microbial bile acid metabolism, was demonstrated at both the genomic and transcriptomic levels (78, 79). Additionally, bacterial species associated with the oral cavity are frequently enriched in gut microbiota in CRC patients, and a model combining data from oral and faecal microbiota was highly predictive of CRC (62).

3 Microbial metabolites as mediators of host-microbe symbiosis in colorectal cancer

Another key interaction between the host and the microbiota is through the production of microbial-derived metabolites (80). Here, we focus on two major products of microbial metabolism, short-chain fatty acids and bile acids, and their role in CRC.

3.1 Short-chain fatty acids

Commensal bacteria contribute to host-microbial homeostasis and resistance to CRC via the production of short-chain fatty acids (SCFAs). SCFAs are fatty acids with less than six carbon atoms and are primarily the product of fermentation of dietary fiber by anaerobic bacteria in the proximal colon (81). The three most common SCFAs are acetate, propionate, and butyrate, with butyrate shown to play a predominant role in CRC (82). The majority of butyrate is produced by bacteria in *Clostridium* clusters XIVa and IV, particularly *Roseburia*/*Eubacterium rectale*-related bacteria in cluster XIVa and *Faecalibacterium prausnitzii* relatives in cluster IV (83). In a screen of butyryl-CoA:acetate CoA-transferase sequences from human faecal samples, 88% of sequences belonged to *E. rectale*, *Roseburia*

faecis, *Eubacterium hallii* and an unnamed species with the remainder coming from uncultured strains (83).

Butyrate is the primary energy source for normal colonic epithelial cells and has been associated with positive health effects, including in CRC (84, 85). Concentrations of SCFAs are highest in the caecum and proximal colon, where the incidence of tumours is low (86). The lowest intracolonic levels of SCFAs are found in the distal colon and rectum, the site of the majority of human CRC. Butyrate was also reduced in a rat model of CRC, where it correlated negatively with tumour mass (87). Moreover, protein feeding increased tumour number in AOM-treated rats which was ameliorated by resistant starch, which is a substrate for microbial butyrate production (88). Mechanisms by which butyrate protect against CRC are presented in Table 2.

Whilst predominantly protective against the development of CRC, butyrate can have pro-tumorigenic effects following CRC onset. One such mechanism involves its ability to act as a histone deacetylase (HDAC) inhibitor *in vivo* where it epigenetically promotes cell proliferation (89). The contrasting effects of butyrate in normal epithelial cells versus CRC cells can be explained by the metabolic fate of intracellular butyrate. The ability to use butyrate as an energy source is lost in malignant colonocytes (67). Instead, these cells perform glycolysis in what is termed the Warburg effect. This causes the accumulation of intracellular butyrate which generates concentrations sufficient to allow butyrate to act as an HDAC inhibitor. This effect in CRC cells is amplified by glucose-induced metabolism of butyrate by ATP citrate lyase to acetyl-CoA, which acts as a histone acetyltransferase in cells exhibiting the Warburg effect (90, 91).

[Table 2]

3.2 Bile acids

Bile acids are endogenous steroid molecules that are conjugated to a glycine or taurine amino acid residue to form bile salts and stored in the gallbladder for post-prandial release into the duodenum to aid lipid digestion. They are derived from cholesterol and are the major route of cholesterol elimination from the body. The major human bile acids are cholic acid (CA) and chenodeoxycholic acid (CDCA), while in mice the majority of CDCA is converted into muricholic acid (MCA) (110). Although most bile salts are reabsorbed in the distal ileum, around 5% escape to the large intestine where they can be modified by intestinal bacteria (111). These bile acids undergo deconjugation of the amino acid residue by *bile salt hydrolase* to form free bile acids, followed by 7α-dehydroxylation to form cytotoxic secondary bile acids, as well as a number of other minor modifications (112). 7α-dehydroxylation of the major human bile acids CA and CDCA forms deoxycholic acid (DCA) and lithocholic acid (LCA) respectively. These modifications can alter the biochemistry and bioactivity of bile acids, as well as their receptor specificities, which affect their role in CRC. The synthesis and microbial metabolism of bile acids are presented in Figure 1.

Secondary bile acids are hydrophobic, cytotoxic molecules and evidence suggests they play a role in CRC. For example, numerous epidemiological studies have highlighted higher faecal bile acid content in populations with increased CRC rates (113-115). Moreover, DCA is higher in patients with colorectal adenomas and was first proposed as a carcinogen in 1940 based on its induction of tumours in mice (116, 117). Bile acids were initially classified as tumour promoters rather than tumour initiators, as studies primarily demonstrated their action when co-administered with chemical carcinogens such as AOM (118, 119). However, the role

of bile acids as aetiologic agents of cancer in their own right is now emerging (120). For example, a diet high in fat and low in fiber is a known risk factor for colon cancer (121). This diet was also associated with increased secondary bile acids, as well as increased glucuronidase deconjugation (121). Also of note, GF rats are generally resistant to chemical carcinogen-induced CRC (122). However, rats treated with the chemical carcinogen methylnitronitrosoguanidine (MNNG) and DCA displayed colonic adenocarcinomas, suggesting microbial production of DCA could play a role in tumorigenesis and may explain, in part, the resistance to CRC observed in GF animals (123).

Bile acids can increase cancer risk by several mechanisms. DCA and CDCA were shown to up-regulate pro-inflammatory cyclooxygenase-2 and its downstream inflammatory product prostaglandin E_2 in a protein kinase C-dependent manner, whilst activating c-Jun and AP-1 (124, 125). Bile acids also generate reactive oxygen and nitrogen species via a detergent effect on cell membranes and activation of inducible nitric oxide synthase (126). Additionally, bile acids may induce apoptosis in the short term but select for apoptosis-resistant cells in the longer term (127). This ability appears to be related to bile acids' hydrophobicity, with the most powerful effect displayed by the most hydrophobic bile acids (128). Indeed, normal cells adjacent to tumour tissue in colon cancer patients were shown to display resistance to bile salt- and bile acid-induced apoptosis, and this is mediated by an up-regulation of the anti-apoptotic protein B-cell lymphoma-extra large (127, 129).

Bile acids can also induce chromosomal abnormalities such as aneuploidy and micronucleus formation (130, 131). In yeast, DCA, LCA, CDCA and CA each induced mitotic chromosome aneuploidy, while tauro- or glyco-conjugated DCA did not (132). Oxidative stress is a well-established source of chromosomal instability and this is a plausible mechanism of bile-acid induced DNA damage and increased CRC risk (133, 134). LCA was also shown to inhibit the repair activity of DNA polymerase β which could exacerbate the consequences of bile acid-induced DNA damage (135). Finally, a proteomic study of CRC cell lines induced with DCA identified alterations in ten proteins involved in DNA repair and cell cycle checkpoints (136).

Bile acids have also been associated with cancer through Farnesoid X Receptor (FXR) signalling (137). Bile acid homeostasis is regulated by FXR, which is a nuclear receptor expressed by liver hepatocytes and small intestine enterocytes (138). *FXR* expression is down-regulated in human colorectal tumours and colon cancer cell lines (139), while $Fxr^{-/-}$ mice are predisposed to multiple cancers, including that of CRC (140, 141). Moreover, administration of tauro-conjugated βMCA, which is an FXR antagonist bile acid, increased stem cell proliferation by activating Wnt signalling, impaired intestinal integrity, accelerated tumour growth, induced dysplastic morphology and chromosome instability, and increased the serum levels of pro-inflammatory cytokines in APC^{MIN} mice (142). FXR agonists, in turn, promoted apoptosis, down-regulated intestinal stem cell genes and inhibited Wnt signalling (143). FXR agonists also delayed tumour progression, reduced tumour multiplicity, proliferation and serum cytokines, and improved intestinal morphology, differentiation, barrier function and bile acid homeostasis (142). Microbial modification of bile acids plays a role in their interaction with FXR, as FXR displays greater affinity for conjugated bile acids, with reducing affinity for CDCA>DCA=LCA>CA (144). As a result, bacterial modification of bile acids can influence their specificity for FXR and hence their influence on cancer risk. FXR has also been demonstrated to modulate the microbiota as FXR antagonism increased the proportion of Bacteroidetes compared to Firmicutes (145, 146). FXR can also suppress expression of pro-inflammatory cytokines (147), to the extent that a synthetic FXR ligand protected mice from DSS-induced colitis (148).

4 Pre- and pro-biotics as modulators of host-microbe symbiosis: implications for colorectal cancer

Clinical trials have provided evidence for the beneficial role of pre- and pro-biotics in CRC (Table 3). One such trial using a combination of pre- and pro-biotics comprising inulin, *Lactobacillus* and *Bifidobacterium* administered to individuals at high risk of CRC development showed that the combination treatment resulted in a decrease in colonic epithelial proliferation, decreased abundance of *Clostridium perfringens* and reduced ability of faecal water to induce necrosis in colon cells *in vitro* (149). Epithelial barrier function, which is deficient in CRC, was also improved (151).

[Figure 1]

This beneficial effect of pre- and pro-biotics has been replicated in several studies (152-155). Moreover, a prebiotic mixture decreased chemotherapy-associated side effects including diarrhoea and enterocolitis in CRC patients (156). Furthermore, the administration of probiotics can have potential cancer preventative effects. For example, a mixture of *Lactobacillus* and *Propionibacterium* administered to healthy subjects reduced faecal levels of the bacterial enzyme β-glucuronidase, which is implicated in the activation of carcinogens in the colon (157).

[Table 3]

In animal studies, *Bifidobacterium longum* has been shown to ameliorate AOM/DMH-induced colon carcinogenesis, an effect that is enhanced by co-administration with the prebiotics inulin and lactulose (175, 176). A similar effect was seen with *Lactobacillus* species, although this effect was absent when probiotic administration was delayed until 9 weeks into DMH-administration, suggesting *Lactobacillus* was only protective in the early stages of tumorigenesis (177, 178).

The ability of probiotics to affect early-stage cancer development could be due to their function as anti-mutagenic agents. For instance, *Lactobacillus casei* gavage attenuated DNA damage induced by MNNG in rat colonic and gastric mucosa, while in another study, a selection of lactic acid bacteria (LAB) inhibited the genotoxic effects of MNNG and DMH in the rat colon (161, 162). Heat treatment eliminated the protective effect of the bacteria in both studies, suggesting that viable bacteria are required for this effect, although the peptidoglycan fraction and whole freeze-dried *L. acidophilus* were also anti-genotoxic. Arimochi *et al*. also demonstrated a reduction in ACF in AOM-treated rats after the administration of *L. acidophilus* and *C. perfringens* (179). In particular, *L. acidophilus* improved DNA repair by DNA methyltransferase. Other potential mechanisms include the ability of LAB to bind dietary mutagens which limits their ability to interact with the colonic epithelium (167, 180). For example, toxic compounds are detoxified by glucuronidation in the liver, but bacterial β-glucuronidase activity may hydrolyse these molecules and liberate carcinogens. The activity of this enzyme was shown to be reduced in AOM- and DMH-treated rats following gavage with the probiotic *B. longum*. This effect was enhanced by co-administration with the prebiotic inulin, possibly as a result of acidification of the intestinal environment and displacement of bacteria expressing β-glucuronidase (181-183).

Probiotic and commensal bacteria, including species that are indigenous to the normal human microbiota, can also provide health benefits by competing with more harmful organisms and preventing them from becoming established in the GI tract (184). LAB have

been shown to inhibit the growth of coliforms in the GI tract and return *E. coli*-infected rats to a normal microbiota composition while reducing β-glucuronidase activity (185). Probiotics can also produce antimicrobial compounds that inhibit enteric pathogens (186, 187).

Chronic inflammation has been shown to promote CRC and this can be ameliorated by probiotic bacteria (188). This can be mediated by the production of anti-inflammatory metabolites such as butyrate (Section 3.1). Some probiotic bacteria have also been shown to suppress the production of inflammatory factors by host immune cells, with *Lactobacillus reuteri* being shown to suppress the production of tumour necrosis factor-alpha (TNFα) and monocyte chemoattractant protein 1 production by lipopolysaccharide-activated monocytes and macrophages (189). A similar anti-inflammatory effect was also observed in rat pups (190). As well as inhibiting pro-tumorigenic inflammation, probiotics may also induce the targeted production of immune-activating cytokines to suppress tumorigenesis. For instance, the *L. casei* strain Shirota, when administered into the intrapleural cavity of tumour-bearing mice, induced the production of interferon gamma, IL-1β, and TNFα, which in turn inhibited tumour growth and increased survival (191).

5 Conclusions

In summary, the gut microbiota is an integral part of normal human physiology. This microbial reservoir of genes and metabolic functions is larger and more dynamic than the human genome, and from this grows a complex symbiosis between microbiota and host. Disruption of this relationship can have widespread negative effects on human health. This chapter has presented evidence of both protective and harmful influences of gut bacteria and their metabolites in CRC, with a particular focus on SCFAs and bile acids. Manipulation of this symbiosis with pre- and pro-biotics has the potential to have considerable health benefits, as we begin to better understand the cross-talk between the gut microbiota and the host in the maintenance of a healthy symbiotic relationship.

Acknowledgements

This publication has emanated from research supported in part by a research grant from Science Foundation Ireland (SFI) under Grant Number SFI/12/RC/2273.

References

1. Group NHW, Peterson J, Garges S, Giovanni M, McInnes P, Wang L, et al. The NIH Human Microbiome Project. Genome Res. 2009;19(12):2317-23.
2. Sekirov I, Russell SL, Antunes LCM, Finlay BB. Gut Microbiota in Health and Disease. Physiological Reviews. 2010;90(3):859-904.
3. Quigley EMM. Gut bacteria in health and disease. Gastroenterol Hepatol (N Y). 2013;9(9):560-9.
4. Patel RM, Lin PW. Developmental biology of gut-probiotic interaction. Gut microbes. 2010;1(3):186-95.
5. Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, et al. Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. Foods (Basel, Switzerland). 2019;8(3).
6. Marteau PR, Vrese Md, Cellier CJ, Schrezenmeir J. Protection from gastrointestinal diseases with the use of probiotics. The American Journal of Clinical Nutrition. 2001;73(2):430s-6s.
7. Food and Agriculture Organization of the United Nations, World Health Organization. Guidelines for the Evaluation of Probiotics in Food. 2002.
8. Wroblewski LE, Peek RM, Jr., Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clin Microbiol Rev. 2010;23(4):713-39.
9. Owyang SY, Luther J, Kao JY. Helicobacter pylori: beneficial for most? Expert review of gastroenterology & hepatology. 2011;5(6):649-51.
10. Pasolli E, Asnicar F, Manara S, Zolfo M, Karcher N, Armanini F, et al. Extensive Unexplored Human Microbiome Diversity Revealed by Over 150,000 Genomes from Metagenomes Spanning Age, Geography, and Lifestyle. Cell. 2019;176(3):649-62.e20.
11. Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proceedings of the National Academy of Sciences of the United States of America. 2007;104(34):13780-5.
12. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. Science (New York, NY). 2005;308(5728):1635-8.
13. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, et al. Metagenomic analysis of the human distal gut microbiome. Science (New York, NY). 2006;312(5778):1355-9.
14. O'Hara AM, Shanahan F. The gut flora as a forgotten organ. EMBO reports. 2006;7(7):688-93.
15. Mandar R, Mikelsaar M. Transmission of mother's microflora to the newborn at birth. Biology of the neonate. 1996;69(1):30-5.
16. de Goffau MC, Lager S, Sovio U, Gaccioli F, Cook E, Peacock SJ, et al. Human placenta has no microbiome but can contain potential pathogens. Nature. 2019;572(7769):329-34.
17. Huurre A, Kalliomaki M, Rautava S, Rinne M, Salminen S, Isolauri E. Mode of delivery - effects on gut microbiota and humoral immunity. Neonatology. 2008;93(4):236-40.
18. Voreades N, Kozil A, Weir TL. Diet and the development of the human intestinal microbiome. Front Microbiol. 2014;5(494).
19. Maskarinec G, Noh JJ. The effect of migration on cancer incidence among Japanese in Hawaii. Ethnicity & disease. 2004;14(3):431-9.
20. Turnbaugh PJ, Backhed F, Fulton L, Gordon JI. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell host & microbe. 2008;3(4):213-23.
21. Nagpal R, Mainali R, Ahmadi S, Wang S, Singh R, Kavanagh K, et al. Gut microbiome and aging: Physiological and mechanistic insights. Nutr Healthy Aging. 2018;4(4):267-85.

22. O'Toole PW, Claesson MJ. Gut microbiota: Changes throughout the lifespan from infancy to elderly. International Dairy Journal. 2010;20(4):281-91.
23. Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature. 2012;488:178.
24. Smith K, McCoy KD, Macpherson AJ. Use of axenic animals in studying the adaptation of mammals to their commensal intestinal microbiota. Seminars in immunology. 2007;19(2):59-69.
25. Tibbs TN, Lopez LR, Arthur JC. The influence of the microbiota on immune development, chronic inflammation, and cancer in the context of aging. Microbial cell (Graz, Austria). 2019;6(8):324-34.
26. Klaasen HL, Van der Heijden PJ, Stok W, Poelma FG, Koopman JP, Van den Brink ME, et al. Apathogenic, intestinal, segmented, filamentous bacteria stimulate the mucosal immune system of mice. Infection and immunity. 1993;61(1):303-6.
27. Mathias A, Pais B, Favre L, Benyacoub J, Corthesy B. Role of secretory IgA in the mucosal sensing of commensal bacteria. Gut microbes. 2014;5(6):688-95.
28. Yap YA, Mariño E. An Insight Into the Intestinal Web of Mucosal Immunity, Microbiota, and Diet in Inflammation. Frontiers in immunology. 2018;9:2617.
29. Hope ME, Hold GL, Kain R, El-Omar EM. Sporadic colorectal cancer – role of the commensal microbiota. FEMS Microbiology Letters. 2005;244(1):1-7.
30. Macarthur M, Hold GL, El-Omar EM. Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. American journal of physiology Gastrointestinal and liver physiology. 2004;286(4):G515-20.
31. Wu J, Li Q, Fu X. Fusobacterium nucleatum Contributes to the Carcinogenesis of Colorectal Cancer by Inducing Inflammation and Suppressing Host Immunity. Translational oncology. 2019;12(6):846-51.
32. Haghi F, Goli E, Mirzaei B, Zeighami H. The association between fecal enterotoxigenic B. fragilis with colorectal cancer. BMC cancer. 2019;19(1):879.
33. Nowacki MR. Cell proliferation in colonic crypts of germ-free and conventional mice--preliminary report. Folia histochemica et cytobiologica. 1993;31(2):77-81.
34. Jahani-Sherafat S, Alebouyeh M, Moghim S, Ahmadi Amoli H, Ghasemian-Safaei H. Role of gut microbiota in the pathogenesis of colorectal cancer; a review article. Gastroenterol Hepatol Bed Bench. 2018;11(2):101-9.
35. Suehiro Y, Sakai K, Nishioka M, Hashimoto S, Takami T, Higaki S, et al. Highly sensitive stool DNA testing of Fusobacterium nucleatum as a marker for detection of colorectal tumours in a Japanese population. Annals of clinical biochemistry. 2017;54(1):86-91.
36. Wong SH, Kwong TNY, Chow T-C, Luk AKC, Dai RZW, Nakatsu G, et al. Quantitation of faecal Fusobacterium improves faecal immunochemical test in detecting advanced colorectal neoplasia. Gut. 2017;66(8):1441-8.
37. Liang Q, Chiu J, Chen Y, Huang Y, Higashimori A, Fang J, et al. Fecal Bacteria Act as Novel Biomarkers for Noninvasive Diagnosis of Colorectal Cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2017;23(8):2061-70.
38. Castellarin M, Warren RL, Freeman JD, Dreolini L, Krzywinski M, Strauss J, et al. Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma. Genome Res. 2012;22(2):299-306.
39. Li Y-Y, Ge Q-X, Cao J, Zhou Y-J, Du Y-L, Shen B, et al. Association of Fusobacterium nucleatum infection with colorectal cancer in Chinese patients. World J Gastroenterol. 2016;22(11):3227-33.
40. Mima K, Cao Y, Chan AT, Qian ZR, Nowak JA, Masugi Y, et al. Fusobacterium nucleatum in Colorectal Carcinoma Tissue According to Tumor Location. Clin Transl Gastroenterol. 2016;7(11):e200-e.

41. Fukugaiti MH, Ignacio A, Fernandes MR, Ribeiro Júnior U, Nakano V, Avila-Campos MJ. High occurrence of Fusobacterium nucleatum and Clostridium difficile in the intestinal microbiota of colorectal carcinoma patients. Braz J Microbiol. 2015;46(4):1135-40.
42. Wei Z, Cao S, Liu S, Yao Z, Sun T, Li Y, et al. Could gut microbiota serve as prognostic biomarker associated with colorectal cancer patients' survival? A pilot study on relevant mechanism. Oncotarget. 2016;7(29):46158-72.
43. Mira-Pascual L, Cabrera-Rubio R, Ocon S, Costales P, Parra A, Suarez A, et al. Microbial mucosal colonic shifts associated with the development of colorectal cancer reveal the presence of different bacterial and archaeal biomarkers. Journal of gastroenterology. 2015;50(2):167-79.
44. Tahara T, Yamamoto E, Suzuki H, Maruyama R, Chung W, Garriga J, et al. Fusobacterium in colonic flora and molecular features of colorectal carcinoma. Cancer research. 2014;74(5):1311-8.
45. Marchesi JR, Dutilh BE, Hall N, Peters WHM, Roelofs R, Boleij A, et al. Towards the human colorectal cancer microbiome. PloS one. 2011;6(5):e20447-e.
46. Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, et al. Genomic analysis identifies association of Fusobacterium with colorectal carcinoma. Genome Res. 2012;22(2):292-8.
47. Burns MB, Lynch J, Starr TK, Knights D, Blekhman R. Virulence genes are a signature of the microbiome in the colorectal tumor microenvironment. Genome medicine. 2015;7(1):55.
48. Gao Z, Guo B, Gao R, Zhu Q, Qin H. Microbiota disbiosis is associated with colorectal cancer. Front Microbiol. 2015;6:20-.
49. Ahn J, Sinha R, Pei Z, Dominianni C, Wu J, Shi J, et al. Human gut microbiome and risk for colorectal cancer. Journal of the National Cancer Institute. 2013;105(24):1907-11.
50. Sinha R, Ahn J, Sampson JN, Shi J, Yu G, Xiong X, et al. Fecal Microbiota, Fecal Metabolome, and Colorectal Cancer Interrelations. PloS one. 2016;11(3):e0152126-e.
51. Viljoen KS, Dakshinamurthy A, Goldberg P, Blackburn JM. Quantitative profiling of colorectal cancer-associated bacteria reveals associations between fusobacterium spp., enterotoxigenic Bacteroides fragilis (ETBF) and clinicopathological features of colorectal cancer. PloS one. 2015;10(3):e0119462.
52. Geng J, Fan H, Tang X, Zhai H, Zhang Z. Diversified pattern of the human colorectal cancer microbiome. Gut pathogens. 2013;5(1):2-.
53. Zhou Y, He H, Xu H, Li Y, Li Z, Du Y, et al. Association of oncogenic bacteria with colorectal cancer in South China. Oncotarget. 2016;7(49):80794-802.
54. Warren RL, Freeman DJ, Pleasance S, Watson P, Moore RA, Cochrane K, et al. Co-occurrence of anaerobic bacteria in colorectal carcinomas. Microbiome. 2013;1(1):16-.
55. Sobhani I, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, Langella P, et al. Microbial dysbiosis in colorectal cancer (CRC) patients. PloS one. 2011;6(1):e16393-e.
56. Balamurugan R, Rajendiran E, George S, Samuel GV, Ramakrishna BS. Real-time polymerase chain reaction quantification of specific butyrate-producing bacteria, Desulfovibrio and Enterococcus faecalis in the feces of patients with colorectal cancer. Journal of gastroenterology and hepatology. 2008;23(8 Pt 1):1298-303.
57. Flemer B, Lynch DB, Brown JMR, Jeffery IB, Ryan FJ, Claesson MJ, et al. Tumour-associated and non-tumour-associated microbiota in colorectal cancer. Gut. 2017;66(4):633-43.
58. Swidsinski A, Khilkin M, Kerjaschki D, Schreiber S, Ortner M, Weber J, et al. Association between intraepithelial Escherichia coli and colorectal cancer. Gastroenterology. 1998;115(2):281-6.
59. Zumkeller N, Brenner H, Zwahlen M, Rothenbacher D. Helicobacter pylori infection and colorectal cancer risk: a meta-analysis. Helicobacter. 2006;11(2):75-80.

60. Kwong TNY, Wang X, Nakatsu G, Chow TC, Tipoe T, Dai RZW, et al. Association Between Bacteremia From Specific Microbes and Subsequent Diagnosis of Colorectal Cancer. Gastroenterology. 2018;155(2):383-90.e8.
61. Gold JS, Bayar S, Salem RR. Association of Streptococcus bovis bacteremia with colonic neoplasia and extracolonic malignancy. Archives of surgery (Chicago, Ill : 1960). 2004;139(7):760-5.
62. Flemer B, Warren RD, Barrett MP, Cisek K, Das A, Jeffery IB, et al. The oral microbiota in colorectal cancer is distinctive and predictive. Gut. 2018;67(8):1454-63.
63. Zackular JP, Baxter NT, Iverson KD, Sadler WD, Petrosino JF, Chen GY, et al. The Gut Microbiome Modulates Colon Tumorigenesis. mBio. 2013;4(6):e00692-13.
64. Kaur K, Saxena A, Debnath I, O'Brien JL, Ajami NJ, Auchtung TA, et al. Antibiotic-mediated bacteriome depletion in Apc(Min/+) mice is associated with reduction in mucus-producing goblet cells and increased colorectal cancer progression. Cancer medicine. 2018;7(5):2003-12.
65. Xiao H, Yin W, Khan MA, Gulen MF, Zhou H, Sham HP, et al. Loss of single immunoglobulin interlukin-1 receptor-related molecule leads to enhanced colonic polyposis in Apc(min) mice. Gastroenterology. 2010;139(2):574-85.
66. Belcheva A, Irrazabal T, Robertson Susan J, Streutker C, Maughan H, Rubino S, et al. Gut Microbial Metabolism Drives Transformation of Msh2-Deficient Colon Epithelial Cells. Cell. 2014;158(2):288-99.
67. Onoue M, Kado S, Sakaitani Y, Uchida K, Morotomi M. Specific species of intestinal bacteria influence the induction of aberrant crypt foci by 1,2-dimethylhydrazine in rats. Cancer Lett. 1997;113(1-2):179-86.
68. Yang Y, Weng W, Peng J, Hong L, Yang L, Toiyama Y, et al. Fusobacterium nucleatum Increases Proliferation of Colorectal Cancer Cells and Tumor Development in Mice by Activating Toll-Like Receptor 4 Signaling to Nuclear Factor-κB, and Up-regulating Expression of MicroRNA-21. Gastroenterology. 2017;152(4):851-66.e24.
69. Ellmerich S, Scholler M, Duranton B, Gosse F, Galluser M, Klein JP, et al. Promotion of intestinal carcinogenesis by Streptococcus bovis. Carcinogenesis. 2000;21(4):753-6.
70. Horie H, Kanazawa K, Okada M, Narushima S, Itoh K, Terada A. Effects of intestinal bacteria on the development of colonic neoplasm: an experimental study. European journal of cancer prevention : the official journal of the European Cancer Prevention Organisation (ECP). 1999;8(3):237-45.
71. Scanlan PD, Shanahan F, Clune Y, Collins JK, O'Sullivan GC, O'Riordan M, et al. Culture-independent analysis of the gut microbiota in colorectal cancer and polyposis. Environmental microbiology. 2008;10(3):789-98.
72. Martin HM, Campbell BJ, Hart CA, Mpofu C, Nayar M, Singh R, et al. Enhanced Escherichia coli adherence and invasion in Crohn's disease and colon cancer. Gastroenterology. 2004;127(1):80-93.
73. Kanazawa K, Konishi F, Mitsuoka T, Terada A, Itoh K, Narushima S, et al. Factors influencing the development of sigmoid colon cancer. Bacteriologic and biochemical studies. Cancer. 1996;77(8 Suppl):1701-6.
74. Hayatsu H, Hayatsu T. Suppressing effect of Lactobacillus casei administration on the urinary mutagenicity arising from ingestion of fried ground beef in the human. Cancer Letters. 1993;73(2):173-9.
75. Ling WH, Korpela R, Mykkänen H, Salminen S, Hänninen O. Lactobacillus Strain GG Supplementation Decreases Colonic Hydrolytic and Reductive Enzyme Activities in Healthy Female Adults. The Journal of Nutrition. 1994;124(1):18-23.
76. Spanhaak S, Havenaar R, Schaafsma G. The effect of consumption of milk fermented by Lactobacillus casei strain Shirota on the intestinal microflora and immune parameters in humans. European Journal of Clinical Nutrition. 1998;52(12):899-907.

77. Velcich A, Yang W, Heyer J, Fragale A, Nicholas C, Viani S, et al. Colorectal cancer in mice genetically deficient in the mucin Muc2. Science (New York, NY). 2002;295(5560):1726-9.
78. Wirbel J, Pyl PT, Kartal E, Zych K, Kashani A, Milanese A, et al. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. Nature Medicine. 2019;25(4):679-89.
79. Thomas AM, Manghi P, Asnicar F, Pasolli E, Armanini F, Zolfo M, et al. Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. Nature Medicine. 2019;25(4):667-78.
80. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites and colorectal cancer. Nature Reviews Microbiology. 2014;12:661.
81. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev. 2001;81(3):1031-64.
82. Hinnebusch BF, Meng S, Wu JT, Archer SY, Hodin RA. The effects of short-chain fatty acids on human colon cancer cell phenotype are associated with histone hyperacetylation. J Nutr. 2002;132(5):1012-7.
83. Louis P, Young P, Holtrop G, Flint HJ. Diversity of human colonic butyrate-producing bacteria revealed by analysis of the butyryl-CoA:acetate CoA-transferase gene. Environmental microbiology. 2010;12(2):304-14.
84. Wu X, Wu Y, He L, Wu L, Wang X, Liu Z. Effects of the intestinal microbial metabolite butyrate on the development of colorectal cancer. J Cancer. 2018;9(14):2510-7.
85. Donohoe DR, Garge N, Zhang X, Sun W, O'Connell TM, Bunger MK, et al. The microbiome and butyrate regulate energy metabolism and autophagy in the mammalian colon. Cell metabolism. 2011;13(5):517-26.
86. Macfarlane GT, Gibson GR, Cummings JH. Comparison of fermentation reactions in different regions of the human colon. The Journal of applied bacteriology. 1992;72(1):57-64.
87. McIntyre A, Gibson PR, Young GP. Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. Gut. 1993;34(3):386-91.
88. Le Leu RK, Brown IL, Hu Y, Morita T, Esterman A, Young GP. Effect of dietary resistant starch and protein on colonic fermentation and intestinal tumourigenesis in rats. Carcinogenesis. 2007;28(2):240-5.
89. Bultman SJ. Interplay between diet, gut microbiota, epigenetic events, and colorectal cancer. Molecular Nutrition & Food Research. 2017;61(1):1500902.
90. Berwick DC, Hers I, Heesom KJ, Moule SK, Tavare JM. The identification of ATP-citrate lyase as a protein kinase B (Akt) substrate in primary adipocytes. The Journal of biological chemistry. 2002;277(37):33895-900.
91. Donohoe DR, Collins LB, Wali A, Bigler R, Sun W, Bultman SJ. The Warburg effect dictates the mechanism of butyrate-mediated histone acetylation and cell proliferation. Mol Cell. 2012;48(4):612-26.
92. Kripke SA, Fox AD, Berman JM, Settle RG, Rombeau JL. Stimulation of intestinal mucosal growth with intracolonic infusion of short-chain fatty acids. JPEN Journal of parenteral and enteral nutrition. 1989;13(2):109-16.
93. Velazquez OC, Seto RW, Bain AM, Fisher J, Rombeau JL. Deoxycholate inhibits in vivo butyrate-mediated BrDU labeling of the colonic crypt. The Journal of surgical research. 1997;69(2):344-8.
94. Hague A, Elder DJ, Hicks DJ, Paraskeva C. Apoptosis in colorectal tumour cells: induction by the short chain fatty acids butyrate, propionate and acetate and by the bile salt deoxycholate. Int J Cancer. 1995;60(3):400-6.
95. Hague A, Manning AM, Hanlon KA, Huschtscha LI, Hart D, Paraskeva C. Sodium butyrate induces apoptosis in human colonic tumour cell lines in a p53-independent

pathway: implications for the possible role of dietary fibre in the prevention of large-bowel cancer. Int J Cancer. 1993;55(3):498-505.
96. Caderni G, Luceri C, Lancioni L, Tessitore L, Dolara P. Slow-release pellets of sodium butyrate increase apoptosis in the colon of rats treated with azoxymethane, without affecting aberrant crypt foci and colonic proliferation. Nutrition and cancer. 1998;30(3):175-81.
97. Hass R, Busche R, Luciano L, Reale E, Engelhardt WV. Lack of butyrate is associated with induction of Bax and subsequent apoptosis in the proximal colon of guinea pig. Gastroenterology. 1997;112(3):875-81.
98. Augeron C, Laboisse CL. Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate. Cancer research. 1984;44(9):3961-9.
99. Taylor CW, Kim YS, Childress-Fields KE, Yeoman LC. Sensitivity of nuclear c-myc levels and induction to differentiation-inducing agents in human colon tumor cell lines. Cancer Lett. 1992;62(2):95-105.
100. Heerdt BG, Houston MA, Augenlicht LH. Short-chain fatty acid-initiated cell cycle arrest and apoptosis of colonic epithelial cells is linked to mitochondrial function. Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research. 1997;8(5):523-32.
101. Gamet L, Daviaud D, Denis-Pouxviel C, Remesy C, Murat JC. Effects of short-chain fatty acids on growth and differentiation of the human colon-cancer cell line HT29. Int J Cancer. 1992;52(2):286-9.
102. Barnard JA, Warwick G. Butyrate rapidly induces growth inhibition and differentiation in HT-29 cells. Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research. 1993;4(6):495-501.
103. Siavoshian S, Blottiere HM, Cherbut C, Galmiche JP. Butyrate stimulates cyclin D and p21 and inhibits cyclin-dependent kinase 2 expression in HT-29 colonic epithelial cells. Biochemical and biophysical research communications. 1997;232(1):169-72.
104. Gibson PR, Moeller I, Kagelari O, Folino M, Young GP. Contrasting effects of butyrate on the expression of phenotypic markers of differentiation in neoplastic and non-neoplastic colonic epithelial cells in vitro. Journal of gastroenterology and hepatology. 1992;7(2):165-72.
105. Wang HB, Wang PY, Wang X, Wan YL, Liu YC. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. Dig Dis Sci. 2012;57(12):3126-35.
106. Wachtershauser A, Stein J. Rationale for the luminal provision of butyrate in intestinal diseases. European journal of nutrition. 2000;39(4):164-71.
107. Campbell JM, Fahey GC, Jr., Wolf BW. Selected indigestible oligosaccharides affect large bowel mass, cecal and fecal short-chain fatty acids, pH and microflora in rats. J Nutr. 1997;127(1):130-6.
108. Rafter JJ, Eng VW, Furrer R, Medline A, Bruce WR. Effects of calcium and pH on the mucosal damage produced by deoxycholic acid in the rat colon. Gut. 1986;27(11):1320-9.
109. Abrahamse SL, Pool-Zobel BL, Rechkemmer G. Potential of short chain fatty acids to modulate the induction of DNA damage and changes in the intracellular calcium concentration by oxidative stress in isolated rat distal colon cells. Carcinogenesis. 1999;20(4):629-34.
110. Li T, Chiang JYL. Bile Acid Signaling in Liver Metabolism and Diseases. Journal of Lipids. 2012;2012:9.
111. Dawson PA, Haywood J, Craddock AL, Wilson M, Tietjen M, Kluckman K, et al. Targeted Deletion of the Ileal Bile Acid Transporter Eliminates Enterohepatic Cycling of Bile Acids in Mice. Journal of Biological Chemistry. 2003;278(36):33920-7.

112. Hill MJ. Bile flow and colon cancer. Mutation Research/Reviews in Genetic Toxicology. 1990;238(3):313-20.
113. Jensen OM, MacLennan R, Wahrendorf J. Diet, bowel function, fecal characteristics, and large bowel cancer in Denmark and Finland. Nutrition and cancer. 1982;4(1):5-19.
114. Cheah PY. Hypotheses for the etiology of colorectal cancer — an overview. Nutrition and cancer. 1990;14(1):5-13.
115. Crowther JS, Drasar BS, Hill MJ, Maclennan R, Magnin D, Peach S, et al. Faecal steroids and bacteria and large bowel cancer in Hong Kong by socio-economic groups. British Journal of Cancer. 1976;34(2):191-8.
116. Bayerdörffer E, Mannes GA, Ochsenkühn T, Dirschedl P, Wiebecke B, Paumgartner G. Unconjugated secondary bile acids in the serum of patients with colorectal adenomas. Gut. 1995;36(2):268-73.
117. Cook JW, Kennaway EL, Kennaway NM. Production of Tumours in Mice by Deoxycholic Acid. Nature. 1940;145(3677):627-.
118. Magnuson BA, Carr I, Bird RP. Ability of Aberrant Crypt Foci Characteristics to Predict Colonic T\imor Incidence in Rats Fed Cholic Acid. Cancer research. 1993;53(19):4499-504.
119. Reddy BS, Narasawa T, Weisburger JH, Wynder EL. Promoting Effect of Sodium Deoxycholate on Colon Adenocarcinomas in Germfree Rats2. JNCI: Journal of the National Cancer Institute. 1976;56(2):441-2.
120. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mutation research. 2005;589(1):47-65.
121. Reddy BS, Weisburger JH, Wynder EL. Effects of high risk and low risk diets for colon carcinogenesis on fecal microflora and steroids in man. J Nutr. 1975;105(7):878-84.
122. Sumi Y, Miyakawa M. Gastrointestinal carcinogenesis in germ-free rats given N-methyl-N'-nitro-N-nitrosoguanidine in drinking water. Cancer research. 1979;39(7 Pt 1):2733-6.
123. Reddy BS, Narasawa T, Weisburger JH, Wynder EL. Promoting effect of sodium deoxycholate on colon adenocarcinomas in germfree rats. Journal of the National Cancer Institute. 1976;56(2):441-2.
124. Glinghammar B, Inoue H, Rafter JJ. Deoxycholic acid causes DNA damage in colonic cells with subsequent induction of caspases, COX-2 promoter activity and the transcription factors NF-kB and AP-1. Carcinogenesis. 2002;23(5):839-45.
125. Zhang F, Subbaramaiah K, Altorki N, Dannenberg AJ. Dihydroxy Bile Acids Activate the Transcription of Cyclooxygenase-2. Journal of Biological Chemistry. 1998;273(4):2424-8.
126. Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mutation Research/Reviews in Mutation Research. 2005;589(1):47-65.
127. Bernstein H, Bernstein C, Payne CM, Dvorak K. Bile acids as endogenous etiologic agents in gastrointestinal cancer. World journal of gastroenterology. 2009;15(27):3329-40.
128. Powell AA, LaRue JM, Batta AK, Martinez JD. Bile acid hydrophobicity is correlated with induction of apoptosis and/or growth arrest in HCT116 cells. The Biochemical journal. 2001;356(Pt 2):481-6.
129. Badvie S, Hanna-Morris A, Andreyev HJ, Cohen P, Saini S, Allen-Mersh TG. A "field change" of inhibited apoptosis occurs in colorectal mucosa adjacent to colorectal adenocarcinoma. Journal of clinical pathology. 2006;59(9):942-6.
130. Jenkins GJ, D'Souza FR, Suzen SH, Eltahir ZS, James SA, Parry JM, et al. Deoxycholic acid at neutral and acid pH, is genotoxic to oesophageal cells through the induction of ROS: The potential role of anti-oxidants in Barrett's oesophagus. Carcinogenesis. 2007;28(1):136-42.
131. Assinder SJ, Upshall A. Mitotic aneuploidy induced by sodium deoxycholate in Aspergillus nidulans. Mutation research. 1982;93(1):101-8.

132. Ferguson LR, Parry JM. Mitotic aneuploidy as a possible mechanism for tumour promoting activity in bile acids. Carcinogenesis. 1984;5(4):447-51.
133. Limoli CL, Giedzinski E. Induction of chromosomal instability by chronic oxidative stress. Neoplasia (New York, NY). 2003;5(4):339-46.
134. Hunt CR, Sim JE, Sullivan SJ, Featherstone T, Golden W, Von Kapp-Herr C, et al. Genomic instability and catalase gene amplification induced by chronic exposure to oxidative stress. Cancer research. 1998;58(17):3986-92.
135. Ogawa A, Murate T, Suzuki M, Nimura Y, Yoshida S. Lithocholic Acid, a Putative Tumor Promoter, Inhibits Mammalian DNA Polymerase β. Japanese Journal of Cancer Research. 1998;89(11):1154-9.
136. Bernstein H, Payne CM, Kunke K, Crowley-Weber CL, Waltmire CN, Dvorakova K, et al. A proteomic study of resistance to deoxycholate-induced apoptosis. Carcinogenesis. 2004;25(5):681-92.
137. Degirolamo C, Modica S, Palasciano G, Moschetta A. Bile acids and colon cancer: Solving the puzzle with nuclear receptors. Trends in Molecular Medicine. 2011;17(10):564-72.
138. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. Science (New York, NY). 1999;284(5418):1362-5.
139. De Gottardi A, Touri F, Maurer CA, Perez A, Maurhofer O, Ventre G, et al. The Bile Acid Nuclear Receptor FXR and the Bile Acid Binding Protein IBABP Are Differently Expressed in Colon Cancer. Digestive Diseases and Sciences. 2004;49(6):982-9.
140. Maran RRM, Thomas A, Roth M, Sheng Z, Esterly N, Pinson D, et al. Farnesoid X Receptor Deficiency in Mice Leads to Increased Intestinal Epithelial Cell Proliferation and Tumor Development. Journal of Pharmacology and Experimental Therapeutics. 2009;328(2):469-77.
141. Kim I, Morimura K, Shah Y, Yang Q, Ward JM, Gonzalez FJ. Spontaneous hepatocarcinogenesis in farnesoid X receptor-null mice. Carcinogenesis. 2007;28(5):940-6.
142. Fu T, Coulter S, Yoshihara E, Oh TG, Fang S, Cayabyab F, et al. FXR Regulates Intestinal Cancer Stem Cell Proliferation. Cell. 2019;176(5):1098-112.e18.
143. Modica S, Murzilli S, Salvatore L, Schmidt DR, Moschetta A. Nuclear Bile Acid Receptor FXR Protects against Intestinal Tumorigenesis. Cancer research. 2008;68(23):9589-94.
144. Ding L, Yang L, Wang Z, Huang W. Bile acid nuclear receptor FXR and digestive system diseases. Acta pharmaceutica Sinica B. 2015;5(2):135-44.
145. Bervoets L, Van Hoorenbeeck K, Kortleven I, Van Noten C, Hens N, Vael C, et al. Differences in gut microbiota composition between obese and lean children: a cross-sectional study. Gut pathogens. 2013;5(1):10.
146. Jiang C, Xie C, Lv Y, Li J, Krausz KW, Shi J, et al. Intestine-selective farnesoid X receptor inhibition improves obesity-related metabolic dysfunction. Nature Communications. 2015;6:10166.
147. Stojancevic M, Stankov K, Mikov M. The impact of farnesoid X receptor activation on intestinal permeability in inflammatory bowel disease. Can J Gastroenterol. 2012;26(9):631-7.
148. Vavassori P, Mencarelli A, Renga B, Distrutti E, Fiorucci S. The bile acid receptor FXR is a modulator of intestinal innate immunity. Journal of immunology (Baltimore, Md : 1950). 2009;183(10):6251-61.
149. Rafter J, Bennett M, Caderni G, Clune Y, Hughes R, Karlsson PC, et al. Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. Am J Clin Nutr. 2007;85(2):488-96.
150. Long SL, Gahan CGM, Joyce SA. Interactions between gut bacteria and bile in health and disease. Molecular Aspects of Medicine. 2017;56:54-65.

151. Soler AP, Miller RD, Laughlin KV, Carp NZ, Klurfeld DM, Mullin JM. Increased tight junctional permeability is associated with the development of colon cancer. Carcinogenesis. 1999;20(8):1425-31.
152. Roessler A, Forssten SD, Glei M, Ouwehand AC, Jahreis G. The effect of probiotics on faecal microbiota and genotoxic activity of faecal water in patients with atopic dermatitis: a randomized, placebo-controlled study. Clinical nutrition (Edinburgh, Scotland). 2012;31(1):22-9.
153. Gianotti L, Morelli L, Galbiati F, Rocchetti S, Coppola S, Beneduce A, et al. A randomized double-blind trial on perioperative administration of probiotics in colorectal cancer patients. World journal of gastroenterology. 2010;16(2):167-75.
154. Xia Y, Yang Z, Chen HQ, Qin HL. [Effect of bowel preparation with probiotics on intestinal barrier after surgery for colorectal cancer]. Zhonghua wei chang wai ke za zhi = Chinese journal of gastrointestinal surgery. 2010;13(7):528-31.
155. Roller M, Clune Y, Collins K, Rechkemmer G, Watzl B. Consumption of prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and Bifidobacterium lactis has minor effects on selected immune parameters in polypectomised and colon cancer patients. The British journal of nutrition. 2007;97(4):676-84.
156. Mego M, Chovanec J, Vochyanova-Andrezalova I, Konkolovsky P, Mikulova M, Reckova M, et al. Prevention of irinotecan induced diarrhea by probiotics: A randomized double blind, placebo controlled pilot study. Complementary therapies in medicine. 2015;23(3):356-62.
157. Ishikawa H, Akedo I, Umesaki Y, Tanaka R, Imaoka A, Otani T. Randomized controlled trial of the effect of bifidobacteria-fermented milk on ulcerative colitis. Journal of the American College of Nutrition. 2003;22(1):56-63.
158. Wollowski I, Rechkemmer G, Pool-Zobel BL. Protective role of probiotics and prebiotics in colon cancer. Am J Clin Nutr. 2001;73(2 Suppl):451s-5s.
159. Pool-Zobel BL, Munzner R, Holzapfel WH. Antigenotoxic properties of lactic acid bacteria in the S. typhimurium mutagenicity assay. Nutrition and cancer. 1993;20(3):261-70.
160. Bodana AR, Rao DR. Antimutagenic activity of milk fermented by Streptococcus thermophilus and Lactobacillus bulgaricus. Journal of dairy science. 1990;73(12):3379-84.
161. Pool-Zobel BL, Bertram B, Knoll M, Lambertz R, Neudecker C, Schillinger U, et al. Antigenotoxic properties of lactic acid bacteria in vivo in the gastrointestinal tract of rats. Nutrition and cancer. 1993;20(3):271-81.
162. Pool-Zobel BL, Neudecker C, Domizlaff I, Ji S, Schillinger U, Rumney C, et al. Lactobacillus- and bifidobacterium-mediated antigenotoxicity in the colon of rats. Nutrition and cancer. 1996;26(3):365-80.
163. Goldin BR, Gorbach SL. The effect of milk and lactobacillus feeding on human intestinal bacterial enzyme activity. The American journal of clinical nutrition. 1984;39(5):756-61.
164. Goldin BR, Gorbach SL, Saxelin M, Barakat S, Gualtieri L, Salminen S. Survival of Lactobacillus species (strain GG) in human gastrointestinal tract. Dig Dis Sci. 1992;37(1):121-8.
165. Benno Y, Mitsuoka T. Impact of Bifidobacterium longum on human fecal microflora. Microbiol Immunol. 1992;36(7):683-94.
166. Bouhnik Y, Flourie B, Andrieux C, Bisetti N, Briet F, Rambaud JC. Effects of Bifidobacterium sp fermented milk ingested with or without inulin on colonic bifidobacteria and enzymatic activities in healthy humans. Eur J Clin Nutr. 1996;50(4):269-73.
167. Orrhage K, Sillerstrom E, Gustafsson JA, Nord CE, Rafter J. Binding of mutagenic heterocyclic amines by intestinal and lactic acid bacteria. Mutation research. 1994;311(2):239-48.

168. Zhang XB, Ohta Y. In vitro binding of mutagenic pyrolyzates to lactic acid bacterial cells in human gastric juice. Journal of dairy science. 1991;74(3):752-7.
169. Morotomi M, Mutai M. In vitro binding of potent mutagenic pyrolysates to intestinal bacteria. Journal of the National Cancer Institute. 1986;77(1):195-201.
170. Lidbeck A, Övervik E, Rafter J, Nord CE, Gustafsson JÅ. Effect of Lactobacillus acidophilus Supplements on Mutagen Excretion in Faeces and Urine in Humans. Microbial Ecology in Health and Disease. 1992;5(1):59-67.
171. Link-Amster H, Rochat F, Saudan KY, Mignot O, Aeschlimann JM. Modulation of a specific humoral immune response and changes in intestinal flora mediated through fermented milk intake. FEMS immunology and medical microbiology. 1994;10(1):55-63.
172. Segal I, Hassan H, Walker AR, Becker P, Braganza J. Fecal short chain fatty acids in South African urban Africans and whites. Diseases of the colon and rectum. 1995;38(7):732-4.
173. Baghurst PA, Baghurst KI, Record SJ. Dietary fibre, non-starch polysaccharides and resistant starch: a review. Food Australia. 1996.
174. Marchetti C, Migliorati G, Moraca R, Riccardi C, Nicoletti I, Fabiani R, et al. Deoxycholic acid and SCFA-induced apoptosis in the human tumor cell-line HT-29 and possible mechanisms. Cancer letters. 1997;114(1-2):97-9.
175. Challa A, Rao DR, Chawan CB, Shackelford L. Bifidobacterium longum and lactulose suppress azoxymethane-induced colonic aberrant crypt foci in rats. Carcinogenesis. 1997;18(3):517-21.
176. Rowland IR, Rumney CJ, Coutts JT, Lievense LC. Effect of Bifidobacterium longum and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. Carcinogenesis. 1998;19(2):281-5.
177. Goldin BR, Gualtieri LJ, Moore RP. The effect of Lactobacillus GG on the initiation and promotion of DMH-induced intestinal tumors in the rat. Nutrition and cancer. 1996;25(2):197-204.
178. Goldin BR, Gorbach SL. Effect of Lactobacillus acidophilus dietary supplements on 1,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. Journal of the National Cancer Institute. 1980;64(2):263-5.
179. Arimochi H, Kinouchi T, Kataoka K, Kuwahara T, Ohnishi Y. Effect of intestinal bacteria on formation of azoxymethane-induced aberrant crypt foci in the rat colon. Biochemical and biophysical research communications. 1997;238(3):753-7.
180. Morotomi M, Mutal M. In Vitro Binding of Potent Mutagenic Pyrolyzates to Intestinal Bacteria. JNCI: Journal of the National Cancer Institute. 1986;77(1):195-201.
181. Kulkarni N, Reddy BS. Inhibitory Effect of Bifidobacterium Iongum Cultures on the Azoxymethane-Induced Aberrant Crypt Foci Formation and Fecal Bacterial β-Glucuronidase. Proceedings of the Society for Experimental Biology and Medicine. 1994;207(3):278-83.
182. Abdelali H, Cassand P, Soussotte V, Daubeze M, Bouley C, Narbonne JF. Effect of dairy products on initiation of precursor lesions of colon cancer in rats. Nutrition and cancer. 1995;24(2):121-32.
183. Rowland IR, Rumney CJ, Coutts JT, Lievense LC. Effect of Bifidobacterium longum and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. Carcinogenesis. 1998;19(2):281-5.
184. Collado MC, Meriluoto J, Salminen S. Role of commercial probiotic strains against human pathogen adhesion to intestinal mucus. Letters in applied microbiology. 2007;45(4):454-60.
185. Sreekumar O, Hosono A. Immediate effect of Lactobacillus acidophilus on the intestinal flora and fecal enzymes of rats and the in vitro inhibition of Escherichia coli in coculture. Journal of dairy science. 2000;83(5):931-9.

186. Spinler JK, Taweechotipatr M, Rognerud CL, Ou CN, Tumwasorn S, Versalovic J. Human-derived probiotic Lactobacillus reuteri demonstrate antimicrobial activities targeting diverse enteric bacterial pathogens. Anaerobe. 2008;14(3):166-71.
187. O'Shea EF, Cotter PD, Stanton C, Ross RP, Hill C. Production of bioactive substances by intestinal bacteria as a basis for explaining probiotic mechanisms: bacteriocins and conjugated linoleic acid. International journal of food microbiology. 2012;152(3):189-205.
188. Drago L. Probiotics and Colon Cancer. Microorganisms. 2019;7(3).
189. Lin YP, Thibodeaux CH, Pena JA, Ferry GD, Versalovic J. Probiotic Lactobacillus reuteri suppress proinflammatory cytokines via c-Jun. Inflammatory bowel diseases. 2008;14(8):1068-83.
190. Liu Y, Fatheree NY, Mangalat N, Rhoads JM. Human-derived probiotic Lactobacillus reuteri strains differentially reduce intestinal inflammation. American journal of physiology Gastrointestinal and liver physiology. 2010;299(5):G1087-96.
191. Matsuzaki T. Immunomodulation by treatment with Lactobacillus casei strain Shirota. International journal of food microbiology. 1998;41(2):133-40.