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REVIEW

Intestinal microbiota and its association with colon cancer and red/processed meat consumption

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Key words

Bacteroides fragilis, bile acids, colorectal cancer, diet, dysbiosis, *Escherichia coli*, *Fusobacterium nucleatum*, microbiota, processed meat, *Streptococcus gallolyticus subsp. gallolyticus* (*Sgg*).

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Abstract

The human colon harbors a high number of microorganisms that were reported to play a crucial role in colorectal carcinogenesis. In the recent decade, molecular detection and metabolomic techniques have expanded our knowledge on the role of specific microbial species in promoting tumorigenesis. In this study, we reviewed the association between microbial dysbiosis and colorectal carcinoma (CRC). Various microbial species and their association with colorectal tumorigenesis and red/processed meat consumption have been reviewed. The literature demonstrated a significant abundance of *Fusobacterium nucleatum*, *Streptococcus bovis/gallolyticus*, *Escherichia coli*, and *Bacteroides fragilis* in patients with adenoma or adenocarcinoma compared to healthy individuals. The mechanisms in which each organism was postulated to promote colon carcinogenesis were collated and summarized in this review. These include the microorganisms' ability to adhere to colon cells; modulate the inhibition of tumor suppressor genes, the activations of oncogenes, and genotoxicity; and activate downstream targets responsible for angiogenesis. The role of these microorganisms in conjugation with meat components including N-nitroso compounds, heterocyclic amines, and heme was also evident in multiple studies. The outcome of this review supports the role of red meat consumption in modulating CRC progression and the possibility of gut microbiome influencing the relationship between CRC and diet. The study also demonstrates that microbiota analysis could potentially complement existing screening methods when detecting colonic lesions.

Introduction

The complex microbiota of the colon and its high exposure to environmental agents has resulted in heightened interest to deduce the interrelationship between colorectal cancer (CRC), microbiota, and diet. Through microbial meta'omics, many studies have attempted to explain the relationship between colorectal carcinogenesis and microbial communities.[1–13] This understanding is not restricted to the interaction between microbial genre and the colon but rather expands to specific relationships between individual microbial species and CRC development.[14] Several studies have proposed different pathogenic effects of the CRC-associated bacteria, which include induction of chronic inflammation, the transformation of host metabolites into carcinogens, expression of toxins with oncogenic properties, and damage of barrier function.[15,16] Likewise, dietary compounds including red and processed meat were deemed to damage the intestinal mucosa and favor cancer development.[17–20] Epidemiological studies have demonstrated that long-term consumption of red and processed meat is coupled with an increased risk of colon cancer.[21] This prompted the International Agency for Research on Cancer, a specialized cancer agency of the World Health Organization, to review 800 relevant scientific pieces of literature and made conclusive evidence that processed meat is carcinogenic to humans, while red meat may be carcinogenic to humans.[22] Molecules found in red and processed meat, including heterocyclic amines, N-nitroso compounds (NOCs), heme, and protein supported this conclusion by providing strong mechanistic evidence of the association.[17–20]

Dysbiosis is a disturbance in the composition, structure, or function of the healthy colonic microbiota that leads to an interruption of the normal microbe-host homeostatic relationship.[23] It remains uncertain whether dysbiosis is the consequence or cause of diet-induced inflammation. Limited information is available on the operation of microbial species in a complex microbial environment and its dynamics in response to diet and the host immune response. In consideration of the multifactorial and polymicrobial etiology of CRC, as well as the availability of reductionist scientific experimental paradigms, this review aims to present the cross-talk of human intestinal microbiota and red meat consumption with the host. In this study, we have discussed the association between CRC, microbiota, and diet through three different frameworks: (i) the role of specific microbes in CRC, (ii) the role of microbial communities in CRC, and (iii) the association of microbiota and red/processed meat in CRC. Due to the limited studies in this area, this review presents a brief description of the role of microbial communities in CRC (Fig. 1).

Methods

Relevant research in the last 30 years about the deregulation of intestinal microbiota and its association with colorectal

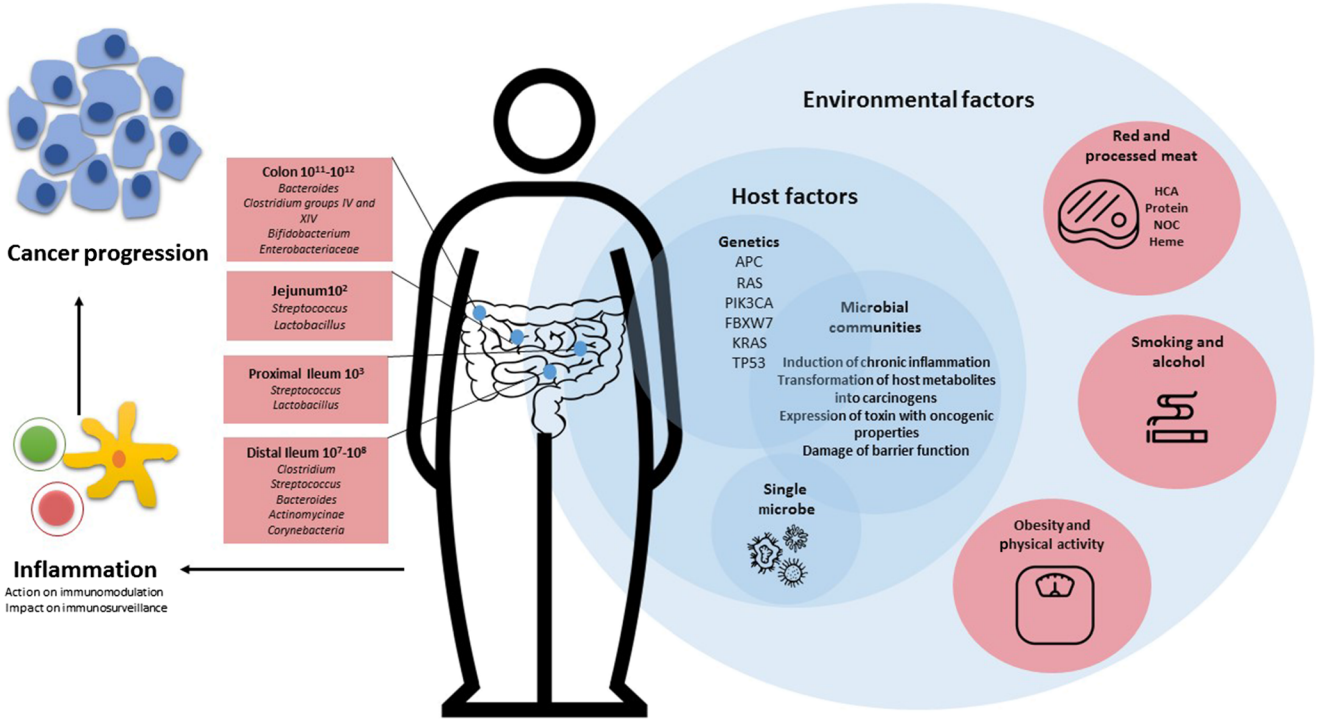

Figure 1 Framework for the factors influencing colon cancer initiation and progression and the microbial composition and concentration in the small and large intestine. Host factors include obesity and fat intake, commensal microbiota, and genetics including genetic, mutations, and polymorphism. The identified involved genes include APC, RAS, PIK3CA, FBXW7, KRAS, and TP53. The microbiota can affect inducing the expression of these genes. Three main frameworks were proposed for the multifactorial polymicrobial nature of CRC: (i) the role of specific microbes in CRC, (ii) the role of microbial communities (acting in synergy) in CRC, and (iii) the role of microbiota and diet in CRC. Several mechanisms are proposed to explain CRC-associated bacteria including (i) induction of chronic inflammation, (ii) transformation of host metabolites into carcinogens, (iii) expression of toxins with oncogenic properties, and (iv) damage of barrier function.[15] Inflammation induced by the factors earlier triggers neoplastic changes leading to carcinogenesis. Microbial composition and concentration were adapted from Balfour.[16] [Color figure can be viewed at wileyonlinelibrary.com]

carcinogenesis was mainly identified using the PubMed database. Articles were also accessed through the University Library Catalogues, MEDLINE, and Google Scholars. The search terms used were "colon and rectal cancer," "microbiota," "meat," and "processed or red." Articles were eligible for consideration in this literature review if they were full text, written from 1951 to the present and in English. In total, 320 journal articles were gathered according to the relevance of their title and publishing date, and approximately 175 articles were thoroughly reviewed. The relevant sources were organized through EndNote Referencing Tool (version X8.2).

Discussion

Microbial dysbiosis has been associated with colon cancer. Metabolic and metagenomic analysis studies suggest that CRC-associated samples have different species compositions compared to control (Table 1). However, inflammation and microbial dysbiosis may not be sufficient alone to promote tumorigenesis. Complex interactions between established risk factors for cancer including genetics, obesity, dietary intake, and alcohol consumption seem to alter the gut microbiota to contribute to colorectal carcinogenesis[24,25] (Fig. 1).

Individual bacterial species and colon cancer risk.

Understanding the bacterial species associated with CRC is essential for developing new, specific, and sensitive molecular tools to help in the early detection of colonic diseases.[13] Moreover, such as in genetic tumor profiles, finding abundant microorganisms in tumors might become a routine test to guide patient prognosis and management. A list of relevant bacterial species and its role in colorectal carcinogenesis are detailed hereafter.

Fusobacterium nucleatum. Genomic methods, including 16 rDNA and shotgun metagenomics analysis, suggest *Fusobacterium nucleatum* as a potential bacterium contributing to CRC pathogenesis. Kostic and colleagues were the first to show that *F. nucleatum* promotes myeloid infiltration of intestinal tumors in $Apc^{Min/+}$ mice and increases the expression of pro-inflammatory genes such as Ptgs2(COX-2), Scyb1(IL8), Il6, Tnf (TNFα), and Mmp3.[26] Subsequent studies including fluorescent *in situ* hybridization (FISH) and quantitative polymerase chain reaction further confirmed the rise of *F. nucleatum* in the colonic mucosa, tissues, and feces of patients with adenomas or adenocarcinomas compared to healthy individuals.[12,27] These results have been consistent throughout different stages of CRCs and across diverse ethnic populations.[12,27]

Table 1 Microbial dysbiosis and its association with colon cancer: The studies from 2012 onwards that looked into changes in the microbiota in different human colon cancer samples

Year/author	Reference	Location	Participants' number	Sample collected	Metadata	Summarized outcome
2019/Thomas *et al.* (cohort 1)	[13]	N/A	Control (24) Adenoma (27) CRC (29)	Fecal samples	+	Abundance of *Fusobacterium nucleatum*, *Parvimonas micra*, *Gemella morbillorum*, *Peptostreptococcus stomatis*, *Solobacterium moorei*, *Clostridium symbiosum*, *Anaerococcus vaginalis*, *Porphyromonas asaccharolytica*, *Bacteroides fragilis*, *Porphyromonas somerae*, *Anaerococcus obesiensis*, *Porphyromonas uenonis*, and *Streptococcus constellatus* in patients compared to control
2019/Thomas *et al.* (cohort 2)	[13]	N/A	Control (28) CRC (32)	Fecal samples	+	Abundance of *Fusobacterium nucleatum*, *Parvimonas micra*, *Gemella morbillorum*, *Peptostreptococcus stomatis*, *Solobacterium moorei*, *Clostridium symbiosum*, *Anaerococcus vaginalis*, *Porphyromonas asaccharolytica*, *Prevotella intermedia*, *Bacteroides fragilis*, *Porphyromonas somerae*, *Anaerococcus obesiensis*, *Porphyromonas uenonis*, *Peptostreptococcus anaerobius*, *Streptococcus constellatus*, and *Granulicatella adiacens* in patients compared to control
2019/Yachida *et al.*	[12]	Left and right colon rectum	Control (251) Adenoma (67) CRC (258)	Fecal samples	+	Abundance in early to late CRC stages: *F. nucleatum*, *Solobacterium moorei*, *Peptostreptococcus stomatis*, *Peptostreptococcus anaerobius*, *Lactobacillus sanfranciscensis*, *Parvimonas micra*, and *Gemella morbillorum* Abundance in early CRC stages: *Atopobium parvulum*, *Actinomyces odontolyticus*, *Desulfovibrio longreachensis*, *Phascolarctobacterium succinatutens*, and *Atopobium parvulum* Species newly associated with CRC: *Colinsella aerofaciens*, *Dorea longicatena*, *Porphyromonas uenonis*, *Selenomonas sputigena*, and *Streptococcus anginosus* Depleted in CRC: *Lachnospira multipara and Eubacterium eligens* Abundance in sulfide-producing bacteria: *Desulfovibrio vietnamensis*, *Desulfovibrio longreachensis*, and *Bilophila wadsworthia*
2017/Hibberd *et al.*	[11]	Mid-portion of the ascending and sigmoid colon	CRC (15)	Cancer tissues specimens/ fecal samples	+	Abundance of *Fusobacterium*, *Selenomonas*, and *Peptostreptococcus* in tumor mucosa compared to healthy muscosa in patients
2017/Yu *et al.*	[1]	N/A	Control (54) CRC (74)	Fecal samples	Limited	Abundance of *Fusobacterium nucleatum*, *Parvimonas micra*, *Gemella morbillorum*, *Peptostreptococcus*

(Continues)

Table 1. (Continued)

Year/author	Reference	Location	Participants' number	Sample collected	Metadata	Summarized outcome
						stomatis, *Solobacterium moorei*, *Clostridium symbiosum*, *Anaerococcus vaginalis*, *Porphyromonas asaccharolytica*, *Prevotella intermedia*, *Bacteroides fragilis*, *Anaerococcus obesiensis*, *Porphyromonas uenonis*, *Peptostreptococcus anaerobius*, *Streptococcus constellatus*, and *Granulicatella adiacens* in patients compared to control
2016/Baxter *et al.*	[2]	N/A	CRC (490)	Fecal samples	Limited	Abundance of *Porphyromonas assaccharolytica*, *Peptostreptococcus stomatis*, *Parvimonas micra*, and *Fusobacterium nucleatum* and lower abundance of potentially beneficial organisms, such as members of the Lachnospiraceae
2016/ Vogtmann *et al.*	[3]	Right and left colon metastasis	Control (52) CRC (52)	Fecal samples	+	Abundance of *Fusobacterium nucleatum*, *Parvimonas micra*, *Gemella morbillorum*, *Peptostreptococcus stomatis*, *Solobacterium moorei*, *Clostridium symbiosum*, *Anaerococcus vaginalis*, *Porphyromonas asaccharolytica*, *Prevotella intermedia*, *Bacteroides fragilis*, *Porphyromonas somerae*, *Anaerococcus obesiensis*, *Porphyromonas uenonis*, *Peptostreptococcus anaerobius*, *Streptococcus constellatus*, and *Granulicatella adiacens* in patients compared to control
2015/Feng *et al.*	[10]	N/A	Control (61) Adenoma (47) CRC (46)	Fecal samples	+	Abundance of Bacteroides, Alistipes, Escherichia, Parvimonas, Bilophila and *Fusobacterium* lower abundance of Ruminococcus, Bifidobacterium, and Streptococcus species in patients compared to control
2014/Zackular *et al.*	[4]	N/A	Control (30) Adenoma (30) CRC (30)	Fecal samples	+	Abundance of Ruminococcaceae, Clostridium, *Pseudomonas*, and Porphyromonadaceae Lower abundance of *Bacteroides*, Lachnospiraceae, Clostridiales, and *Clostridium* in patients compared to control
2014/Zeller *et al.*	[8]	N/A	Control (61) Adenoma (42) CRC (53)	Fecal sample	–	Abundance of Fusobacteria, Proteobacteria, and Bacteroidetes Lower abundance of Actinobacteria and Firmicutes in patients compared to control
2013/Wu *et al.*	[9]	N/A	Control (19) CRC (20)	Fecal samples	Limited	Abundance of *Fusobacteria*, *Enterococcaceae*, *Eubacteriaceae*, *Staphylococcaceae*, and *Clostridiales* in patients compared to control
2013/Weir *et al.*	[7]	Rectum, sigmoid, and ascending colon	Control (10) CRC (11)	Fecal samples	+	Abundance of Bacteroides and Prevotella in patients compared to control Abundance of *Akkermansia muciniphila* and Citrobacter farmer in patients compared to control

(Continues)

Table 1. (Continued)

Year/author	Reference	Location	Participants' number	Sample collected	Metadata	Summarized outcome
2012/ Chen *et al.*	[6]	Rectum for mucosal adherent microbial composition N/A for other colorectal carcinoma tissues	Control (56) CRC (46)	Cancer tissues/feces/ rectal swabs	Limited	Two Prevotella species were absent in patients Significant variation in microbial composition in intestinal lumen (i.e., feces) and cancerous tissue (i.e., swab) in patients Abundance of Erysipelotrichaceae, Prevotellaceae, Coriobacteriaceae, and Peptostreptococcaceae and lower abundance of Bacteroidetes and Proteobacteria in fecal samples of patients with colorectal cancers Abundance of Lactobacillales in cancerous tissue and decreased abundance of *Faecalibacterium* in patients compared to control Lower abundance of *Bifidobacterium*, *Faecalibacterium*, and *Blautia*were and an increased abundance of *Fusobacterium*, *Porphyromonas*, *Peptostreptococcus*, and *Mogibacterium* in mucosa-adherent microbiota from a rectal swab
2012/Wang *et al.*	[5]	N/A	Control (56) CRC (46)	Feces	Limited	Abundance of *Bacteroidetes Bacteroides*, *Firmicutes Roseburia*, *Bacteroidetes Alistipes*, *Firmicutes Eubacterium*, *Proteobacteria Parasuterella* patients compared to control, Lower abundance of *Bacteroidetes Porphyromonas*, *Proteobacteria Escherichia/Shigella*, *Firmicutes Enterococcus*, *Firmicutes Streptococcus*, and *Firmicutes Peptostreptococcus in patients compared to control*

Samples included cancer tissue, feces, and rectal swabs. Other than age and genetics, the relationship between microbial dysbiosis and colon cancer is affected by health-compromising factors such as weight, dietary intake, and alcohol consumption. Metadata refers to data collection from the participants on variables including weight, food and alcohol consumption, age, gender, ethnicity, and meat consumption.

Recent studies also suggest that *F. nucleatum* levels are valuable for CRC diagnosis.[28] However, Kostic *et al*. revealed that the abundance of *F. nucleatum* could be persistent in both healthy and CRC hosts suggesting that *F. nucleatum* detection alone is not robust enough as a biomarker for CRC.[26] Investigation of human colonic tissues has revealed complex mechanisms in which *F. nucleatum* promotes a pro-tumorigenic microenvironment. Yang *et al*. incubated CRC cell lines with *F. nucleatum* in mice and analyzed their microRNA (miRNA) expression patterns.[29] Results demonstrated that *F. nucleatum* upregulates inflammatory factors and microRNA21 through toll-like receptors.[29] *F. nucleatum* was also seen to affect the downstream targets of miR21, including the oncoprotein RAS P21 Protein Activator 1 (RASA1) and tumor suppressor programmed cell death protein 4 (Pdcd4).[29] Experimental evidence suggests that the aberrant expression of RASA1 in CRC leads to the activation of RAS-mitogen-activated protein kinase (MAPK) cascade.[30] Therefore, *F. nucleatum*'s upregulation of miR21 is strongly correlated with the activation of the MAPK cascade, which could, in turn, contributes to CRC development[29] (Fig. 2a,b).

Abed and colleagues have identified that polysaccharide D-galactors-b (1-3)-*N*-acetyl-D-galactosamine (Gal-GalNAc), an early biomarker for colon carcinogenesis, was overexpressed in CRC tissues compared to normal colonic tissues.[31] The same study has also noted an increased number of *F. nucleatum* binding to CRC tissues, as opposed to the normal colon. The high levels of *F. nucleatum* were related to the high expression of Gal-GalNAc, which binds to fusobacterial lectin Fap2 in CRC tissues.[31] The immunological consequences of *F. nucleatum* translocation to tumor sites remains unclear. Gur *et al*.[32] have demonstrated that Fap2 of the bacteria facilitates immune evasion and thereby promotes tumor growth and progression (Fig. 3b). This is achieved through binding *to* T-cell immunoglobulin and ITIM domain expressed on natural killer cells and T cells.[32] Further research targeting the pathological effects of the TGIT-Fap2 is essential as inhibiting this interaction has potential therapeutic implications in CRCs (Fig. 3).

Experiments by Rubinstein *et al*.[33] demonstrated a relationship between FadA component of *F. nucleatum* and E-cadherin, a cell adhesion molecule and a tumor suppressor that functions through the β-catenin signaling pathway (Fig. 3a). In the presence of *F. nucleatum*, the FadA portion interacts with E-cadherin inhibiting its tumor suppression activity and activating β-catenin signaling pathway in a similar mechanism as that of other growth factors such as endothelial growth factor and vascular endothelial growth factor. This will, in turn, result in the activation of oncogenes, Wnt genes, and inflammatory genes[33] (Fig. 4).

Recent studies support the potential role of *F. nucleatum* in mediating the association between diet and colorectal neoplasms.[34] Interestingly, foods rich in red and processed meat were associated with *F. nucleatum* positive tumors but not with *F. nucleatum* negative tumors.[34] Also, this association was stronger for proximal colon cancers compared to distal CRCs.[34] The altered levels of *F. nucleatum* and its association with CRCs across different

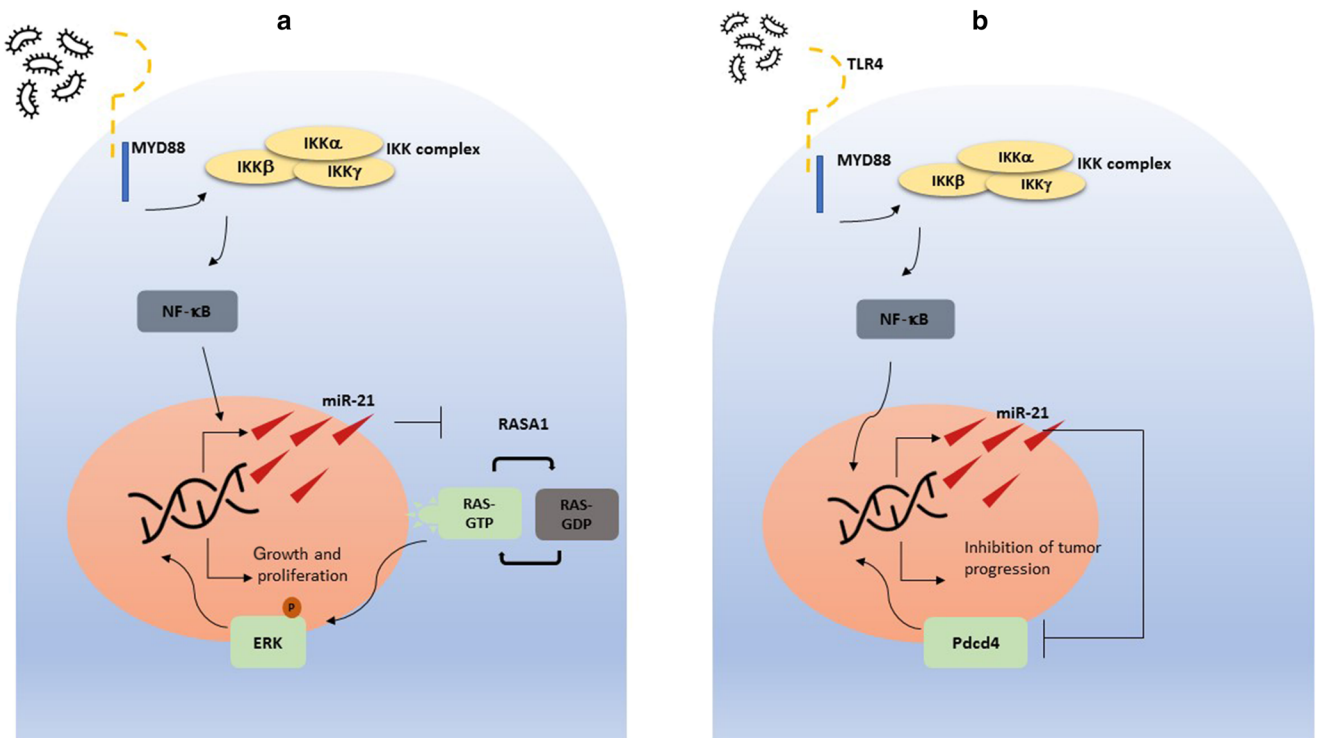

Figure 2 *Fusobacterium nucleatum* induces tumor growth and proliferation. *Fusobacterium nucleatum* activates NFκB signaling pathway through toll-like receptors (TLR). This upregulates the transcription of miR-21, which is then hypothesized to act in two different mechanisms: (a) activation of RAS which phosphorylates transcription factor ERK thereby increasing growth and proliferation and (b) inhibition of Pdcd4 which is responsible for the inhibition of tumor promotion and progression. [Color figure can be viewed at wileyonlinelibrary.com]

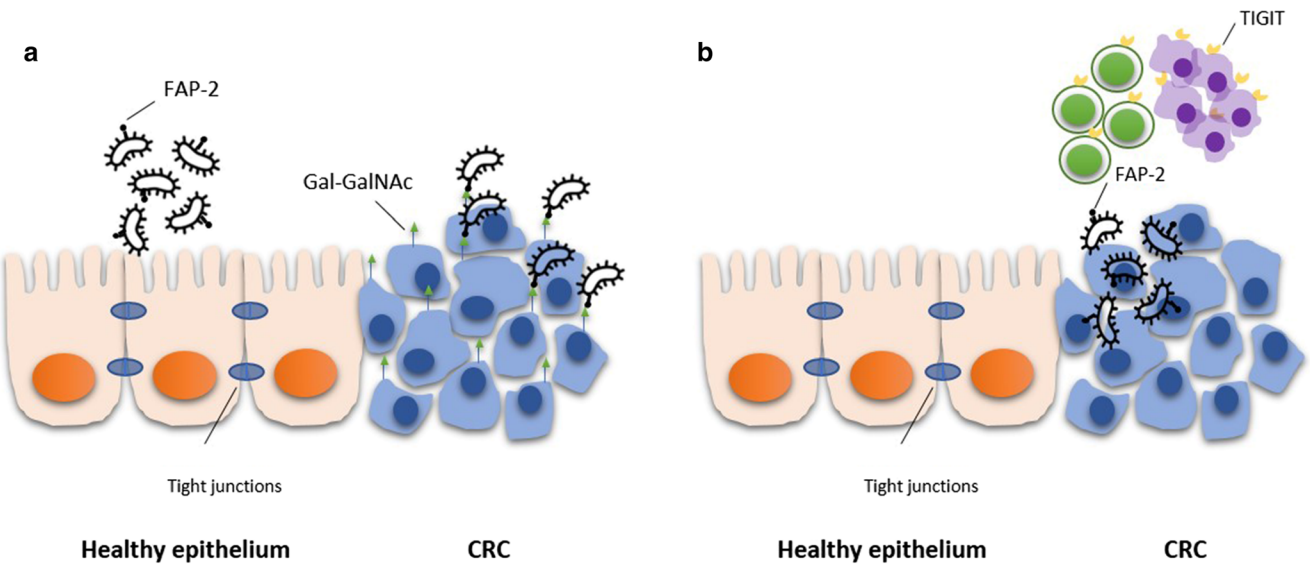

Figure 3 Tumor immune tolerance and *Fusobacterium nucleatum*. (a) Galactose and N-acetyl-D-galactosamine (Gal-GalNAc) is overexpressed on tumor cells. This increases the binding of Gal-GalNAc with *F. nucleatum* protein FAP-2. (b) the increase of *F. nucleatum* in the tumor microenvironment promotes its escape from immune cells. The binding of FAP-2 with TIGIT (T cell immunoglobulin and ITIM domain) inhibits the function of natural killer cells (purple) and T cells (green). [Color figure can be viewed at wileyonlinelibrary.com]

populations could be attributed to differences in the diet.[27] Phenotypically, *F. nucleatum* has the ability to ferment undigested proteins to produce hydrogen sulfide,[35] which could induce carcinogenesis in the colon.[36]

Streptococcus gallolyticus subsp. gallolyticus (Sgg). Since 1951, clinical studies linked the presence of *Streptococcus bovis* biotype I, renamed *S. gallolyticus subsp. gallolyticus* (*Sgg*), to colon carcinoma.[37] Recently, Kumar *et al.*[38] established the tumor-promoting role of *Sgg* that involves specific bacterial and host factors. The strong association between *Sgg* and CRC indicates that the bacterium may have specific pathogenic traits that aid in the promotion and spread of cancer.

S. bovis/gallolyticus can adhere to colon epithelial surfaces and can bind to heparin sulfate proteoglycans on malignant cells through its histone-like protein A[39] or to components of the extracellular matrix such as collagen.[40] The bacterium pili demonstrate phase-variable expression, which is an advantageous feature for its colonization around tissues and evasion of the host immune responses.[41] The pili are also highly immunogenic and hence anti-pilins IgG could potentially be an ideal serological diagnostic tool in patients with early adenomas.[42]

Upon binding to tissues with a malignant phenotype, multiple *in vitro* experiments demonstrated that *S. bovis/gallolyticus* proteins stimulate the production of inflammatory cytokines.[43] These inflammatory cytokines, in turn, mediate the production of free radicals and thereby the expression of nitric oxide enzyme (NOS2) producing nitric oxide (NO).[44,45] In the colonic epithelial cells, NO was seen to directly regulate oncogenes or tumor suppressor genes to promote its carcinogenic properties.[46] The NO, produced by *S. bovis/gallolyticus*, can activate NFκB, an inflammation-induced carcinogenesis transcription factor. This mediates angiogenesis through activation of vascular endothelial growth factors, and cellular proliferation through COX-2 production and c-Myc and cyclin-D activation[46] (Fig. 5).

Studies have also revealed that the production of cell proliferation markers following the bacteria colonization increases the risk of colonic adenomas by 50%, which provides further evidence on the role of *Sgg* "as promoter/propagator of colorectal carcinoma rather than just a consequence of the colorectal tumours."[43] Aymeric *et al.*[47] have suggested that the bacterium benefits from tumor metabolites and can kill closely related enterococci commensals through an SGG-specific bacteriocin, gallocin, thereby allowing a better colonization niche. Gallocin activity is improved in the presence of secondary bile acids, an established risk factor for CRC.[47] It was also shown that the presence of premalignant conditions and APC gene mutation enhances *Sgg* colonization in a gallocin-dependent manner.[47] In addition, the Wnt pathway activation, one of the earliest signaling alternation in CRC, decreases the expression of bile acid apical transporter gene thereby establishing a new link between Wnt pathway activation and high levels of secondary bile acid.[47] Furthermore, *Sgg* colonization, accompanied by APC mutation and increased carcinogenic secondary bile acids, could potentially be part of a tumorigenesis triangle.[42]

Recent studies have indicated that *Sgg* is a passenger and cancer-promoting bacterium and may thus act as a marker for CRC screening.[42] A most recent study by Thomas *et al.*[13] have further confirmed *S. gallolyticus* as a potential marker for CRC. However, because it requires premalignant conditions for colonization, *Sgg* might not induce carcinogenesis but rather accelerates its pathogenic progression.[42] Also, the evidence of the bacteria in the transformation of aberrant crypts to adenoma and cancer could aid in the early detection of colorectal lesions and thereby act as a potential target in colon carcinogenesis.[42,47]

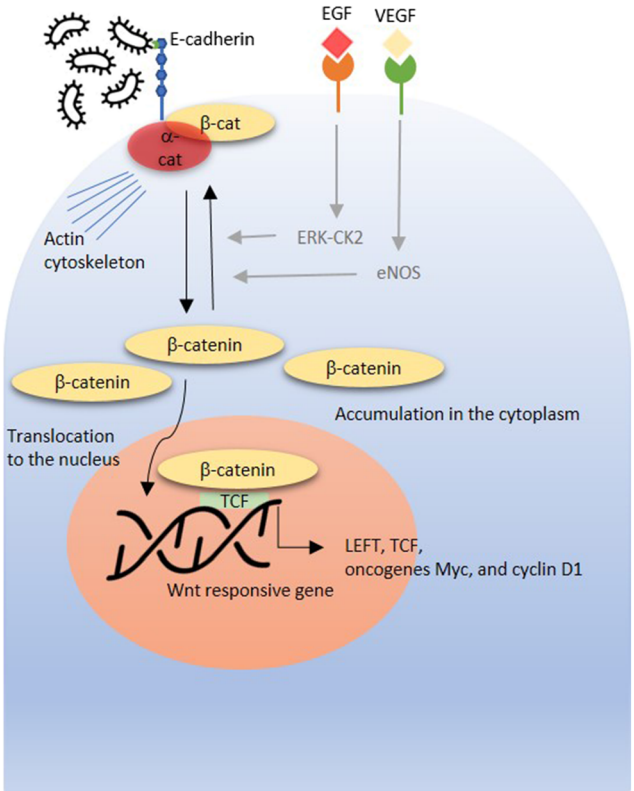

Figure 4 *Fusobacterium nucleatum* mediates the expression of oncogenes. The interaction between the FadA component of *F. nucleatum* and E-cadherin stimulates a carcinogenic pathway that is often induced via endothelial growth factors (EGF) and vascular endothelial growth factors (VEGF). This pathway induces the dissociation of β-catenin from α-catenin and translocates it into the nucleus for β-catenin-regulated transcription. This results in the activation of oncogenes, Wnt genes, and inflammatory genes, a phenotype that is consistent with adenomatous polyposis coli (APC) mutation. [Color figure can be viewed at wileyonlinelibrary.com]

Escherichia coli. Certain *Escherichia coli* strains including B2 genotoxic *E. coli*, and enteropathogenic *E. coli*, or tightly adherent *E. coli* frequently colonize the colorectal mucosa.[48] During carcinogenesis, the colonic epithelial changes mainly the loss of tight junctions increase the internalization of *E. coli* to the tumor tissues.[49] Inflammatory mediators, including interferon and tumor necrosis factor, in the carcinogenic microenvironment, upregulate the expression of carcinoembryonic antigen-related cell adhesion molecule 6.[49] Upregulation of carcinoembryonic antigen-related cell adhesion molecule 6 could lead to the adhesion, invasion, and multiplication of *E. coli* type 1 pili in the colonic epithelium,[49] which further promotes tumor survival.

Arthur *et al.*[14] provided the first evidence of the prevalence of *E. coli* strains of B2 phylotype in CRCs compared to healthy individuals. In the B2 phylogroup, the expression of polyketide synthase (pks) island containing genotoxin colibactin peptide is thought to induce double-strand breaks in DNA.[48] The defined genotoxic factors such as cytolethal distending toxin or undefined genotoxic factors[49] can also modulate the enterocyte transformation and induce inflammatory reactions in the intestinal epithelium.[50] An alternative hypothesis suggests that rather than a carcinogen, colibactin is a bacteriocin that kills commensals, which gives the phylotype a selective advantage.[51]

Bacteroides fragilis. The adherence of enterotoxigenic *Bacteroides fragilis* (ETBF) to colonic epithelial cells and its impact on carcinogenesis are yet to be proven. ETBF can cause direct DNA damage and modulate gene expression through multiple gene signaling pathways. Wu *et al.*[52] demonstrated that mice heterozygous for the APC gene (*Apc* $^{Min/+}$) experience increased adenocarcinoma growth rate and distribution when incubated with ETBF. This is due to the selective TH17 response, which directly contributes to ETBF-induced tumorigenesis.[52] The colonization of the colonic epithelial cells with ETBF as well as the expression of *B. fragilis* toxin (BFT) have led to signal transduction that cleaves E-cadherin.[53] This results in the release of β-catenin, which activates tyrosine kinases, MAPKS, and NFκB.[54] Because BFT mucosal exposure activates Wnt/β-catenin and NFκB signaling pathways and promotes the expression of proto-oncogenes including MYC, *B. fragilis* might act as a single most important risk factor for CRCs.[54] Serological detection of anti-BFT antibodies might be useful in detecting the rise of ETBF levels and thereby detecting subclinical colonic damages early on.

The role of microbial communities in colorectal carcinoma. Even though each of the microorganisms mentioned earlier plays a mechanistic role in certain CRC cases, none of them is present in every patient with colorectal carcinomas. The gut microbiota is quite complicated, and hence, it is potentially simplistic to consider one species solely responsible for CRC without considering the interplay among other microbial species and between the microbe and the host.

Multiple studies investigated the potential of gut metagenomic and metabolomic parameters as diagnostic markers for CRCs. Detection of single biomarkers such as fadA, bft, and the pks island in sequenced fecal metagenomes varied broadly with respect to abundance, significance, and cross-study consistency.[55] Also, combination models that considered species, genes, and metabolites all together for CRC detection outperformed models that considered each factor independently.[12] The progression of CRC is reported to be linked with the presence of cancer-associated microorganisms and the microbial communities.[56–58] Wong *et al.*[56] showed that fecal microbiota from patients with CRC promotes tumorigenesis in germ-free and conventional mice. Other studies demonstrated the harboring of microbial communities in the colon in biofilm-like structures.[57,58] These biofilm structures were seen to induce CRC in murine models regardless as to whether the biofilms were from healthy individuals or patients with CRCs.[58] Hence, it is postulated that predicting CRC from a single microorganism, metagenomic, or metabolic dataset results in reduced accuracy.

The possibility of sequential microbial exposure in promoting CRC is still unclear. *S. gallolyticus* has been proposed to promote carcinogenesis via adhesion and colonization around tumorous tissues by the members of *Fusobacterium* spp.[59] A recent study by Dejea *et al.*[57] showed that mice colonized with colibactin *E. coli* and enterotoxigenic *B. fragilis* demonstrated a rise in interleukin-17 in the colon and DNA damage with faster tumor

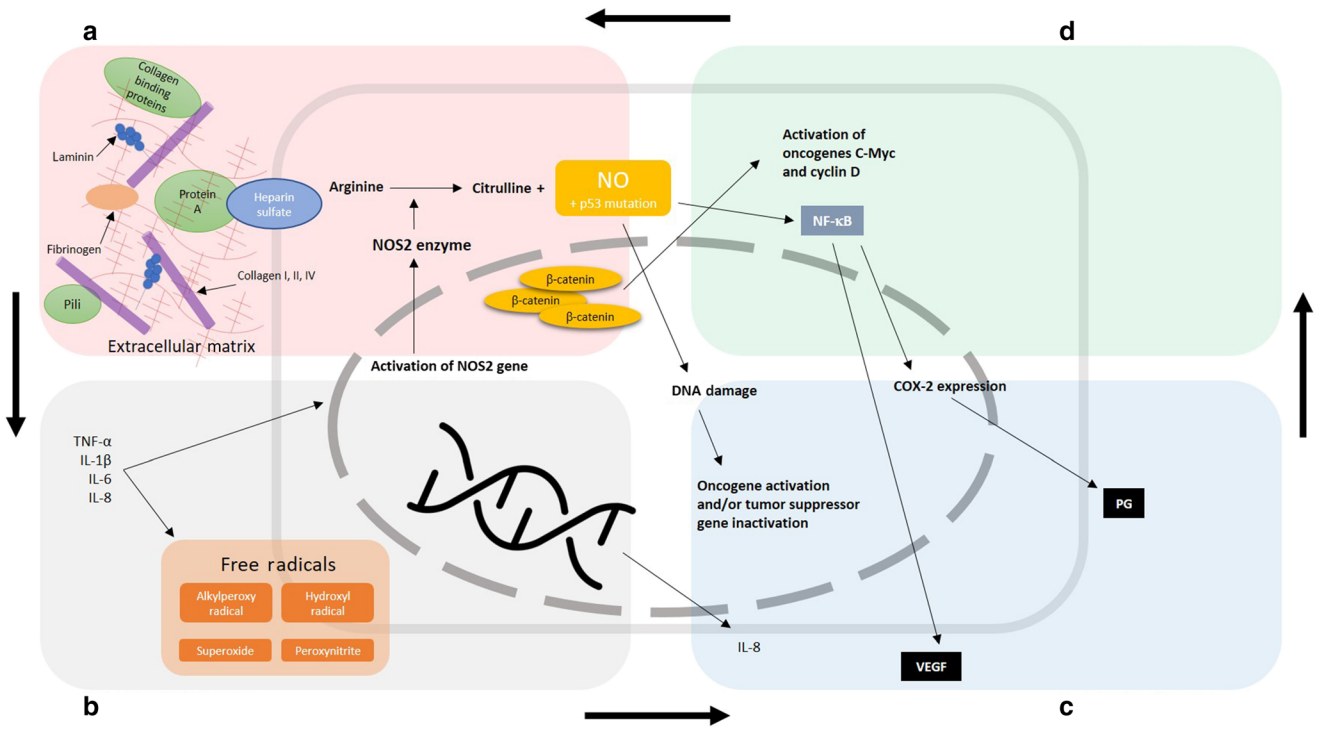

Figure 5 *Streptococcus bovis/gallolyticus* adheres to the epithelium, initiates inflammation, and promotes angiogenesis and proliferation. The role of *Streptococcus bovis/gallolytics* in promoting different stages of carcinogenesis is demonstrated. (a) *Streptococcus bovis/gallolyticus*, through a histone-like protein A and collagen-binding proteins, can bind respectively to heparin sulfate proteoglycans on malignant cells and collagens I, II, III, and IV in the extracellular matrix. (b) The adherence of the bacterium to malignant cells stimulates the production of inflammatory cytokines, which induce carcinogenic free radical damage and the expression of inducible nitric oxide enzyme (NOS2) coded by NOS2 genes. (c) The activation of NOS enzymes promotes nitric oxide (NO) production thereby inducing oncogene activation and tumor suppressor gene inactivation. NO also facilitates the production of vascular endothelial growth factors through the activation of NF-B. (d) NO, bacterial proteins, and inflammatory mediators induce COX-2 mediated prostaglandins production, which mediates cellular proliferation. The bacterium also increases *β*-catenin levels which induce cellular proliferation through c-Myc and cyclin-D activation. [Color figure can be viewed at wileyonlinelibrary.com]

progression and higher mortality compared to mice colonized with either strain alone. The co-abundance of *F. nucleatum* and *Solobacterium moori* in intramucosal carcinoma might also indicate contributions of both species to initiate events of CRC.[12]

Furthermore, the metagenomic and metabolic analysis revealed disease-specific shifts in gut microbial composition, gene abundance, and metabolites of gut microbiota that are distinct to each CRC stage.[12,13,55] These were reproducible across different cohorts and can overcome technical and geographical study differences.[55] This strongly supports the potential of polymicrobial CRC screenings and diagnostics that are sufficiently robust, sensitive, and cost-effective for pre-colonoscopy screening tests.[55] Prospective studies on the effectiveness of these biomarkers in identifying individuals at elevated risk of CRC are needed.

Diet, microbiota, and colon cancer. A diet rich in red and processed meat has been linked to colorectal carcinogenesis.[22] The intricate metabolic and inflammatory mechanisms underlying the diet-cancer association remains unclear. However, it is postulated that dietary components or their microbial metabolism are strong determinants of gut microbiota function and composition. Microbial dysbiosis can induce changes in host gene expression and inflammatory responses, thereby promoting an oncogenic microenvironment. In this section, we review the mechanistic evidence regarding the associations between microbes, red/processed meat, and colon cancers. The major carcinogenic factors associated with red and processed meat consumption include heme compounds, heterocyclic amines, NOCs, and undigested proteins (Fig. 6). Apart from their direct carcinogenic effect, these molecules can modify gut microbiota and consequently affect gene expressions and colorectal epithelial cell homeostasis to favor CRC development.[17–20]

We reviewed a large body of research that attempts to establish a link between diet, microbes, and CRC. This section will discuss various carcinogenic molecules from red/processed meat and their potential interaction with microorganisms in the colorectum.

Heterocyclic amines. The heterocyclic aromatic amines (HCAs) are considered as a potential carcinogen and are generated from amino acids in red meat under high temperatures.[60] Studies demonstrated that β-Glucuronidase bacteria could release mutagenic intermediates from HCA.[17,61] The dietary carcinogen 2-amino-3-methyl-3*H*-imidazo[4,5-f]quinoline (IQ), one of the most

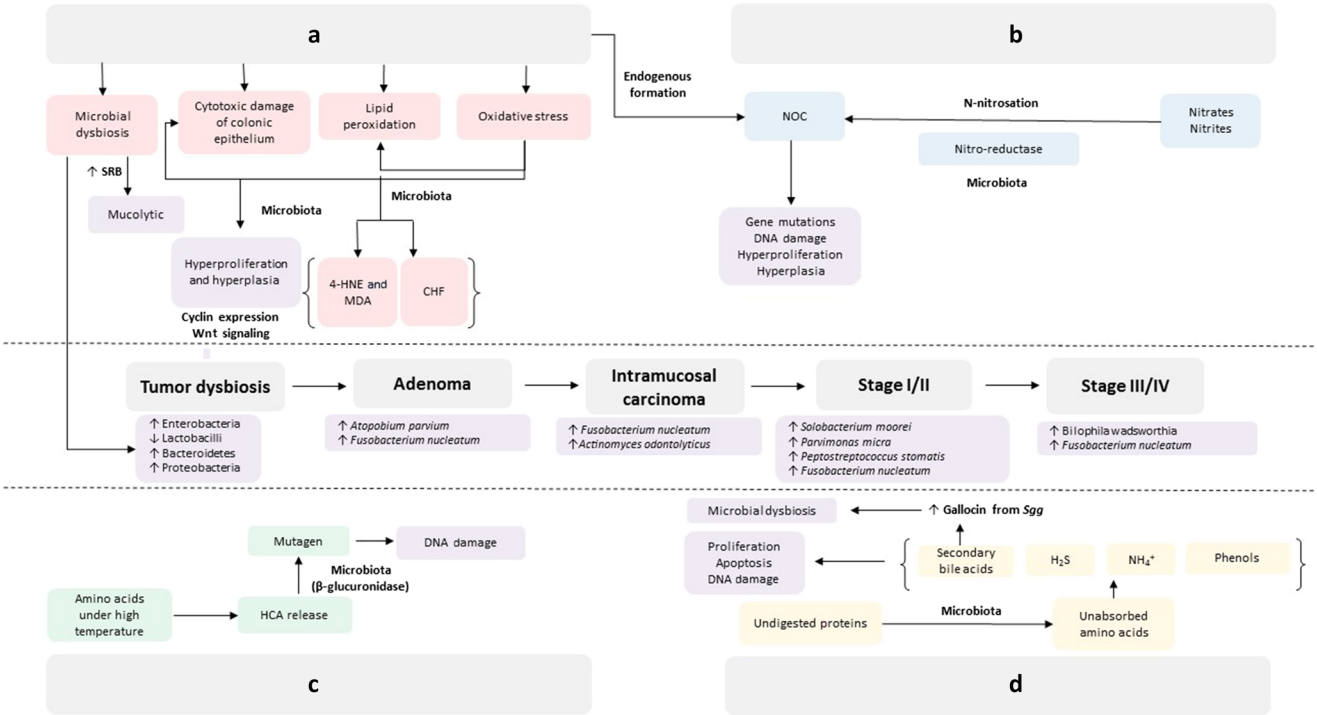

Figure 6 Microbiota, red/processed meat, and colon cancer. The microbial composition differs in relationship to colorectal cancer progression. Processed/red meat is seen to mediate CRC progression. (a) Heme promotes carcinogenesis through inducing epithelial damage and reducing cell death through pentraxin downregulation. This leads to compensatory hyperproliferation which is mediated by the microbiota. Heme induces lipid peroxidation which produces a malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), carcinogenic aldehydes. Heme also promotes microbial dysbiosis and increases sulfide-producing bacteria (SPB) which damages the mucosa thereby exposing the epithelium to carcinogens. Lastly, heme promotes the endogenous formation of N-nitroso compounds (NOCs). (b) NOCs are also found in food containing nitrates. The microbiota increases the activity of nitroreductase, which converts nitrates to NOC, a carcinogen. (c) Heterocyclic amines (HCA) are generated from amino acids in meat under high temperatures. β-Glucuronidase bacteria can release mutagenic intermediates from HCA. (d) Undigested proteins: bacterial fermentation products of red meat proteins such as hydrogen sulfide, ammonia, secondary bile acids, and phenolic compounds seem to increase CRC risk. Secondary bile acids increase the production of gallocin, a bactericidin (produced by *Sgg*) which promotes dysbiosis. [Color figure can be viewed at wileyonlinelibrary.com]

widespread HCAs in the human diet, causes direct DNA damage upon activation by gut microsomal enzymes.[61] Also, incubation of human feces with heterocyclic amine IQ under anaerobic conditions leads to the formation of 2-amino-3,6-dihydro-3-methyl-7*H*-imidazo(4,5)-fquinoline-7-one (HOIQ).[62] HOIQ is a direct-acting genotoxin even without microsomal activation as opposed to IQ. Though there is limited evidence, Humblot *et al.*[63] have identified *E. coli* as one of the species with such genotoxic potential. These findings suggest that there might be a potential role for certain microbial species in mediating the association between red meat consumption and CRC progression.

Heme. Extensive research has been conducted to determine a causal effect between heme in red meat and colon cancer. Firstly, heme was noted to increase the risk of colon cancer by inducing epithelial damage and reducing cell death through downregulating pentraxin, a protein which inhibits apoptosis.[64,65] The damage to the epithelial surface leads to compensatory hyperproliferation of epithelial cells in the intestinal crypts and increases CRC risk.[66] The hyperproliferation effect was only evident in mice models, whereby the microbiota was preserved.[64] Also, the absence of microbiota, through antibiotic admission, was seen to stop the differential expression of oncogenes, tumor suppressors, and cell turnover genes thereby preventing the effect of heme toxicity on the epithelium.[64] Besides, heme increases reactive oxygen species production, which induces cytotoxicity, lipid peroxidation, and redox modification of lipids and proteins.[64,66] All these factors are previously reported to be linked with colorectal carcinogenesis.[64,66]

The heme iron-induced lipid peroxidation can catalyzes the formation of aldehydes including malondialdehyde and 4-hydroxynonenal.[18] Previous studies have detected these molecules in fecal samples as thiobarbituric acid reactive substances.[67] Malondialdehyde binds to DNA to form mutagenic adducts whereas 4-hydroxynonenal promotes apoptosis of healthy cells.[18] In a study by Martin *et al.*,[67] the rats were given diets rich in heme and resulted in increased fecal thiobarbituric acid reactive substances. This was later suppressed by antibiotic treatment. Hence, the intestinal microbiota is involved in the enhancement of lipoperoxidation by heme iron. Lipid peroxides can also react with heme to form cytotoxic heme factor.[68] This reaction is only present in the colon, suggesting the role of microbiota in promoting cytotoxicity in the colonic epithelium.[68]

Several studies have confirmed the association between dietary heme and microbial dysbiosis.[69] Schepens *et al.*[69] have shown that dietary heme (0.5 μmol/g diet) promotes the growth of Gram-negative enterobacteria in rats and have decreased the growth of Gram-positive lactobacilli in fecal samples, thereby increasing CRC risk. The number of fecal enterobacteria including *E. coli* was also noted to be raised by 10-folds in heme-fed rats compared to the controls.[69] In another study, Bacteroidetes including *B. fragilis* and Proteobacteria including *E. coli* were overexpressed in heme fed rodents and facilitated heme-induced hyperproliferation and hyperplasia.[70] These microbiota changes contribute to a greater risk of carcinogenesis as discussed in the previous sections. Furthermore, heme-based diets could increase the levels of sulfide-producing mucin degrading microbiota such as *Akkermansia muciniphila*.[64] This can in turn damage the colonic mucus layer and can promote cytotoxic heme factor induced epithelial degradation in the colon.[64]

N-nitroso compounds. N-nitroso compounds (NOC) are found in food containing nitrites and nitrates as well as food that has been exposed to nitrogen oxide.[20] NOCs can act as DNA alkylating agents and can induce gene mutations, which are responsible for cell proliferation and differentiation.[20] In addition, heme derived from red meat can promote the endogenous formation of NOCs and promote carcinogenesis[71] Therefore, it has been hypothesized that a diet with high levels of NOCs increases the risk of CRCs.[20] The nitro groups, from the diet, can be reduced to an aromatic amine in the large intestine, a reaction knowns as N-nitrosation.[20] The activity of nitroreductases was found to be highly dependent on the colonic microbiota, which is influenced by the diet.[20] Diets rich in meat and low in fiber were seen to increase the nitroreductase activity of intestinal bacteria,[72] whereas probiotic bacteria including *Lactobacillus casei* and *Lactobacillus acidophilus* reduce the nitroreductase activity in the intestine and thereby lessen the risk of CRC.[73]

Protein. Fermentation products of red meat proteins such as hydrogen sulfide, ammonia, secondary bile acids, and phenolic compounds seem to increase CRC risk.[36] Heme itself was seen to promote the production of these bacterial fermentation products.[74] Like heme, hydrogen sulfide has genotoxic and mucolytic properties and can damage the epithelium, induce chronic inflammation, and influence epithelial proliferation/differentiation.[36] Sulfide-reducing bacteria (SRB) can produce hydrogen sulfide through fermentation of sulfur-containing amino acids from abundant proteins in the red meat.[74] Even though proteins are digested in the small intestine, substantial endogenous, and exogenous nitrogenous compounds reach the large intestine whereby they mix with the microbiota, undergo proteolysis, and release amino acids.[74] These amino acids are then fermented by colonic bacteria to release hydrogen sulfide. This fermentation process is achieved by Bacteroides, which are significantly increased by dietary heme, as well as Clostridium, Streptococcus, Propionibacterium, and Bifidobacterium.[74] Notably, other SRB such as *Atopobium parvulum* and *Actinomycosis odontolyticus* showed significant association with multiple polypoid adenomas and/or intramucosal carcinomas in the colorectum.[12] Metagenomic analyses also demonstrated a co-abundance of these two species in early stages of CRCs and a strong correlation of *A. parvulum* with Streptococcus spp.[12] *A. parvulum* has been shown to create a network of SRB bacteria through its relationships with Streptococcus.[12] Dissimilatory sulfate reductase subunit A (dsrA), which is responsible for the production of genotoxic hydrogen sulfide, was reported to be present in the later stages of CRC,[12] while *Fusobacterium* spp., an SRB, found to be consistently increased across all CRC stages. Increases in H_2S-producing bacteria even in the early stage of CRC suggests the interplay between microbiota, diet, and CRC.

Fermentation products, other than hydrogen sulfide, include ammonia and phenolic compounds, and toxic substances that promote DNA damage and affect the homeostasis and renewal of colonic epithelial cells.[75] After a week of supplementation with protein-rich foods, excessive ammonia alters cell viability and nucleic acid synthesis and promote cancer cell growth *in vitro*.[75] Another study by Davila *et al.*[74] have shown that phenol, the end product of tyrosine and phenylalanine degradation, reacts with the nitrites to produce p-diazoquinone, a chemical compound that possesses strong mutagenic activity *in vitro*.

Secondary bile acids are another meat-dependent microbiome factor that can contribute to carcinogenesis. Deoxycholate, a secondary bile acid, is elevated in multiple polypoid adenomas, which could correlate to the rise of *Bilophila wadsworthia* in early CRC stages.[12] *B. wadsworthia*, alongside other intestinal microbiota, is known to contribute to the conversion of primary to secondary bile acids. One of the products of this pathway, deoxycholate, has the potential to induce DNA damage and mutations. Recently, Yachida *et al.*[12] have demonstrated that *B. wadsworthia* is positively correlated with the intake of dietary protein and meat. Furthermore, carcinoma-enriched bacteria that produce short-chain fatty acids through amino acid fermentation, and/or bacteria that metabolize bile acids, showed a positive correlation with the consumption of red meat.[10] The elevated production of secondary bile acids and short-chain fatty acids from CRC metagenomes suggest a metabolic link between cancer-associated gut microbes and fat-rich and meat-rich diet.

Challenges. While a large body of research points towards individual microbes as the protagonist for CRC, there is limited evidence on the absolute quantification and its relationship with colon cancer progression. Also, the *in vivo* confirmation of the pathogenic synergy between individual microbes and colorectal carcinogenesis is not well investigated. Relying on mucosal samples, rather than fecal, may provide a more inclusive assessment of microbiota changes: bacterial adherence to the epithelium and biofilm formation.[11] The use of different samples (fecal and tissue) across the studies might have limited the reproducibility of the results, which may explain the inconsistency among certain studies (Table 1). Host genetic polymorphism, diet, and environmental factors are also contributing to the major sources of discrepancies when comparing microbial profiles.[10] Ensuring a consistent age group and collecting metadata on host parameters, including family and medical history, tumor microenvironment, and medication history, have accounted for confounding factors in multiple studies.

Conclusion

This study attempts to summarize the literature findings specific to the association of relevant microorganisms, microbial dysbiosis, and the consumption of red/processed meat with CRC. The utilization of different omic observational approaches to compare human colon cancer mucosa and fecal samples with that of healthy individuals are more suited to translate microbial changes in CRC. Specific microorganisms including *F. nucleatum*, *S. bovis/gallolyticus*, *E. coli*, and *B. fragilis* were abundant in the colonic mucosa and feces of patients with adenoma or adenocarcinoma. *F. nucleatum* can upregulate the production of inflammatory factors and activate signaling pathways that inhibit tumor suppressor genes in the colorectum. The Fap2 component of the bacterium also facilitates immune evasion and tumor growth. Multiple research suggested that *S. bovis/gallolyticus* can adhere to epithelial surfaces, induce free radical damage, directly regulate oncogenes or tumor suppressor genes, and mediate cellular proliferation in the colorectum. Similar effects were seen by *E. coli* and *B. fragilis*. Despite extensive research on the specific microorganism, the possibility of sequential microbial exposure in promoting CRC remains unclear. Red and processed meat were found to have multiple carcinogenic molecules, which are inducible by these microorganisms. Deducing a strong association with regard to specific species, microbial communities, or ingested red/processed meat is all potential approaches for improving the cancer risk and providing target points for diagnosis, treatment, and therapy. Analysis of the microbiome is also critical in understanding how these interactions influence the development of colon cancer.

References

1 Yu J, Feng Q, Wong SH *et al*. Metagenomic analysis of faecal microbiome as a tool towards targeted non-invasive biomarkers for colorectal cancer. *Gut* 2017; **66**: 70–8.
2 Baxter NT, Ruffin MT, Rogers MA, Schloss PD. Microbiota-based model improves the sensitivity of fecal immunochemical test for detecting colonic lesions. *Genome Med* 2016; **8**: 37.
3 Vogtmann E, Hua X, Zeller G *et al*. Colorectal cancer and the human gut microbiome: reproducibility with whole-genome shotgun sequencing. *PLoS ONE* 2016; **11**: e0155362.
4 Zackular JP, Rogers MA, Ruffin MT, Schloss PD. The human gut microbiome as a screening tool for colorectal cancer. *Cancer Prev Res (Phila)* 2014; **7**: 1112–21.
5 Wang T, Cai G, Qiu Y *et al*. Structural segregation of gut microbiota between colorectal cancer patients and healthy volunteers. *ISME J* 2012; **6**: 320–9.
6 Moschetta A, Chen W, Liu F, Ling Z, Tong X, Xiang C. Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. *PLoS ONE* 2012; **7**: e39743.
7 Weir TL, Manter DK, Sheflin AM, Barnett BA, Heuberger AL, Ryan EP. Stool microbiome and metabolome differences between colorectal cancer patients and healthy adults. *PLoS ONE* 2013; **8**: e70803.
8 Zeller G, Tap J, Voigt AY *et al*. Potential of fecal microbiota for early-stage detection of colorectal cancer. *Mol Syst Biol* 2014; **10**: 766.
9 Wu N, Yang X, Zhang R *et al*. Dysbiosis signature of fecal microbiota in colorectal cancer patients. *Microb Ecol* 2013; **66**: 462–70.
10 Feng Q, Liang S, Jia H *et al*. Gut microbiome development along the colorectal adenoma-carcinoma sequence. *Nat Commun* 2015; **6**: 6528.
11 Hibberd AA, Lyra A, Ouwehand AC *et al*. Intestinal microbiota is altered in patients with colon cancer and modified by probiotic intervention. *BMJ Open Gastroenterol* 2017; **4**: e000145.
12 Yachida S, Mizutani S, Shiroma H *et al*. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat Med* 2019; **25**: 968–76.
13 Thomas AM, Manghi P, Asnicar F *et al*. Metagenomic analysis of colorectal cancer datasets identifies cross-cohort microbial diagnostic signatures and a link with choline degradation. *Nat Med* 2019; **25**: 667–78.
14 Arthur JC, Jobin C. The struggle within: microbial influences on colorectal cancer. *Inflamm Bowel Dis* 2011; **17**: 396–409.
15 Gagniere J, Raisch J, Veziant J *et al*. Gut microbiota imbalance and colorectal cancer. *World J Gastroenterol* 2016; **22**: 501–18.
16 Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008; **134**: 577–94.
17 Zhang J, Lacroix C, Wortmann E *et al*. Gut microbial beta-glucuronidase and glycerol/diol dehydratase activity contribute to dietary heterocyclic amine biotransformation. *BMC Microbiol* 2019; **19** () (no pagination: 99.
18 Corpet DE. Red meat and colon cancer: should we become vegetarians, or can we make meat safer? *Meat Sci* 2011; **89**: 310–6.
19 IJssennagger N, Derrien M, van Doorn GM *et al*. Dietary heme alters microbiota and mucosa of mouse colon without functional changes in host-microbe cross-talk. *PLoS ONE* 2012; **7** (no pagination: e49868.
20 Bingham SA, Hughes R, Cross AJ. Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. *J Nutr* 2002; **132**: 3522S–5S.
21 Norat T, Lukanova A, Ferrari P, Riboli E. Meat consumption and colorectal cancer risk: dose-response meta-analysis of epidemiological studies. *Int J Cancer* 2002; **98**: 241–56.
22 Bouvard V, Loomis D, Guyton KZ *et al*. Carcinogenicity of consumption of red and processed meat. *Lancet Oncol* 2015; **16**: 1599–600.
23 Sobhani I, Tap J, Roudot-Thoraval F *et al*. Microbial dysbiosis in colorectal cancer (CRC) patients. *PLoS ONE* 2011; **6**: e16393.
24 Saus E, Iraola-Guzman S, Willis JR, Brunet-Vega A, Gabaldon T. Microbiome and colorectal cancer: roles in carcinogenesis and clinical potential. *Mol Aspects Med* 2019; **69**: 93–106.
25 Johnson CM, Wei C, Ensor JE *et al*. Meta-analyses of colorectal cancer risk factors. *Cancer Causes Control* 2013; **24**: 1207–22.
26 Kostic AD, Chun E, Robertson L *et al*. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* 2013; **14**: 207–15.
27 Kostic AD, Gevers D, Pedamallu CS *et al*. Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. *Genome Res* 2012; **22**: 292–8.
28 Peng B-J, Cao C-Y, Li W *et al*. Diagnostic performance of intestinal *Fusobacterium nucleatum* in colorectal cancer. *Chin Med J (Engl)* 2018; **131**: 1349–56.
29 Yang Y, Weng W, Peng J *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-kappaB, and up-regulating expression of microRNA-21. *Gastroenterology* 2017; **152**: 851–66 e24.
30 Kent OA, Mendell JT, Rottapel R. Transcriptional regulation of miR-31 by oncogenic KRAS mediates metastatic phenotypes by repressing RASA1. *Mol Cancer Res* 2016; **14**: 267–77.
31 Abed J, Emgard JE, Zamir G *et al*. Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. *Cell Host Microbe* 2016; **20**: 215–25.
32 Gur C, Ibrahim Y, Isaacson B *et al*. Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* 2015; **42**: 344–55.
33 Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by

modulating E-cadherin/beta-catenin signaling via its FadA adhesin. *Cell Host Microbe* 2013; **14**: 195–206.
34 Liu L, Tabung FK, Zhang X *et al*. Diets that promote colon inflammation associate with risk of colorectal carcinomas that contain *Fusobacterium nucleatum*. *Clin Gastroenterol Hepatol* 2018; **16**: 1622–31 e3.
35 Basic A, Blomqvist M, Dahlen G, Svensater G. The proteins of *Fusobacterium* spp. involved in hydrogen sulfide production from L-cysteine. *BMC Microbiol* 2017; **17**: 61.
36 Deplancke B, Gaskins HR. Hydrogen sulfide induces serum-independent cell cycle entry in nontransformed rat intestinal epithelial cells. *FASEB J* 2003; **17**: 1310–2.
37 Mc CW, Mason JM 3rd. Enterococcal endocarditis associated with carcinoma of the sigmoid: report of a case. *J Med Assoc State Ala* 1951; **21**: 162–6.
38 Kumar R, Herold JL, Schady D *et al*. *Streptococcus gallolyticus subsp gallolyticus* promotes colorectal tumor development. *PLoS Pathog* 2017; **13**.
39 Boleij A, Schaeps RM, de Kleijn S *et al*. Surface-exposed histone-like protein a modulates adherence of *Streptococcus gallolyticus* to colon adenocarcinoma cells. *Infect Immun* 2009; **77**: 5519–27.
40 Sillanpaa J, Nallapareddy SR, Qin X *et al*. A collagen-binding adhesin, Acb, and ten other putative MSCRAMM and pilus family proteins of *Streptococcus gallolyticus subsp. gallolyticus* (*Streptococcus bovis* group, biotype I). *J Bacteriol* 2009; **191**: 6643–53.
41 Wessels MR, Danne C, Dubrac S, Trieu-Cuot P, Dramsi S. Single cell stochastic regulation of pilus phase variation by an attenuation-like mechanism. *PLoS Pathog* 2014; **10**: e1003860.
42 Pasquereau-Kotula E, Martins M, Aymeric L, Dramsi S. Significance of *Streptococcus gallolyticus subsp. gallolyticus* association with colorectal cancer. *Front Microbiol* 2018; **9**: 614.
43 Ellmerich S, Djouder N, Scholler M, Klein JP. Production of cytokines by monocytes, epithelial and endothelial cells activated by *Streptococcus bovis*. *Cytokine* 2000; **12**: 26–31.
44 Janeway CATP. *Immunobiology: The Immune System in Health and Disease*. New York And London: Garland Publishing Inc, 1997.
45 Kanwar JR. Recent advances on the roles of NO in cancer and chronic inflammatory disorders. *Curr Med Chem* 2009; **16**: 2373–94.
46 Schetter AJ, Heegaard NH, Harris CC. Inflammation and cancer: interweaving microRNA, free radical, cytokine and p53 pathways. *Carcinogenesis* 2010; **31**: 37–49.
47 Aymeric L, Donnadieu F, Mulet C *et al*. Colorectal cancer specific conditions promote *Streptococcus gallolyticus* gut colonization. *Proc Natl Acad Sci* 2018; **115**: E283–91.
48 Cuevas-Ramos G, Petit CR, Marcq I, Boury M, Oswald E, Nougayrede JP. *Escherichia coli* induces DNA damage in vivo and triggers genomic instability in mammalian cells. *Proc Natl Acad Sci U S A* 2010; **107**: 11537–42.
49 Buc E, Dubois D, Sauvanet P *et al*. High prevalence of mucosa-associated *E. coli* producing cyclomodulin and genotoxin in colon cancer. *PLoS ONE* 2013; **8**: e56964.
50 Swidsinski A, Khilkin M, Kerjaschki D *et al*. Association between intraepithelial *Escherichia coli* and colorectal cancer. *Gastroenterology* 1998; **115**: 281–6.
51 Vizcaino MI, Engel P, Trautman E, Crawford JM. Comparative metabolomics and structural characterizations illuminate colibactin pathway-dependent small molecules. *J Am Chem Soc* 2014; **136**: 9244–7.
52 Wu S, Rhee KJ, Albesiano E *et al*. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat Med* 2009; **15**: 1016–22.
53 Wu S, Lim KC, Huang J, Saidi RF, Sears CL. *Bacteroides fragilis* enterotoxin cleaves the zonula adherens protein, E-cadherin. *Proc Natl Acad Sci U S A* 1998; **95**: 14979–84.
54 Kim JM, Jung HY, Lee JY, Youn J, Lee CH, Kim KH. Mitogen-activated protein kinase and activator protein-1 dependent signals are essential for *Bacteroides fragilis* enterotoxin-induced enteritis. *Eur J Immunol* 2005; **35**: 2648–57.
55 Wirbel J, Pyl PT, Kartal E *et al*. Meta-analysis of fecal metagenomes reveals global microbial signatures that are specific for colorectal cancer. *Nat Med* 2019; **25**: 679–89.
56 Wong SH, Zhao L, Zhang X *et al*. Gavage of fecal samples from patients with colorectal cancer promotes intestinal carcinogenesis in germ-free and conventional mice. *Gastroenterology* 2017; **153**: 1621–33 e6.
57 Dejea CM, Fathi P, Craig JM *et al*. Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science* 2018; **359**: 592–7.
58 Tomkovich S, Dejea CM, Winglee K *et al*. Human colon mucosal biofilms from healthy or colon cancer hosts are carcinogenic. *J Clin Invest* 2019; **130**: 1699–712.
59 Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects. *Nat Rev Microbiol* 2012; **10**: 575–82.
60 Jagerstad M, Skog K, Grivas S, Olsson K. Formation of heterocyclic amines using model systems. *Mutat Res* 1991; **259**: 219–33.
61 Humblot C, Murkovic M, Rigottier-Gois L *et al*. Glucuronidase in human intestinal microbiota is necessary for the colonic genotoxicity of the food-borne carcinogen 2-amino-3-methylimidazo[4,5-f]quinoline in rats. *Carcinogenesis* 2007; **28**: 2419–25.
62 Carman RJ, Van Tassell RL, Kingston DGI, Bashir M, Wilkins TD. Conversion of IQ, a dietary pyrolysis carcinogen to a direct-acting mutagen by normal intestinal bacteria of humans. *Mutat Res Genet Toxicol* 1988; **206**: 335–42.
63 Humblot C, Combourieu B, Vaisanen ML, Furet JP, Delort AM, Rabot S. 1H nuclear magnetic resonance spectroscopy-based studies of the metabolism of food-borne carcinogen 2-amino-3-methylimidazo[4,5-f] quinoline by human intestinal microbiota. *Appl Environ Microbiol* 2005; **71**: 5116–23.
64 Ijssennagger N, Belzer C, Hooiveld GJ *et al*. Gut microbiota facilitates dietary heme-induced epithelial hyperproliferation by opening the mucus barrier in colon. *Proc Natl Acad Sci U S A* 2015; **112**: 10038–43.
65 Van Der Meer-Van Kraaij C, Van Lieshout EM, Kramer E, Van Der Meer R, Keijer J. Mucosal pentraxin (*Mptx*), a novel rat gene 10-fold down-regulated in colon by dietary heme. *FASEB J* 2003; **17**: 1277–85.
66 Ijssennagger N, Rijnierse A, de Wit NJ *et al*. Dietary heme induces acute oxidative stress, but delayed cytotoxicity and compensatory hyperproliferation in mouse colon. *Carcinogenesis* 2013; **34**: 1628–35.
67 Martin OCB, Lin C, Naud N *et al*. Antibiotic suppression of intestinal microbiota reduces heme-induced lipoperoxidation associated with colon carcinogenesis in rats. *Nutr Cancer* 2015; **67**: 119–25.
68 Sesink AL, Termont DS, Kleibeuker JH, Van der Meer R. Red meat and colon cancer: the cytotoxic and hyperproliferative effects of dietary heme. *Cancer Res* 1999; **59**: 5704–9.
69 Schepens MA, Vink C, Schonewille AJ, Dijkstra G, van der Meer R, Bovee-Oudenhoven IM. Dietary heme adversely affects experimental colitis in rats, despite heat-shock protein induction. *Nutrition* 2011; **27**: 590–7.
70 IJssennagger N, Derrien M, van Doorn GM *et al*. Dietary heme alters microbiota and mucosa of mouse colon without functional changes in host-microbe cross-talk. *PLoS ONE* 2012; **7**: e49868.
71 Amanda Jane Cross JRAP, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. *Cancer Res* 2003; **63**: 2358–60.
72 Gorbach BR, Gorbach SL. The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. *J Natl Cancer Inst* 1976; **57**: 371–5.

73 Verma A, Shukla G. Probiotics *Lactobacillus rhamnosus GG*, *Lactobacillus acidophilus* suppresses DMH-induced procarcinogenic fecal enzymes and preneoplastic aberrant crypt foci in early colon carcinogenesis in Sprague Dawley rats. *Nutr Cancer* 2013; **65**: 84–91.
74 Davila AM, Blachier F, Gotteland M *et al*. Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. *Pharmacol Res* 2013; **68**: 95–107.
75 Visek WJ. Diet and cell growth modulation by ammonia. *Am J Clin Nutr* 1978; **31**: S216–20.