Three specific gut bacteria in the occurrence and development of colorectal cancer: a concerted effort (#82720)

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Three specific gut bacteria in the occurrence and development of colorectal cancer: a concerted effort

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Colorectal cancer (CRC), which develops from gradual evolution of tubular adenomas and serrated polyps in the colon and rectum, has poor prognosis and a high mortality rate. In addition to genetics, lifestyle, and chronic diseases, intestinal integrity and microbiota (which facilitate digestion, metabolism, and immune regulation) could promote CRC development. For example, enterotoxigenic *Bacteroides fragilis*, genotoxic *Escherichia coli (pks + E. coli)*, and *Fusobacterium nucleatum*, members of the intestinal flora, are highly correlated in CRC. This review describes the roles and mechanisms of these three bacteria in CRC development. Their interaction during CRC initiation and progression has also been proposed.

Three specific gut bacteria in the occurrence and development of colorectal cancer: a concerted effort

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Abstract

Colorectal cancer (CRC), which develops from gradual evolution of tubular adenomas and serrated polyps in the colon and rectum, has poor prognosis and a high mortality rate. In addition to genetics, lifestyle, and chronic diseases, intestinal integrity and microbiota (which facilitate digestion, metabolism, and immune regulation) could promote CRC development. For example, enterotoxigenic *Bacteroides fragilis*, genotoxic *Escherichia coli (pks + E. coli)*, and *Fusobacterium nucleatum*, members of the intestinal flora, are highly correlated in CRC. This review describes the roles and mechanisms of these three bacteria in CRC development. Their interaction during CRC initiation and progression has also been proposed.

Keywords: gut bacteria, enterotoxigenic *Bacteroides fragilis*, genotoxic *Escherichia coli, Fusobacterium nucleatum,* mechanisms.

1. Introduction

Colorectal cancer (CRC) is a serious threat to human health, with more than 1.9 million new cases worldwide and 935,000 deaths in 2020(Sung *et al.*, 2021). Globally, CRC ranks third in cancer incidence and second in mortality(Sung et al., 2021). According to incidence data from the Cancer Registries and mortality data from the National Center for Health Statistics, CRC ranks second in morbidity and mortality among all cancers in the United States, with approximately 147,950 people diagnosed with CRC and 53,200 deaths from the disease, both in men (78,300 cases and 28,630 deaths) and women (69,650 cases and 24,570 deaths)

(Kindler&Shulman, 2001;Siegel *et al.*, 2020;Siegel;Miller&Jemal, 2020). CRC incidence rate has also continued to rise in China in the last two years (Cao *et al.*, 2021a;Sung et al., 2021). Epidemiological data further suggest that the incidence of CRC in adults under the age of 50 is on the increase (Keum&Giovannucci, 2019).

In general, CRC is characterized by localized abnormal cells or growths, which accumulate in the gut mucosa to form protruding benign polyps(Tan *et al.*, 2013). Previous studies have shown that genetic mutations and immune disorders, the main features of CRC, were closely related to lifestyle, the environment and genetics (Punt;Koopman&Vermeulen, 2017;Zhou&Sonnenberg, 2018;Janney;Powrie&Mann, 2020;Calibasi-Kocal *et al.*, 2021;Choi *et al.*, 2021;Joh *et al.*, 2021;Lopez;Bleich&Arthur, 2021;Naghshi *et al.*, 2021). However, the specific mechanism of CRC pathogenesis remains unclear, and this presents challenges for its prevention and treatment. Therefore, identification of its etiology and pathogen is regarded as the key in addressing CRC.

It is widely reported that the composition of gut bacteria in CRC patients is significantly different from healthy individuals. *Clostridium, Bacteroides*, *Dermatobacteria* and *Proteus* were enriched in CRC patients, whereas *Pachylocycetes* and *Actinomycetes* were the prominent bacteria in healthy individuals (Yang *et al.*, 2019). The types and abundance of intestinal flora are also known to vary significantly depending on the location and progression of the tumor (Claesson *et al.*, 2011;Biagi *et al.*, 2016;Wilmanski *et al.*, 2021). Furthermore, intestinal dysbacteriosis, which is mainly characterized by an increase in the abundance of harmful

bacteria such as enterotoxigenic *Bacteroides fragilis* (ETBF), polyketone compound synthase *E. coli* (*pks*+ *E. coli*), and *Fusobacterium nucleatum* (*F. nucleatum*), and a decrease in the abundance of beneficial bacteria such as *Clostridium* sp. and *Bifidobacterium sp*. has been associated with CRC (Tilg *et al.*, 2018;Bundgaard-Nielsen *et al.*, 2019;Garrett, 2019;Saus *et al.*, 2019;Wirbel *et al.*, 2019;Pleguezuelos-Manzano *et al.*, 2020;Ternes *et al.*, 2020;Zhao&Zhao, 2021). Specific intestinal flora including ETBF played an important role in the development of inflammatory bowel disease (IBD), an important factor driving the formation of CRC (Choi *et al.*, 2017;Kang&Martin, 2017). Further research found that the abundance of harmful bacteria such as *F. nucleatum* increased during the evolution of multiple polypoidomas to intramucosal carcinoma and more advanced lesions (Yachida *et al.*, 2019). Thus, species type and abundance of the intratumor flora varied with the progression of the CRC. Regardless of whether this manifestation is a "cause" or an "effect" of CRC, understanding the correlation between key microflora and CRC could provide an important basis for diagnosis and disease interventions. The current review summarizes the roles and mechanisms of the most closely related bacteria(based on literature): ETBF, *pks* + *E. coli*, and *F. nucleatum* in the occurrence and development of CRC.

2. The role and mechanism of ETBF in CRC pathogenesis

2.1. BFT- a major virulence factor

Bacteroides fragilis belong to the genus *Bacteroides*, and can be divided into enterotoxigenic *Bacteroides fragilis* and non-enterotoxigenic *Bacteroides fragilis* (NTBF)

according to their ability to secrete the *Bacteroides fragilis* toxin (BFT) (Sears;Geis&Housseau, 2014). The main differences between ETBF and NTBF are listed as follows: (1) *Bacteroides fragilis* toxin pathogenicity islands (BFT PAI) are present in the genome of ETBF; (2) type VI secretion system (t6ss) is produced by several NTBF strains. Isogenic NTBF mutants lacking key components of the type VI secretion system (T6SS) allow ETBF colonization; and (3) the ETBF biofilm activity is stronger than that of NTBF (Russell;Peterson&Mougous, 2014;Russell *et al.*, 2014;Pierce&Bernstein, 2016). These studies also reported the expression of the BFT gene in the colonic mucosa of patients with advanced CRC. The results of an outpatient CRC screening based on *bft* detection showed more than 85.7% *bft* positive rate in the mucosa and as high as 100% in the mucosa of patients with advanced CRC; hence, it was speculated that this toxin might be a risk factor for CRC (Franco *et al.*, 1997;Kato *et al.*, 2000;Boleij *et al.*, 2015;Jasemi *et al.*, 2020). The high *bft* detection rate and the occurrence of three main subtypes of this gene: *bft*-1, *bft*-2, and *bft*-3 in CRC has gained research attention (Franco et al., 1997). The homology of amino acids between these three subtypes is 87%-96%, and their differences in histology and biological activity were obvious (Franco et al., 1997;Kato et al., 2000;Wu *et al.*, 2002;Boleij et al., 2015;Jasemi et al., 2020). Firstly, the difference in abundance of the subtypes was *bft*-1 > *bft*-3 > *bft*-2 in CRC patients and *bft*-2 > *bft*-3 > *bft*-1 in healthy human tissues (Jasemi et al., 2020). Secondly, results of the activity verification test in HT29 cells showed *bft*-3 > *bft*-1 > *bft*-2(Franco et al., 1997). Notably, the half-life of *bft*-2 was longer than *bft*-1, although its biological activity was lower. NTBF did not contain *bft* but polysaccharide A (PSA), which had a significant inhibitory effect on the formation of CRC (Lee *et al.*, 2018b). In an *in vitro* co-culture

of ETBF and NTBF, the growth of ETBF was inhibited by proteins secreted from NTBF (Pierce&Bernstein, 2016). However, in the microenvironment of precancerous colon polyps, NTBF induced the production of pro-inflammatory cytokines, and thus may also play a role in the early stages of the disease (Kordahi *et al.*, 2021). The results from co-cultivation of these two kinds of bacteria in a CRC environment as well as is the probiotic effect of NTBF remain to be ascertained.

2.2. Activation of the Wnt/β-catenin pathway

It is well known that Wnt/*β*-catenin (a canonical Wingless-related integration –Wnt signalling pathway) plays a crucial role in the regulation of embryonic development and carcinogenesis (Muralidhar *et al.*, 2019). *β*-catenin is pivotal in the Wnt signalling pathway and mediates cell adhesion by interacting with E-cadherin at cell junctions (MacDonald;Tamai&He, 2009). BFT was the first bacterial effector reported to activate *β*-catenin-dependent gene expression(Wu *et al.*, 2003). As shown in **Fig. 1a**, the BFT receptor (BFT-r) upon exposure and interaction with colonic epithelial cells (CECs) binds to a BFT toxin, leading to cleavage and dislocation of the extracellular structure of the transmembrane glycoprotein E-cadherin (mediated by presenilin-1/γ-secretase), and its complete degradation. As the structure of E-cadherin changes, *β*-catenin (which is normally bound to the E-cadherin intracellular domain) dissociates, causing the abnormally expressed *β*-catenin to escape the regulation of the adenomatous colon polyp (APC) protein *β*-catenin enters the nucleus to form a complex with Transcription Factor 4 (TCF4). The nuclear gene c-Myc is then activated and the CECs become

119 cancerous (Wu et al., 2003).

120 Based on the above evidence, one could infer that BFT-induced degradation of E-cadherin
121 and the dissociation of *β*-catenin are critical factors in activating the Wnt/*β*-catenin pathway.
122 However, whether BFT is the only virulence factor acting in this process or not is still not clear.
123 The occurrence of alternative BFT receptors, their structures, and mechanisms in cancer
124 development (as well as their similarity to the known BFT mechanism) also remain to be fully
125 elucidated. Furthermore, ETBF induced the anti-apoptotic protein cIAP2 and the polyamine
126 catalyst spermine oxidase (SMO) through *bft*; *bft* triggered ROS production, leading to DNA
127 damage and cell proliferation (Wu et al., 2003;Wu *et al.*, 2004;Kim;Lee&Kim, 2008;Dejea *et al.*,
128 2018). These findings confirm the carcinogenicity of ETBF, which occurs via direct interaction
129 with CECs.

130 ***2.3. Occurrence of inflammation***

131 Inflammation, especially long-term chronic inflammation in the colon, correlates strongly
132 with the occurrence of CRC (this is known as colitis-associated CRC (CAC)) (Hirano *et al.*,
133 2020). Several reports suggest that Th17 cells and interleukin-17 (IL-17) are involved in the
134 occurrence of various inflammations and tumors. According to retrospective studies, IL-17 was
135 significantly elevated in both the colonic mucosa and sera from IBD patients with pre-CRC
136 symptoms; further etiological studies found a close relation to BFT exposure (Wu et al.,
137 2003;Kim;Lee&Kim, 2008;Dejea;Wick&Sears, 2013;Boleij et al., 2015;Chung *et al.*,

2018;Dejea et al., 2018). The discovery of IL-17 as an important regulator of the NF-κB (a vital inflammatory response regulator, which is also closely related to the occurrence of IBD) pathway was recently reported by Chung et al (Chung et al., 2018). Generally, mucosal immune response mediated by Th17 is triggered when BFT targets CECs (namely, IL-17 met IL-17-r located on the surface of CECs) resulting in the activation of the NF-κB pathway(Chung et al., 2018). Activation of NF-κB can then trigger the expression of CXCL chemokines, which directly promote pre-tumor cells and infiltrate the distal colon, leading to carcinogenesis. Interestingly Savkovic *et al*, showed NF-κB-induced secretion of pro-inflammatory factors and chemokines such as IL-8 and TNF-α, this promoted the recruitment of neutrophils and other immune cells to the colonic mucosa (Savkovic;Koutsouris&Hecht, 1996).

STAT3, another significant inflammatory mediator, is also associated with CAC and sporadic CRC (Grivennikov *et al.*, 2009). Recent studies by Chung et. al., showed that activation of the STAT3 pathway play a critical role in the occurrence and development of CRC; although not independently (Chung et al., 2018). In the mechanism of STAT3-mediated inflammatory signalling, binding of cytokines IL-6, IL-10, IL-11, and IL-23 to their receptors precedes the activation of the JAK signalling pathway (an essential part of this event). Afterwards, the phosphorylated STAT3 is translocated to the nucleus to regulate gene expression, inhibit apoptosis, and promote cell proliferation and tumor formation. Findings from *in vitro* and *in vivo* experiments confirmed the concurrent activation of STAT3 in mucosal immune cells and CECs during ETBF colonization (Wick *et al.*, 2014).

158 In addition, inflammatory signalling pathways in mucosal immune cells could be triggered
159 upon exposure to ETBF, resulting in IL-6(Gargalionis;Papavassiliou&Papavassiliou, 2021).
160 Whether ETBF acted on immune cells directly or induced immune cells through CECs was still
161 inconclusive from this study. However, it is clear that inflammatory cells, cytokines, and
162 inflammatory signalling pathways play a key role in ETBF-mediated inflammation, a major
163 cause of carcinogenesis in CECs. Th17, neutrophils, and CECs could also interact to promote
164 ETBF-mediated inflammation of the mucosa, even though the initiating cell remains unclear.
165 Furthermore, *miR-149-3p* could be released from exosomes to mediate cell-to-cell
166 communication by regulating differentiation of Th17 cells (Cao *et al.*, 2021b). Thus, mucosal
167 immune responses mediated by Th17 and triggered by ETBF could play a vital role in the
168 pathogenesis of inflammatory CRC. However, the origin of Th17 (whether derived from *miR-*
169 *149-3p* or alternative sources) remains to be confirmed by further experiments. Based on the
170 existing evidence, the authors speculate (shown in **Fig. 1b**) that ETBF invasion of CECs triggers
171 the release of warning signals (cytokines) from CECs for the recruitment of neutrophils. The
172 neutrophils also signal for the mobilization of Th17 cells, which cooperate with the former for
173 CECs, inducing the cells to become cancerous.

174 ***2.4. High expression of BFAL1***

175 A recent study found that lncRNA1 (*Bacteroides fragilis*-associated lncRNA1, BFAL1) was
176 abnormally elevated in CRC cells and tissues exposed to ETBF (Bao *et al.*, 2019). Clinically, the
177 high expression of BFAL1 in CRC tissues and the high abundance of ETBF indicates a poor

prognosis in CRC patients. The proposed mechanism (shown in **Fig. 1c**) suggests ETBF-induced overexpression of lncRNA-BFAL1 in CECs. Therefore, ETBF could bind to *miR-155-5p* and *miR-200a-3p* competitively, resulting in activation of the mammalian target of the rapamycin complex 1(mTORC1) pathway. The mTORC1 signalling pathway, closely related to the occurrence and development of tumors, was deregulated in about 50% of human malignant tumors (Shorning *et al.*, 2020) and promoted further tumor growth (Bao et al., 2019). More so, ETBF could induce the development of CRC cells from tumor Cancer stem-like cells (CSCs), via activating toll-like receptor 4 (TLR4), and promoting the expression of Jumonji domain-containing 2B (JMJD2B) through T cell nuclear factor 5 (NFAT5) stimulation (**Fig. 1c**). The subsequent demethylation of H3K9me3, up-regulation of NANOG and enhancement of the stemness in CRC cells has been proven (Liu *et al.*, 2020).

Thus, ETBF an exogenous pathogenic factor could, play a crucial role CRC (especially CAC) initiation, while endogenous carcinogenesis caused by epigenetic changes could accelerate the disease progression in the advanced stage. Notably, most of the current findings are based on the different subtypes of ETBF. As mentioned earlier, the influence of the different subtypes on the diverse mechanisms of carcinogenicity needs to be studied in depth.

3. Role and mechanism of pks + E. coli in the pathogenesis of CRC

3.1. Mutations in genes

An increase in the abundance of colonic mucosa-associated *E. coli* with the *pks* gene has

been observed in IBD, familial adenomatous polyposis (FAP), and CRC patients, compared to healthy individuals (Arthur *et al.*, 2012;Prorok-Hamon *et al.*, 2014;Dejea et al., 2018). Macrogenomic sequencing results also showed that *pks* cluster was enriched in the colon tissues of CRC patients (Wirbel et al., 2019). According to Nougayrede et al, infection of Hela cells with *E. coli* (which produce these genotoxins) resulted in DNA interstrand cross-linking (ICLs) and double-strand breaks (DSBs), and subsequently led to megaloblastosis and cell cycle arrest (Nougayrede *et al.*, 2006). Exposure to *pks* + *E. coli* caused more single base substitutions (SBSs) in the host gene, with a bias towards T>N substitutions preferentially occurring at the base of the intermediate ATA (also called SBS-*pks*), this bacteria also induced a characteristic small indel signature (ID-*pks*) of a single T deletion on the T homopolymer (Lee-Six *et al.*, 2019;Pleguezuelos-Manzano et al., 2020;Li, 2021). In addition, cancerous organs of CRC patients often exhibit genomic instability (Chromosomal instability, CIN) (Scully, 2010;Cancer Genome Atlas, 2012). Another study demonstrated this genomic instability after four-hour exposure of primary intestinal epithelial cells to *pks* + *E. coli* (Nougayrede et al., 2006). Interestingly, the appearance of CIN was not regulated by the Wnt signalling pathway, rather,CIN exhibited a "hit and run" mechanism (Iftekhar *et al.*, 2021). Mutations in single bases and CIN are among the commonly observed types of genetic mutations in CRC cases; however, the mechanism of their involvement is not yet clear. Nevertheless, the pathogenic effect of *E. coli* toxins on host DNA is a complex process of damage and repair (shown in **Fig. 2**). The "contribution" of *E. coli* toxins to host mutations may provide a new basis for unravelling this mechanism.

3.2. Ubiquitination of P53

Gene mutations in the P53 pathway are considered early biological events in CRC (Calibasi-Kocal et al., 2021;Choi et al., 2021;Joh et al., 2021;Lopez;Bleich&Arthur, 2021). In CECs, *pks+ E. coli* induced alterations in catalytic P53C-terminal class ubiquitination. In this mechanism, *E. coli* genotoxin induced *miR-20a-5p* expression via the c-Myc transcription factor and up-regulated the expression of *miR-20a-5p* bound to the Sentrin-specific protease 1(SENP1) mRNA 3'UTR. This led to the latter's translational silencing and, thus, P53 SUMOylation (the SENP1 protein is a known key protein in catalytic P53C-terminal ubiquitination) (Iftekhar et al., 2021). Moreover, the occurrence of C-terminal ubiquitination of P53 led to the phosphorylation of hepatocyte growth factor (HGF) and its receptor; this promoted tumor growth, while inactivating *miR-34* (Cougnoux *et al.*, 2014;Dalmasso *et al.*, 2014;Iftekhar et al., 2021). Likewise, findings from a clinical study, where HGF expression was significantly increased in *pks+ E. coli*-infected tissues compared to non-infected biopsy specimens, confirmed the occurrence of this mechanism (Cougnoux et al., 2014). These authors identified HGF production as a key determinant of CRC progression; a marker of poor prognosis and a therapeutic target in CRC. Survey data also showed that *miR-34a* and *miR-34b/c* were silent in 75% and 99% of disseminated CRC samples, respectively (Vogt *et al.*, 2011;Wu *et al.*, 2014). *miR-34* inhibits proliferation of in situ and tumor-derived cells (He *et al.*, 2007), and all three isoforms (*miR-34a/b/c*) have been shown to inhibit adenoma formation (Jiang&Hermeking, 2017). *miR-34a* also affects the development of the epithelial-mesenchymal transition (EMT) inhibitory effect

(Raver-Shapira *et al.*, 2007;Siemens *et al.*, 2011). Furthermore, regulation and activation of *miR-34* by the P53 pathway has been confirmed (Kim *et al.*, 2011a). Thus, upon P53 ubiquitination, *miR-34* could be inactivated, losing its inhibitory effect on the proliferation of in situ and tumor-derived cells. The proposed summary on the role of P53 ubiquitination in CRC (shown in **Fig. 2**) indicates that c-Myc, a target of *pks+ E. coli* genotoxins, is key in causing P53 heterozygosity and ultimately promoting tumorigenesis. *miR-20a-5p* and *miR-34* may be important factors in c-Myc regulation.

4. Role and mechanism of F. nucleatum in the pathogenesis of CRC

4.1. Suppression of immunity and proliferation of tumor cells

The occurrence of CRC has been closely associated with the of *F. nucleatum*, a bacterium that is native to the human mouth (McIlvanna *et al.*, 2021), which promotes the proliferation of cancer cells in the gut (Bullman *et al.*, 2017;Yu *et al.*, 2017;Garrett, 2019). Previous studies found that *F. nucleatum* promotes the development of CRC through three main pathways: (i) activation of downstream oncogenic signals in cancer cells; (ii) inhibition of immune cell activation; and (iii) promotion of tumor metabolism (Hong *et al.*, 2021). The involvement of *F. nucleatum* in CRC progression begins with adhesion and invasion of vascular endothelial cells. *F. nucleatum* invades vascular endothelial cells through the binding of FadA (virulence factor for *F. nucleatum*) to its vascular endothelial cell surface receptor CDH5 (a member of the cadherin superfamily (Xu *et al.*, 2007)). Upon entering the vasculature *F. nucleatum* colonizes the intestinal epithelial cells; a process that is also dependent on the action of FadA and the presence

of E-cadherin on the surface of CECs (Rubinstein *et al.*, 2013). E-cadherin is an important member of the calcium-dependent cell adhesion glycoprotein family, which contains a transmembrane structural domain and a highly conserved cytoplasmic tail that binds to other cytoplasmic components, such as *β*-catenin. E-cadherin exerts its tumor-suppressive activity through Wnt/*β*-catenin signalling. Therefore, the binding of FadA to E-cadherin, which promotes CRC cell proliferation and leads to tumorigenesis, activates Wnt/*β*-catenin signalling (Rubinstein et al., 2013).

It is evident that FadA (exists in two forms, secretory and non-secretory) plays a major role in *F. nucleatum* migration and intestinal colonization. Notably, *mFadA* - the secretory form of FadA could not bind to E-cadherin. Although immune evasion is one of the known hallmarks of cancer, its mechanism is unclear (Hanahan&Weinberg, 2011). Interestingly, the lethal effect of natural killer (NK) cells in a tumor microenvironment was inhibited by *F. nucleatum*, which also exerted a significant inhibitory effect on immune cells, such as T cells derived from hTIGIT expressed in human NK cells (Stanietsky *et al.*, 2009). The activation of hTIGIT mainly inhibited the induction of NK cells, and other immune cells (Stanietsky et al., 2009), while *F. nucleatum* assisted tumor cells to achieve immune evasion through specific binding of the adhesion protein Fap2 to hTIGIT to inhibit its activity (Gur *et al.*, 2015). Furthermore, Fap2 mediated the binding of *F. nucleatum* to Gal-GalNAc overexpressed in CRC, and this explain the recruitment of *F. nucleatum* to colon tumor sites (Abed *et al.*, 2016). It is worth noting that Fap2 is non-specific to Gal-GalNAc. Thus, Fap2 might be an important factor in the *F. nucleatum* -

mediated immune evasion mechanism in CRC.

4.2. Liberated glycolysis

High glycolysis is closely associated with poor prognosis in patients with CRC. This is because cancer cells depend on energy supplementation for growth; therefore disturbances in energy metabolism, particularly abnormal glycolysis, are regarded as hallmarks of cancer (Hanahan&Weinberg, 2011). Enhanced glycolysis in CRC produces large amounts of lactic acid, which accelerates the acidification of the tumor microenvironment (Boedtkjer&Pedersen, 2020). Epigenetic alterations affected CRC progression with the involvement of lncRNAs in a wide range of biological processes, including epigenetic modifications (Chen, 2016). Glycolytic process in the tumor microenvironment was regulated by lncRNA ENO1- IT1; a regulator of ENO1 expression, mainly via formation of the KAT7/ENO1-IT1 complex with KAT7(Abed et al., 2016). KAT7 belongs to the MYST protein family and is a histone acetyltransferase that regulates cell proliferation during cancer development. As a vital glycolytic enzyme, ENO1 catalyzed the conversion of 2-phosphoglycerate to phosphoenolpyruvate (PEP) (Didiasova;Schaefer&Wygrecka, 2019). Clinical studies have also shown that the expression of lncRNA ENO1-IT1 is significantly up-regulated in cancer patients with high levels of *F. nucleatum (Hong et al., 2021)*. However, since ENO1-IT1 is mainly located in the nucleus of CRC cells, the connection between these two is unclear. Further studies have shown that the effect of *F. nucleatum* on ENO1-IT1 is mainly via the transcription factor SP1 (Parhi *et al.*, 2020). SP1 is known to bind directly to the promoter region of ENO1-IT1, which could be

298 closely associated with glycolysis (Ke *et al.*, 2012). Although SPI was activated by *F. nucleatum*
299 (Martin-Gallausiaux *et al.*, 2018), the mechanism of action is not clear (see **Fig. 3**).

300 5. Correlation between gut microbiota and epigenetics in CRC

301 The findings presented in the previous Sections as well as existing literature highlight the
302 inextricable link between intestinal flora and CRC epigenetic changes, irrespective of the role
303 played by *B. fragilis*-associated *miR-149-3p*, *pks+ E. coli*-associated *miR-20a-5p*, or lncRNA
304 ENO1-IT1. Hence, the correlation between gut microbiota and CRC epigenetics in existing
305 reports (using CRC-related epigenetic changes as clues) has been explored here. Accordingly,
306 the mechanisms of epigenetic regulation in CRC mainly include: (1) microRNAs (miRNAs) and
307 non-coding RNAs; (2) DNA methylation of CpG island; (3) post-translational modification of
308 histones; and (4) localization, occupation and remodelling of nucleosome. Their specific
309 association with the intestinal flora is discussed in the subsequent Subsections.

310 *5.1. Role of miRNAs and lncRNAs in CRC epigenetics*

311 *In vivo and in vitro* studies have shown that while CRC-associated miRNAs and lncRNAs
312 are closely related to the imbalance of some specific gut microbiota, CRC-associated intestinal
313 bacteria can also cause abnormal expression of miRNAs (Cougnoux et al., 2014;Zhao *et al.*,
314 2020;Cao et al., 2021b). Furthermore ETBF promoted CRC cell proliferation *in vitro* and *in vivo*
315 by downregulating *miR-149-3p* expression (Cao et al., 2021b); *pks+ E. coli* (on the other hand)
316 up-regulated *miR-20a-5p* expression to promote tumor growth (Iftekhar et al., 2021).

317 *F. nucleatum* also promoted CRC cell proliferation and tumorigenesis by upregulating *miR-21*
318 expression (Yang *et al.*, 2017). In a clinical study by Feng et. al., upregulation of *miR-4474/4717*
319 expression was observed in CRC tissues (Feng *et al.*, 2019). More so, exosomes from
320 *F. nucleatum* -infected CRC cells selectively possessed *miR-1246/92b-3p/27a-3p* (consequently
321 promoting tumor migration in a lab-based study) (Wang *et al.*, 2021). The above findings
322 demonstrate the influence of intestinal bacteria on the progression of CRC via the regulation of
323 miRNAs. Indeed, miRNAs also regulate CRC development independently by influencing the
324 colonization and proliferation of intestinal bacteria. Existing studies have found that both
325 endogenous and exogenous *miR-139-5p* exert inhibitory effect on the colonization and
326 proliferation of *F. nucleatum*, consequently inhibiting the development and progression of CRC
327 (Zhao et al., 2020). However, relatively fewer studies have been conducted in this regard.
328 Furthermore, evidence of the effects of miRNAs on ETBF and *pks*+ *E. coli*, this might be
329 influenced by CRC progression, is still lacking.

330 The association between lncRNA and CRC development has been reported as well as its
331 pronounced up-regulation of XLOC006844, LOC152578 and XLOC000303 in CRC, using gene
332 chips through multi-stage validation (Shi *et al.*, 2015;Wang *et al.*, 2016;Hibner;Kimsa-
333 Furdzik&Francuz, 2018;Liu *et al.*, 2019;Pan *et al.*, 2019). Another comparative study of serum
334 samples from 71 CRC patients and 70 healthy individuals found significantly increased levels of
335 lncRNAs RP11-462C24.1, LOC285194, and Nbla12061 in CRC patients; the levels of all three
336 lncRNAs were significantly reduced in patients after surgical removal of the tumors (Wang et al.,

2016). Silencing lncRNA CRNDE-7 in vivo significantly attenuated the growth of CRC tumor (Sun *et al.*, 2021). However, the role of lncRNAs in mediating gut microbiota-related CRC development is still unclear. Moreover, the carcinogenicity of CRC-associated ETBF was mediated by lncRNA1 (BFAL1) (Bao et al., 2019). *F. nucleatum* also promoted glycolysis and tumorigenesis of CRC by targeting lncRNA-intron transcript 1 (ENO1-IT1) (Hong et al., 2021). The pathogenicity of lncRNAs on CRC-related intestinal flora was also observed (Hong et al., 2021). It is noteworthy that there are no existing reports (to date) on the interaction of lncRNAs with *pks+ E. coli*. Nonetheless, the commonality of IncRNAs to both ETBF and *F. nucleatum* indicate its potential as a diagnostic and/or therapeutic targets in CRC.

5.2. DNA methylation and histone modification

Alterations in DNA methylation patterns and modifications of histone have been widely reported in the etiology of cancer. Abnormal DNA hyper methylation of tumor suppressor genes ANO1, Fut4, Gas2I, Polg, Runx3, Gata2, and Hoxa5 were found in the tumors of ETBF-infected ApcΔ716/Min mice: this was also observed in human at the same time (Kim *et al.*, 2011b;Maiuri *et al.*, 2017). Other studies also found a significant increase in the mutation rate of AMER1 and ATM genes in CRC patients with a high abundance of *F nucleatum* (Lennard;Goosen&Blackburn, 2016;Lee *et al.*, 2018a). The high abundance of colonized Fusobacterium could lead to a significant increase in methylation of CpG island, resulting in up-regulation of oncogenes such as REG3A, REG1A, and REG1P (Lennard;Goosen&Blackburn, 2016;Lee et al., 2018a). An increase in the number of total nucleosome in the blood also

coincided with increasing tumor progression and burden (Krude, 1995;Rahier *et al.*, 2017). According to previous studies, changes in DNA methylation patterns could cause marked changes in histone modifications (Gezer *et al.*, 2015). A correlation was also observed among histone in nucleosomes. Methylation of histones in nucleosomes, such as H3K27me3 and H4K20me3, is considered as a biomarker of CRC (Gezer et al., 2015). Moreover, high methylation of promoters and a sudden increase in the number of nucleosomes were the main effects observed when tumor suppressor gene CDH1 was silenced in CRC cells (Hesson *et al.*, 2014); this were closely related to their corresponding miRNAs and lncRNAs(Li *et al.*, 2019). However, the involvement of intestinal bacteria in this process is unclear. A regulatory axis (bacteria-miRNA/lncRNA-nucleosome histone methylation-CRC) could explain the occurrence and development of CRC; however, more studies are needed to confirm this hypothesis.

5.3. Cooperation among intestinal flora in CRC development

In addition to their peculiar mechanisms in CRC development, the commonalities observed among these three intestinal bacteria (already discussed in the previous Sections) are noteworthy. For instance, both BFT (an EBTF virulence factor) and FadA (an *F. nucleatum* virulence factor) activate the Wnt/β-catenin pathway by interacting with E-cadherin; E-cadherin normally complexes with β-catenin in the cytoplasm. This could highlight E-cadherin on intestinal epithelial cells as a common target of ETBF and *F. nucleatum*, suggesting competitive carcinogenesis between the two mechanisms. Hence, considering the carcinogenic role of these two bacteria in CRC development, E-cadherin can be investigated further for its potential in

CRC drug discovery. Moreover, the transcription factor c-Myc, which is induced by BFT-mediated β-catenin/TCFA complex formation, also serves as a key target in P53 ubiquitination and tumorigenesis by *pks+E. coli*.What seems interesting is the rather concerted manner in which these three specific intestinal bacteria contribute to the occurrence and development of CRC. As proposed in **Fig. 4**, this mutual interaction towards carcinogenesis could be initiated by inflammation-mediated degradation of the intestinal epithelial layer by ETBF, and pathogenic effect on stromal cells, in the early stages of CRC development. This mucosal damage affects the integrity of the intestine (a robust mucosal layer protects the epithelium against pathogens) as well as its ecology. The essential role of mucin glycans in defining the microbiota has also been documented (Larsson *et al.*, 2009;Hansson, 2012). Thus, the mucosal damage and resulting ecological imbalance could provide the optimum environment for the subsequent occupation of *pks+E. coli*, leading to carcinogenesis. *pks+ E. coli* causes genetic mutations in the intestinal epithelial cells, and this could recruit *F. nucleatum* to the disease site. *F. nucleatum* promotes stemness and proliferation of cancer cells via Fap2-mediated immune evasion, contributing mainly to advanced CRC. This proposal highlights the most dominant bacteria in each stage of CRC development, not neglecting the possibility that two, or even all three, bacteria could be engaged at any stage of the disease.

6. Conclusions

The development of pathogen-associated diseases is a process of diverse interactions between host and pathogen. From the etiological perspective, all three bacteria -ETBF, *pks+ E.*

coli, and *F. nucleatum*- possess carcinogenic properties, but their contributions at each stage of CRC may vary. Therefore, ascertaining their mechanisms and/or commonalities in disease development could facilitate the identification of key diagnostic and therapeutic markers. From the host's perspective, CRC development is dominated by the activities of CECs, immune cells and their cytokines, and epigenetic factors. Nonetheless, the mucus layer, cell junction proteins, and CEC together constitute a physical barrier to the carcinogenicity of pathogenic microorganisms. Key epigenetic regulatory factors might also provide new ideas for the screening of clinical drug targets, whereas effectors could be the basis for the discovery of diagnostic targets.

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There is no raw data; this is a literature review.

1. include a rationale for why it is needed.

The manuscript was edited for proper English language, grammar, punctuation, spelling by one or more of the highly qualified native English speaking editors. In this paper, the roles and mechanisms of three bacteria -ETBF, *pks+ E. coli*, and *F. nucleatum* in CRC development are

described. The interaction among these three bacterial genera, from the onset of the disease to its progression and their significance to the disease process, has also been deduced.

2. describe the audience it is intended for.

The audience it is intended for researchers in related fields such as gastrointestinal diseases, intestinal flora, and colorectal cancer, etc.

REFERENCE

Abed J.,Emgard J. E.,Zamir G.,Faroja M.,Almogy G.,Grenov A.,Sol A.,Naor R.,Pikarsky E.,Atlan K. A.,Mellul A.,Chaushu S.,Manson A. L.,Earl A. M.,Ou N.,Brennan C. A.,Garrett W. S.,Bachrach G. 2016. Fap2 Mediates Fusobacterium nucleatum Colorectal Adenocarcinoma Enrichment by Binding to Tumor-Expressed Gal-GalNAc. *Cell Host Microbe,* 20**,** 215-25.doi: https://doi.org/ 10.1016/j.chom.2016.07.006.

Arthur J. C.,Perez-Chanona E.,Muhlbauer M.,Tomkovich S.,Uronis J. M.,Fan T. J.,Campbell B. J.,Abujamel T.,Dogan B.,Rogers A. B.,Rhodes J. M.,Stintzi A.,Simpson K. W.,Hansen J. J.,Keku T. O.,Fodor A. A.,Jobin C. 2012. Intestinal inflammation targets cancer-inducing activity of the microbiota. *Science,* 338**,** 120-3.doi: https://doi.org/ 10.1126/science.1224820.

Bao Y.,Tang J.,Qian Y.,Sun T.,Chen H.,Chen Z.,Sun D.,Zhong M.,Chen H.,Hong J.,Chen Y.,Fang J. Y. 2019. Long noncoding RNA BFAL1 mediates enterotoxigenic Bacteroides fragilis-related carcinogenesis in colorectal cancer via the RHEB/mTOR pathway. *Cell Death Dis,* 10**,** 675.doi: https://doi.org/ 10.1038/s41419-019-1925-2.

Biagi E.,Franceschi C.,Rampelli S.,Severgnini M.,Ostan R.,Turroni S.,Consolandi C.,Quercia S.,Scurti M.,Monti D.,Capri M.,Brigidi P.,Candela M. 2016. Gut Microbiota and Extreme Longevity. *Curr Biol,* 26**,** 1480-5.doi: https://doi.org/ 10.1016/j.cub.2016.04.016.

Boedtkjer E.,Pedersen S. F. 2020. The Acidic Tumor Microenvironment as a Driver of Cancer. *Annu Rev Physiol,* 82**,** 103-126.doi: https://doi.org/ 10.1146/annurev-physiol-021119-034627.

Boleij A.,Hechenbleikner E. M.,Goodwin A. C.,Badani R.,Stein E. M.,Lazarev M. G.,Ellis B.,Carroll K. C.,Albesiano E.,Wick E. C.,Platz E. A.,Pardoll D. M.,Sears C. L. 2015. The Bacteroides fragilis toxin gene is prevalent in the colon mucosa of colorectal cancer patients. *Clin Infect Dis,* 60**,** 208-15.doi: https://doi.org/ 10.1093/cid/ciu787.

Bullman S.,Pedamallu C. S.,Sicinska E.,Clancy T. E.,Zhang X.,Cai D.,Neuberg D.,Huang K.,Guevara F.,Nelson T.,Chipashvili O.,Hagan T.,Walker M.,Ramachandran A.,Diosdado B.,Serna G.,Mulet N.,Landolfi S.,Ramon Y. Cajal S.,Fasani R.,Aguirre A. J.,Ng K.,Elez E.,Ogino S.,Tabernero J.,Fuchs C. S.,Hahn W. C.,Nuciforo P.,Meyerson M. 2017. Analysis of Fusobacterium persistence and antibiotic response in colorectal cancer. *Science,* 358**,** 1443-1448.doi: https://doi.org/ 10.1126/science.aal5240.

Bundgaard-Nielsen C.,Baandrup U. T.,Nielsen L. P.,Sorensen S. 2019. The presence of bacteria

varies between colorectal adenocarcinomas, precursor lesions and non-malignant tissue. *BMC Cancer,* 19**,** 399.doi: https://doi.org/ 10.1186/s12885-019-5571-y.

Calibasi-Kocal G.,Mashinchian O.,Basbinar Y.,Ellidokuz E.,Cheng C. W.,Yilmaz O. H. 2021. Nutritional Control of Intestinal Stem Cells in Homeostasis and Tumorigenesis. *Trends Endocrinol Metab,* 32**,** 20-35.doi: https://doi.org/ 10.1016/j.tem.2020.11.003.

Cancer Genome Atlas Network 2012. Comprehensive molecular characterization of human colon and rectal cancer. *Nature,* 487**,** 330-7.doi: https://doi.org/ 10.1038/nature11252.

Cao W.,Chen H. D.,Yu Y. W.,Li N.,Chen W. Q. 2021a. Changing profiles of cancer burden worldwide and in China: a secondary analysis of the global cancer statistics 2020. *Chin Med J (Engl),* 134**,** 783-791.doi: https://doi.org/ 10.1097/CM9.0000000000001474.

Cao Y.,Wang Z.,Yan Y.,Ji L.,He J.,Xuan B.,Shen C.,Ma Y.,Jiang S.,Ma D.,Tong T.,Zhang X.,Gao Z.,Zhu X.,Fang J. Y.,Chen H.,Hong J. 2021b. Enterotoxigenic Bacteroidesfragilis Promotes Intestinal Inflammation and Malignancy by Inhibiting Exosome-Packaged miR-149-3p. *Gastroenterology,* 161**,** 1552-1566 e12.doi: https://doi.org/ 10.1053/j.gastro.2021.08.003.

Chen L. L. 2016. Linking Long Noncoding RNA Localization and Function. *Trends Biochem Sci,* 41**,** 761-772.doi: https://doi.org/ 10.1016/j.tibs.2016.07.003.

Choi C. R.,Bakir I. A.,Hart A. L.,Graham T. A. 2017. Clonal evolution of colorectal cancer in IBD. *Nat Rev Gastroenterol Hepatol,* 14**,** 218-229.doi: https://doi.org/ 10.1038/nrgastro.2017.1.

Choi J.,Jia G.,Wen W.,Shu X. O.,Zheng W. 2021. Healthy lifestyles, genetic modifiers, and colorectal cancer risk: a prospective cohort study in the UK Biobank. *Am J Clin Nutr,* 113**,** 810-820.doi: https://doi.org/ 10.1093/ajcn/nqaa404.

Chung L.,Thiele Orberg E.,Geis A. L.,Chan J. L.,Fu K.,DeStefano Shields C. E.,Dejea C. M.,Fathi P.,Chen J.,Finard B. B.,Tam A. J.,McAllister F.,Fan H.,Wu X.,Ganguly S.,Lebid A.,Metz P.,Van Meerbeke S. W.,Huso D. L.,Wick E. C.,Pardoll D. M.,Wan F.,Wu S.,Sears C. L.,Housseau F. 2018. Bacteroides fragilis Toxin Coordinates a Pro-carcinogenic Inflammatory Cascade via Targeting of Colonic Epithelial Cells. *Cell Host Microbe,* 23**,** 203-214 e5.doi: https://doi.org/ 10.1016/j.chom.2018.01.007.

Claesson M. J.,Cusack S.,O'Sullivan O.,Greene-Diniz R.,de Weerd H.,Flannery E.,Marchesi J. R.,Falush D.,Dinan T.,Fitzgerald G.,Stanton C.,van Sinderen D.,O'Connor M.,Harnedy N.,O'Connor K.,Henry C.,O'Mahony D.,Fitzgerald A. P.,Shanahan F.,Twomey C.,Hill C.,Ross R. P.,O'Toole P. W. 2011. Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc Natl Acad Sci U S A,* 108 Suppl 1**,** 4586-91.doi: https://doi.org/ 10.1073/pnas.1000097107.

Cougnoux A.,Dalmasso G.,Martinez R.,Buc E.,Delmas J.,Gibold L.,Sauvanet P.,Darcha C.,Dechelotte P.,Bonnet M.,Pezet D.,Wodrich H.,Darfeuille-Michaud A.,Bonnet R. 2014. Bacterial genotoxin colibactin promotes colon tumour growth by inducing a senescence-associated secretory phenotype. *Gut,* 63**,** 1932-42.doi: https://doi.org/ 10.1136/gutjnl-2013-305257.

Dalmasso G.,Cougnoux A.,Delmas J.,Darfeuille-Michaud A.,Bonnet R. 2014. The bacterial

genotoxin colibactin promotes colon tumor growth by modifying the tumor microenvironment. *Gut Microbes,* 5**,** 675-80.doi: https://doi.org/ 10.4161/19490976.2014.969989.

Dejea C. M.,Fathi P.,Craig J. M.,Boleij A.,Taddese R.,Geis A. L.,Wu X.,DeStefano Shields C. E.,Hechenbleikner E. M.,Huso D. L.,Anders R. A.,Giardiello F. M.,Wick E. C.,Wang H.,Wu S.,Pardoll D. M.,Housseau F.,Sears C. L. 2018. Patients with familial adenomatous polyposis harbor colonic biofilms containing tumorigenic bacteria. *Science,* 359**,** 592-597.doi: https://doi.org/ 10.1126/science.aah3648.

Dejea C.,Wick E.,Sears C. L. 2013. Bacterial oncogenesis in the colon. *Future Microbiol,* 8**,** 445-60.doi: https://doi.org/ 10.2217/fmb.13.17.

Didiasova M.,Schaefer L.,Wygrecka M. 2019. When Place Matters: Shuttling of Enolase-1 Across Cellular Compartments. *Front Cell Dev Biol,* 7**,** 61.doi: https://doi.org/ 10.3389/fcell.2019.00061.

Feng Y. Y.,Zeng D. Z.,Tong Y. N.,Lu X. X.,Dun G. D.,Tang B.,Zhang Z. J.,Ye X. L.,Li Q.,Xie J. P.,Mao X. H. 2019. Alteration of microRNA-4474/4717 expression and CREB-binding protein in human colorectal cancer tissues infected with Fusobacterium nucleatum. *PLoS One,* 14**,** e0215088.doi: https://doi.org/ 10.1371/journal.pone.0215088.

Franco A. A.,Mundy L. M.,Trucksis M.,Wu S.,Kaper J. B.,Sears C. L. 1997. Cloning and characterization of the Bacteroides fragilis metalloprotease toxin gene. *Infect Immun,* 65**,** 1007-13.doi: https://doi.org/ 10.1128/IAI.65.3.1007-1013.1997.

Gargalionis A. N.,Papavassiliou K. A.,Papavassiliou A. G. 2021. Targeting STAT3 Signaling Pathway in Colorectal Cancer. *Biomedicines,* 9**,** 1016.doi: https://doi.org/ 10.3390/biomedicines9081016.

Garrett W. S. 2019. The gut microbiota and colon cancer. *Science,* 364**,** 1133-1135.doi: https://doi.org/ 10.1126/science.aaw2367.

Gezer U.,Yoruker E. E.,Keskin M.,Kulle C. B.,Dharuman Y.,Holdenrieder S. 2015. Histone Methylation Marks on Circulating Nucleosomes as Novel Blood-Based Biomarker in Colorectal Cancer. *Int J Mol Sci,* 16**,** 29654-62.doi: https://doi.org/ 10.3390/ijms161226180.

Grivennikov S.,Karin E.,Terzic J.,Mucida D.,Yu G. Y.,Vallabhapurapu S.,Scheller J.,Rose-John S.,Cheroutre H.,Eckmann L.,Karin M. 2009. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. *Cancer Cell,* 15**,** 103-13.doi: https://doi.org/ 10.1016/j.ccr.2009.01.001.

Gur C.,Ibrahim Y.,Isaacson B.,Yamin R.,Abed J.,Gamliel M.,Enk J.,Bar-On Y.,Stanietsky-Kaynan N.,Coppenhagen-Glazer S.,Shussman N.,Almogy G.,Cuapio A.,Hofer E.,Mevorach D.,Tabib A.,Ortenberg R.,Markel G.,Miklic K.,Jonjic S.,Brennan C. A.,Garrett W. S.,Bachrach G.,Mandelboim O. 2015. Binding of the Fap2 protein of Fusobacterium nucleatum to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity,* 42**,** 344-355.doi: https://doi.org/ 10.1016/j.immuni.2015.01.010.

Hanahan D.,Weinberg R. A. 2011. Hallmarks of cancer: the next generation. *Cell,* 144**,** 646-

74.doi: https://doi.org/ 10.1016/j.cell.2011.02.013.
Hansson G. C. 2012. Role of mucus layers in gut infection and inflammation. *Curr Opin Microbiol,* 15**,** 57-62.doi: https://doi.org/ 10.1016/j.mib.2011.11.002.
He L.,He X.,Lim L. P.,de Stanchina E.,Xuan Z.,Liang Y.,Xue W.,Zender L.,Magnus J.,Ridzon D.,Jackson A. L.,Linsley P. S.,Chen C.,Lowe S. W.,Cleary M. A.,Hannon G. J. 2007. A microRNA component of the p53 tumour suppressor network. *Nature,* 447**,** 1130-4.doi: https://doi.org/ 10.1038/nature05939.
Hesson L. B.,Sloane M. A.,Wong J. W.,Nunez A. C.,Srivastava S.,Ng B.,Hawkins N. J.,Bourke M. J.,Ward R. L. 2014. Altered promoter nucleosome positioning is an early event in gene silencing. *Epigenetics,* 9**,** 1422-30.doi: https://doi.org/ 10.4161/15592294.2014.970077.
Hibner G.,Kimsa-Furdzik M.,Francuz T. 2018. Relevance of MicroRNAs as Potential Diagnostic and Prognostic Markers in Colorectal Cancer. *Int J Mol Sci,* 19.doi: https://doi.org/ 10.3390/ijms19102944.
Hirano T.,Hirayama D.,Wagatsuma K.,Yamakawa T.,Yokoyama Y.,Nakase H. 2020. Immunological Mechanisms in Inflammation-Associated Colon Carcinogenesis. *Int J Mol Sci,* 21.doi: https://doi.org/ 10.3390/ijms21093062.
Hong J.,Guo F.,Lu S. Y.,Shen C.,Ma D.,Zhang X.,Xie Y.,Yan T.,Yu T.,Sun T.,Qian Y.,Zhong M.,Chen J.,Peng Y.,Wang C.,Zhou X.,Liu J.,Liu Q.,Ma X.,Chen Y. X.,Chen H.,Fang J. Y. 2021. F. nucleatum targets lncRNA ENO1-IT1 to promote glycolysis and oncogenesis in colorectal cancer. *Gut,* 70**,** 2123-2137.doi: https://doi.org/ 10.1136/gutjnl-2020-322780.
Iftekhar A.,Berger H.,Bouznad N.,Heuberger J.,Boccellato F.,Dobrindt U.,Hermeking H.,Sigal M.,Meyer T. F. 2021. Genomic aberrations after short-term exposure to colibactin-producing E. coli transform primary colon epithelial cells. *Nat Commun,* 12**,** 1003.doi: https://doi.org/ 10.1038/s41467-021-21162-y.
Janney A.,Powrie F.,Mann E. H. 2020. Host-microbiota maladaptation in colorectal cancer. *Nature,* 585**,** 509-517.doi: https://doi.org/ 10.1038/s41586-020-2729-3.
Jasemi S.,Emaneini M.,Fazeli M. S.,Ahmadinejad Z.,Nomanpour B.,Sadeghpour Heravi F.,Sechi L. A.,Feizabadi M. M. 2020. Toxigenic and non-toxigenic patterns I, II and III and biofilm-forming ability in Bacteroides fragilis strains isolated from patients diagnosed with colorectal cancer. *Gut Pathog,* 12**,** 28.doi: https://doi.org/ 10.1186/s13099-020-00366-5.
Jiang L.,Hermeking H. 2017. miR-34a and miR-34b/c Suppress Intestinal Tumorigenesis. *Cancer Res,* 77**,** 2746-2758.doi: https://doi.org/ 10.1158/0008-5472.CAN-16-2183.
Joh H. K.,Lee D. H.,Hur J.,Nimptsch K.,Chang Y.,Joung H.,Zhang X.,Rezende L. F. M.,Lee J. E.,Ng K.,Yuan C.,Tabung F. K.,Meyerhardt J. A.,Chan A. T.,Pischon T.,Song M.,Fuchs C. S.,Willett W. C.,Cao Y.,Ogino S.,Giovannucci E.,Wu K. 2021. Simple Sugar and Sugar-Sweetened Beverage Intake During Adolescence and Risk of Colorectal Cancer Precursors. *Gastroenterology,* 161**,** 128-142 e20.doi: https://doi.org/ 10.1053/j.gastro.2021.03.028.

Kang M.,Martin A. 2017. Microbiome and colorectal cancer: Unraveling host-microbiota interactions in colitis-associated colorectal cancer development. *Semin Immunol,* 32**,** 3-13.doi: https://doi.org/ 10.1016/j.smim.2017.04.003.

Kato N.,Liu C. X.,Kato H.,Watanabe K.,Tanaka Y.,Yamamoto T.,Suzuki K.,Ueno K. 2000. A new subtype of the metalloprotease toxin gene and the incidence of the three bft subtypes among Bacteroides fragilis isolates in Japan. *FEMS Microbiol Lett,* 182**,** 171-6.doi: https://doi.org/ 10.1111/j.1574-6968.2000.tb08892.x.

Ke X.,Fei F.,Chen Y.,Xu L.,Zhang Z.,Huang Q.,Zhang H.,Yang H.,Chen Z.,Xing J. 2012. Hypoxia upregulates CD147 through a combined effect of HIF-1alpha and Sp1 to promote glycolysis and tumor progression in epithelial solid tumors. *Carcinogenesis,* 33**,** 1598-607.doi: https://doi.org/ 10.1093/carcin/bgs196.

Keum N.,Giovannucci E. 2019. Global burden of colorectal cancer: emerging trends, risk factors and prevention strategies. *Nat Rev Gastroenterol Hepatol,* 16**,** 713-732.doi: https://doi.org/ 10.1038/s41575-019-0189-8.

Kim J. M.,Lee J. Y.,Kim Y. J. 2008. Inhibition of apoptosis in Bacteroides fragilis enterotoxin-stimulated intestinal epithelial cells through the induction of c-IAP-2. *Eur J Immunol,* 38**,** 2190-9.doi: https://doi.org/ 10.1002/eji.200838191.

Kim N. H.,Kim H. S.,Kim N. G.,Lee I.,Choi H. S.,Li X. Y.,Kang S. E.,Cha S. Y.,Ryu J. K.,Na J. M.,Park C.,Kim K.,Lee S.,Gumbiner B. M.,Yook J. I.,Weiss S. J. 2011a. p53 and microRNA-34 are suppressors of canonical Wnt signaling. *Sci Signal,* 4**,** ra71.doi: https://doi.org/ 10.1126/scisignal.2001744.

Kim Y. H.,Lee H. C.,Kim S. Y.,Yeom Y. I.,Ryu K. J.,Min B. H.,Kim D. H.,Son H. J.,Rhee P. L.,Kim J. J.,Rhee J. C.,Kim H. C.,Chun H. K.,Grady W. M.,Kim Y. S. 2011b. Epigenomic analysis of aberrantly methylated genes in colorectal cancer identifies genes commonly affected by epigenetic alterations. *Ann Surg Oncol,* 18**,** 2338-47.doi: https://doi.org/ 10.1245/s10434-011-1573-y.

Kindler H. L.,Shulman K. L. 2001. Metastatic colorectal cancer. *Curr Treat Options Oncol,* 2**,** 459-71.doi: https://doi.org/ 10.1007/s11864-001-0068-7.

Kordahi M. C.,Stanaway I. B.,Avril M.,Chac D.,Blanc M. P.,Ross B.,Diener C.,Jain S.,McCleary P.,Parker A.,Friedman V.,Huang J.,Burke W.,Gibbons S. M.,Willis A. D.,Darveau R. P.,Grady W. M.,Ko C. W.,DePaolo R. W. 2021. Genomic and functional characterization of a mucosal symbiont involved in early-stage colorectal cancer. *Cell Host Microbe,* 29**,** 1589-1598 e6.doi: https://doi.org/ 10.1016/j.chom.2021.08.013.

Krude T. 1995. Chromatin. Nucleosome assembly during DNA replication. *Curr Biol,* 5**,** 1232-4.doi: https://doi.org/ 10.1016/s0960-9822(95)00245-4.

Larsson J. M.,Karlsson H.,Sjovall H.,Hansson G. C. 2009. A complex, but uniform O-glycosylation of the human MUC2 mucin from colonic biopsies analyzed by nanoLC/MSn. *Glycobiology,* 19**,** 756-66.doi: https://doi.org/ 10.1093/glycob/cwp048.

Lee-Six H.,Olafsson S.,Ellis P.,Osborne R. J.,Sanders M. A.,Moore L.,Georgakopoulos N.,Torrente F.,Noorani A.,Goddard M.,Robinson P.,Coorens T. H. H.,O'Neill L.,Alder C.,Wang J.,Fitzgerald R. C.,Zilbauer M.,Coleman N.,Saeb-Parsy K.,Martincorena

I.,Campbell P. J.,Stratton M. R. 2019. The landscape of somatic mutation in normal colorectal epithelial cells. *Nature,* 574**,** 532-537.doi: https://doi.org/ 10.1038/s41586-019-1672-7.

Lee D. W.,Han S. W.,Kang J. K.,Bae J. M.,Kim H. P.,Won J. K.,Jeong S. Y.,Park K. J.,Kang G. H.,Kim T. Y. 2018a. Association Between Fusobacterium nucleatum, Pathway Mutation, and Patient Prognosis in Colorectal Cancer. *Ann Surg Oncol,* 25**,** 3389-3395.doi: https://doi.org/ 10.1245/s10434-018-6681-5.

Lee Y. K.,Mehrabian P.,Boyajian S.,Wu W. L.,Selicha J.,Vonderfecht S.,Mazmanian S. K. 2018b. The Protective Role of Bacteroides fragilis in a Murine Model of Colitis-Associated Colorectal Cancer. *mSphere,* 3**,** e00587-18.doi: https://doi.org/ 10.1128/mSphere.00587-18.

Lennard K. S.,Goosen R. W.,Blackburn J. M. 2016. Bacterially-Associated Transcriptional Remodelling in a Distinct Genomic Subtype of Colorectal Cancer Provides a Plausible Molecular Basis for Disease Development. *PLoS One,* 11**,** e0166282.doi: https://doi.org/ 10.1371/journal.pone.0166282.

Li J.,Ma S.,Lin T.,Li Y.,Yang S.,Zhang W.,Zhang R.,Wang Y. 2019. Comprehensive Analysis of Therapy-Related Messenger RNAs and Long Noncoding RNAs as Novel Biomarkers for Advanced Colorectal Cancer. *Front Genet,* 10**,** 803.doi: https://doi.org/ 10.3389/fgene.2019.00803.

Li V. S. W. 2021. Modelling intestinal inflammation and infection using 'mini-gut' organoids. *Nat Rev Gastroenterol Hepatol,* 18**,** 89-90.doi: https://doi.org/ 10.1038/s41575-020-00391-4.

Liu H.,Ye D.,Chen A.,Tan D.,Zhang W.,Jiang W.,Wang M.,Zhang X. 2019. A pilot study of new promising non-coding RNA diagnostic biomarkers for early-stage colorectal cancers. *Clin Chem Lab Med,* 57**,** 1073-1083.doi: https://doi.org/ 10.1515/cclm-2019-0052.

Liu Q. Q.,Li C. M.,Fu L. N.,Wang H. L.,Tan J.,Wang Y. Q.,Sun D. F.,Gao Q. Y.,Chen Y. X.,Fang J. Y. 2020. Enterotoxigenic Bacteroides fragilis induces the stemness in colorectal cancer via upregulating histone demethylase JMJD2B. *Gut Microbes,* 12**,** 1788900.doi: https://doi.org/ 10.1080/19490976.2020.1788900.

Lopez L. R.,Bleich R. M.,Arthur J. C. 2021. Microbiota Effects on Carcinogenesis: Initiation, Promotion, and Progression. *Annu Rev Med,* 72**,** 243-261.doi: https://doi.org/ 10.1146/annurev-med-080719-091604.

MacDonald B. T.,Tamai K.,He X. 2009. Wnt/beta-catenin signaling: components, mechanisms, and diseases. *Dev Cell,* 17**,** 9-26.doi: https://doi.org/ 10.1016/j.devcel.2009.06.016.

Maiuri A. R.,Peng M.,Podicheti R.,Sriramkumar S.,Kamplain C. M.,Rusch D. B.,DeStefano Shields C. E.,Sears C. L.,O'Hagan H. M. 2017. Mismatch Repair Proteins Initiate Epigenetic Alterations during Inflammation-Driven Tumorigenesis. *Cancer Res,* 77**,** 3467-3478.doi: https://doi.org/ 10.1158/0008-5472.CAN-17-0056.

Martin-Gallausiaux C.,Beguet-Crespel F.,Marinelli L.,Jamet A.,Ledue F.,Blottiere H. M.,Lapaque N. 2018. Butyrate produced by gut commensal bacteria activates TGF-beta1 expression through the transcription factor SP1 in human intestinal epithelial cells. *Sci*

Rep, 8**,** 9742.doi: https://doi.org/ 10.1038/s41598-018-28048-y.
McIlvanna E.,Linden G. J.,Craig S. G.,Lundy F. T.,James J. A. 2021. Fusobacterium nucleatum and oral cancer: a critical review. *BMC Cancer,* 21**,** 1212.doi: https://doi.org/ 10.1186/s12885-021-08903-4.
Muralidhar S.,Filia A.,Nsengimana J.,Pozniak J.,O'Shea S. J.,Diaz J. M.,Harland M.,Randerson-Moor J. A.,Reichrath J.,Laye J. P.,van der Weyden L.,Adams D. J.,Bishop D. T.,Newton-Bishop J. 2019. Vitamin D-VDR Signaling Inhibits Wnt/beta-Catenin-Mediated Melanoma Progression and Promotes Antitumor Immunity. *Cancer Res,* 79**,** 5986-5998.doi: https://doi.org/ 10.1158/0008-5472.CAN-18-3927.
Naghshi S.,Sadeghian M.,Nasiri M.,Mobarak S.,Asadi M.,Sadeghi O. 2021. Association of Total Nut, Tree Nut, Peanut, and Peanut Butter Consumption with Cancer Incidence and Mortality: A Comprehensive Systematic Review and Dose-Response Meta-Analysis of Observational Studies. *Adv Nutr,* 12**,** 793-808.doi: https://doi.org/ 10.1093/advances/nmaa152.
Nougayrede J. P.,Homburg S.,Taieb F.,Boury M.,Brzuszkiewicz E.,Gottschalk G.,Buchrieser C.,Hacker J.,Dobrindt U.,Oswald E. 2006. Escherichia coli induces DNA double-strand breaks in eukaryotic cells. *Science,* 313**,** 848-51.doi: https://doi.org/ 10.1126/science.1127059.
Pan H.,Pan J.,Song S.,Ji L.,Lv H.,Yang Z. 2019. Identification and development of long non-coding RNA-associated regulatory network in colorectal cancer. *J Cell Mol Med,* 23**,** 5200-5210.doi: https://doi.org/ 10.1111/jcmm.14395.
Parhi L.,Alon-Maimon T.,Sol A.,Nejman D.,Shhadeh A.,Fainsod-Levi T.,Yajuk O.,Isaacson B.,Abed J.,Maalouf N.,Nissan A.,Sandbank J.,Yehuda-Shnaidman E.,Ponath F.,Vogel J.,Mandelboim O.,Granot Z.,Straussman R.,Bachrach G. 2020. Breast cancer colonization by Fusobacterium nucleatum accelerates tumor growth and metastatic progression. *Nat Commun,* 11**,** 3259.doi: https://doi.org/ 10.1038/s41467-020-16967-2.
Pierce J. V.,Bernstein H. D. 2016. Genomic Diversity of Enterotoxigenic Strains of Bacteroides fragilis. *PLoS One,* 11**,** e0158171.doi: https://doi.org/ 10.1371/journal.pone.0158171.
Pleguezuelos-Manzano C.,Puschhof J.,Rosendahl Huber A.,van Hoeck A.,Wood H. M.,Nomburg J.,Gurjao C.,Manders F.,Dalmasso G.,Stege P. B.,Paganelli F. L.,Geurts M. H.,Beumer J.,Mizutani T.,Miao Y.,van der Linden R.,van der Elst S.,Genomics England Research Consortium,Garcia K. C.,Top J.,Willems R. J. L.,Giannakis M.,Bonnet R.,Quirke P.,Meyerson M.,Cuppen E.,van Boxtel R.,Clevers H. 2020. Mutational signature in colorectal cancer caused by genotoxic pks(+) E. coli. *Nature,* 580**,** 269-273.doi: https://doi.org/ 10.1038/s41586-020-2080-8.
Prorok-Hamon M.,Friswell M. K.,Alswied A.,Roberts C. L.,Song F.,Flanagan P. K.,Knight P.,Codling C.,Marchesi J. R.,Winstanley C.,Hall N.,Rhodes J. M.,Campbell B. J. 2014. Colonic mucosa-associated diffusely adherent afaC+ Escherichia coli expressing lpfA and pks are increased in inflammatory bowel disease and colon cancer. *Gut,* 63**,** 761-70.doi: https://doi.org/ 10.1136/gutjnl-2013-304739.
Punt C. J.,Koopman M.,Vermeulen L. 2017. From tumour heterogeneity to advances in precision

703 treatment of colorectal cancer. *Nat Rev Clin Oncol,* 14**,** 235-246.doi: https://doi.org/
704 10.1038/nrclinonc.2016.171.
705 Rahier J. F.,Druez A.,Faugeras L.,Martinet J. P.,Gehenot M.,Josseaux E.,Herzog M.,Micallef
706 J.,George F.,Delos M.,De Ronde T.,Badaoui A.,D'Hondt L. 2017. Circulating
707 nucleosomes as new blood-based biomarkers for detection of colorectal cancer. *Clin*
708 *Epigenetics,* 9**,** 53.doi: https://doi.org/ 10.1186/s13148-017-0351-5.
709 Raver-Shapira N.,Marciano E.,Meiri E.,Spector Y.,Rosenfeld N.,Moskovits N.,Bentwich Z.,Oren
710 M. 2007. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis.
711 *Mol Cell,* 26**,** 731-43.doi: https://doi.org/ 10.1016/j.molcel.2007.05.017.
712 Rubinstein M. R.,Wang X.,Liu W.,Hao Y.,Cai G.,Han Y. W. 2013. Fusobacterium nucleatum
713 promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via
714 its FadA adhesin. *Cell Host Microbe,* 14**,** 195-206.doi: https://doi.org/
715 10.1016/j.chom.2013.07.012.
716 Russell A. B.,Peterson S. B.,Mougous J. D. 2014. Type VI secretion system effectors: poisons
717 with a purpose. *Nat Rev Microbiol,* 12**,** 137-48.doi: https://doi.org/ 10.1038/nrmicro3185.
718 Russell A. B.,Wexler A. G.,Harding B. N.,Whitney J. C.,Bohn A. J.,Goo Y. A.,Tran B. Q.,Barry
719 N. A.,Zheng H.,Peterson S. B.,Chou S.,Gonen T.,Goodlett D. R.,Goodman A.
720 L.,Mougous J. D. 2014. A type VI secretion-related pathway in Bacteroidetes mediates
721 interbacterial antagonism. *Cell Host Microbe,* 16**,** 227-236.doi: https://doi.org/
722 10.1016/j.chom.2014.07.007.
723 Saus E.,Iraola-Guzman S.,Willis J. R.,Brunet-Vega A.,Gabaldon T. 2019. Microbiome and
724 colorectal cancer: Roles in carcinogenesis and clinical potential. *Mol Aspects Med,* 69**,**
725 93-106.doi: https://doi.org/ 10.1016/j.mam.2019.05.001.
726 Savkovic S. D.,Koutsouris A.,Hecht G. 1996. Attachment of a noninvasive enteric pathogen,
727 enteropathogenic Escherichia coli, to cultured human intestinal epithelial monolayers
728 induces transmigration of neutrophils. *Infect Immun,* 64**,** 4480-7.doi: https://doi.org/
729 10.1128/iai.64.11.4480-4487.1996.
730 Scully R. 2010. The spindle-assembly checkpoint, aneuploidy, and gastrointestinal cancer. *N*
731 *Engl J Med,* 363**,** 2665-6.doi: https://doi.org/ 10.1056/NEJMe1008017.
732 Sears C. L.,Geis A. L.,Housseau F. 2014. Bacteroides fragilis subverts mucosal biology: from
733 symbiont to colon carcinogenesis. *J Clin Invest,* 124**,** 4166-72.doi: https://doi.org/
734 10.1172/JCI72334.
735 Shi J.,Li X.,Zhang F.,Zhang C.,Guan Q.,Cao X.,Zhu W.,Zhang X.,Cheng Y.,Ou K.,Chen Q.,Hu
736 S. 2015. Circulating lncRNAs associated with occurrence of colorectal cancer
737 progression. *Am J Cancer Res,* 5**,** 2258-65.doi: https://doi.org/
738 Shorning B. Y.,Dass M. S.,Smalley M. J.,Pearson H. B. 2020. The PI3K-AKT-mTOR Pathway
739 and Prostate Cancer: At the Crossroads of AR, MAPK, and WNT Signaling. *Int J Mol*
740 *Sci,* 21**,** 4507.doi: https://doi.org/ 10.3390/ijms21124507.
741 Siegel R. L.,Miller K. D.,Goding Sauer A.,Fedewa S. A.,Butterly L. F.,Anderson J. C.,Cercek
742 A.,Smith R. A.,Jemal A. 2020. Colorectal cancer statistics, 2020. *CA Cancer J Clin,* 70**,**
743 145-164.doi: https://doi.org/ 10.3322/caac.21601.

744 Siegel R. L.,Miller K. D.,Jemal A. 2020. Cancer statistics, 2020. *CA Cancer J Clin,* 70**,** 7-30.doi:
745 https://doi.org/ 10.3322/caac.21590.
746 Siemens H.,Jackstadt R.,Hunten S.,Kaller M.,Menssen A.,Gotz U.,Hermeking H. 2011. miR-34
747 and SNAIL form a double-negative feedback loop to regulate epithelial-mesenchymal
748 transitions. *Cell Cycle,* 10**,** 4256-71.doi: https://doi.org/ 10.4161/cc.10.24.18552.
749 Stanietsky N.,Simic H.,Arapovic J.,Toporik A.,Levy O.,Novik A.,Levine Z.,Beiman M.,Dassa
750 L.,Achdout H.,Stern-Ginossar N.,Tsukerman P.,Jonjic S.,Mandelboim O. 2009. The
751 interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc*
752 *Natl Acad Sci U S A,* 106**,** 17858-63.doi: https://doi.org/ 10.1073/pnas.0903474106.
753 Sun J.,Jia H.,Bao X.,Wu Y.,Zhu T.,Li R.,Zhao H. 2021. Tumor exosome promotes Th17 cell
754 differentiation by transmitting the lncRNA CRNDE-h in colorectal cancer. *Cell Death*
755 *Dis,* 12**,** 123.doi: https://doi.org/ 10.1038/s41419-020-03376-y.
756 Sung H.,Ferlay J.,Siegel R. L.,Laversanne M.,Soerjomataram I.,Jemal A.,Bray F. 2021. Global
757 Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide
758 for 36 Cancers in 185 Countries. *CA Cancer J Clin,* 71**,** 209-249.doi: https://doi.org/
759 10.3322/caac.21660.
760 Tan C. L.,Tan S. H.,So J. B.,Petersson F. 2013. Muco-submucosal elongated polyps of the
761 gastrointestinal tract: a case series and a review of the literature. *World J Gastroenterol,*
762 19**,** 1845-9.doi: https://doi.org/ 10.3748/wjg.v19.i11.1845.
763 Ternes D.,Karta J.,Tsenkova M.,Wilmes P.,Haan S.,Letellier E. 2020. Microbiome in Colorectal
764 Cancer: How to Get from Meta-omics to Mechanism? *Trends Microbiol,* 28**,** 401-
765 423.doi: https://doi.org/ 10.1016/j.tim.2020.01.001.
766 Tilg H.,Adolph T. E.,Gerner R. R.,Moschen A. R. 2018. The Intestinal Microbiota in Colorectal
767 Cancer. *Cancer Cell,* 33**,** 954-964.doi: https://doi.org/ 10.1016/j.ccell.2018.03.004.
768 Vogt M.,Munding J.,Gruner M.,Liffers S. T.,Verdoodt B.,Hauk J.,Steinstraesser L.,Tannapfel
769 A.,Hermeking H. 2011. Frequent concomitant inactivation of miR-34a and miR-34b/c by
770 CpG methylation in colorectal, pancreatic, mammary, ovarian, urothelial, and renal cell
771 carcinomas and soft tissue sarcomas. *Virchows Arch,* 458**,** 313-22.doi: https://doi.org/
772 10.1007/s00428-010-1030-5.
773 Wang C.,Yu J.,Han Y.,Li L.,Li J.,Li T.,Qi P. 2016. Long non-coding RNAs LOC285194, RP11-
774 462C24.1 and Nbla12061 in serum provide a new approach for distinguishing patients
775 with colorectal cancer from healthy controls. *Oncotarget,* 7**,** 70769-70778.doi:
776 https://doi.org/ 10.18632/oncotarget.12220.
777 Wang X.,Liu J.,Wang D.,Feng M.,Wu X. 2021. Epigenetically regulated gene expression
778 profiles reveal four molecular subtypes with prognostic and therapeutic implications in
779 colorectal cancer. *Brief Bioinform,* 22**,** bbaa309.doi: https://doi.org/ 10.1093/bib/bbaa309.
780 Wick E. C.,Rabizadeh S.,Albesiano E.,Wu X.,Wu S.,Chan J.,Rhee K. J.,Ortega G.,Huso D.
781 L.,Pardoll D.,Housseau F.,Sears C. L. 2014. Stat3 activation in murine colitis induced by
782 enterotoxigenic Bacteroides fragilis. *Inflamm Bowel Dis,* 20**,** 821-34.doi: https://doi.org/
783 10.1097/MIB.0000000000000019.
784 Wilmanski T.,Diener C.,Rappaport N.,Patwardhan S.,Wiedrick J.,Lapidus J.,Earls J. C.,Zimmer

785 A.,Glusman G.,Robinson M.,Yurkovich J. T.,Kado D. M.,Cauley J. A.,Zmuda J.,Lane N.
786 E.,Magis A. T.,Lovejoy J. C.,Hood L.,Gibbons S. M.,Orwoll E. S.,Price N. D. 2021. Gut
787 microbiome pattern reflects healthy ageing and predicts survival in humans. *Nat Metab,*
788 3**,** 274-286.doi: https://doi.org/ 10.1038/s42255-021-00348-0.
789 Wirbel J.,Pyl P. T.,Kartal E.,Zych K.,Kashani A.,Milanese A.,Fleck J. S.,Voigt A. Y.,Palleja
790 A.,Ponnudurai R.,Sunagawa S.,Coelho L. P.,Schrotz-King P.,Vogtmann E.,Habermann
791 N.,Nimeus E.,Thomas A. M.,Manghi P.,Gandini S.,Serrano D.,Mizutani S.,Shiroma
792 H.,Shiba S.,Shibata T.,Yachida S.,Yamada T.,Waldron L.,Naccarati A.,Segata N.,Sinha
793 R.,Ulrich C. M.,Brenner H.,Arumugam M.,Bork P.,Zeller G. 2019. Meta-analysis of fecal
794 metagenomes reveals global microbial signatures that are specific for colorectal cancer.
795 *Nat Med,* 25**,** 679-689.doi: https://doi.org/ 10.1038/s41591-019-0406-6.
796 Wu S.,Dreyfus L. A.,Tzianabos A. O.,Hayashi C.,Sears C. L. 2002. Diversity of the
797 metalloprotease toxin produced by enterotoxigenic Bacteroides fragilis. *Infect Immun,* 70**,**
798 2463-71.doi: https://doi.org/ 10.1128/IAI.70.5.2463-2471.2002.
799 Wu S.,Morin P. J.,Maouyo D.,Sears C. L. 2003. Bacteroides fragilis enterotoxin induces c-Myc
800 expression and cellular proliferation. *Gastroenterology,* 124**,** 392-400.doi: https://doi.org/
801 10.1053/gast.2003.50047.
802 Wu S.,Powell J.,Mathioudakis N.,Kane S.,Fernandez E.,Sears C. L. 2004. Bacteroides fragilis
803 enterotoxin induces intestinal epithelial cell secretion of interleukin-8 through mitogen-
804 activated protein kinases and a tyrosine kinase-regulated nuclear factor-kappaB pathway.
805 *Infect Immun,* 72**,** 5832-9.doi: https://doi.org/ 10.1128/IAI.72.10.5832-5839.2004.
806 Wu X. D.,Song Y. C.,Cao P. L.,Zhang H.,Guo Q.,Yan R.,Diao D. M.,Cheng Y.,Dang C. X.
807 2014. Detection of miR-34a and miR-34b/c in stool sample as potential screening
808 biomarkers for noninvasive diagnosis of colorectal cancer. *Med Oncol,* 31**,** 894.doi:
809 https://doi.org/ 10.1007/s12032-014-0894-7.
810 Xu M.,Yamada M.,Li M.,Liu H.,Chen S. G.,Han Y. W. 2007. FadA from Fusobacterium
811 nucleatum utilizes both secreted and nonsecreted forms for functional oligomerization for
812 attachment and invasion of host cells. *J Biol Chem,* 282**,** 25000-9.doi: https://doi.org/
813 10.1074/jbc.M611567200.
814 Yachida S.,Mizutani S.,Shiroma H.,Shiba S.,Nakajima T.,Sakamoto T.,Watanabe H.,Masuda
815 K.,Nishimoto Y.,Kubo M.,Hosoda F.,Rokutan H.,Matsumoto M.,Takamaru H.,Yamada
816 M.,Matsuda T.,Iwasaki M.,Yamaji T.,Yachida T.,Soga T.,Kurokawa K.,Toyoda A.,Ogura
817 Y.,Hayashi T.,Hatakeyama M.,Nakagama H.,Saito Y.,Fukuda S.,Shibata T.,Yamada T.
818 2019. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes
819 of the gut microbiota in colorectal cancer. *Nat Med,* 25**,** 968-976.doi: https://doi.org/
820 10.1038/s41591-019-0458-7.
821 Yang Y.,Misra B. B.,Liang L.,Bi D.,Weng W.,Wu W.,Cai S.,Qin H.,Goel A.,Li X.,Ma Y. 2019.
822 Integrated microbiome and metabolome analysis reveals a novel interplay between
823 commensal bacteria and metabolites in colorectal cancer. *Theranostics,* 9**,** 4101-4114.doi:
824 https://doi.org/ 10.7150/thno.35186.
825 Yang Y.,Weng W.,Peng J.,Hong L.,Yang L.,Toiyama Y.,Gao R.,Liu M.,Yin M.,Pan C.,Li

826 H.,Guo B.,Zhu Q.,Wei Q.,Moyer M. P.,Wang P.,Cai S.,Goel A.,Qin H.,Ma Y. 2017.
827 Fusobacterium nucleatum Increases Proliferation of Colorectal Cancer Cells and Tumor
828 Development in Mice by Activating Toll-Like Receptor 4 Signaling to Nuclear Factor-
829 kappaB, and Up-regulating Expression of MicroRNA-21. *Gastroenterology,* 152**,** 851-
830 866 e24.doi: https://doi.org/ 10.1053/j.gastro.2016.11.018.
831 Yu T.,Guo F.,Yu Y.,Sun T.,Ma D.,Han J.,Qian Y.,Kryczek I.,Sun D.,Nagarsheth N.,Chen
832 Y.,Chen H.,Hong J.,Zou W.,Fang J. Y. 2017. Fusobacterium nucleatum Promotes
833 Chemoresistance to Colorectal Cancer by Modulating Autophagy. *Cell,* 170**,** 548-563
834 e16.doi: https://doi.org/ 10.1016/j.cell.2017.07.008.
835 Zhao L.,Zhao N. 2021. Demonstration of causality: back to cultures. *Nat Rev Gastroenterol*
836 *Hepatol,* 18**,** 97-98.doi: https://doi.org/ 10.1038/s41575-020-00400-6.
837 Zhao Y.,Tao Q.,Li S.,Zheng P.,Liu J.,Liang X. 2020. Both endogenous and exogenous miR-139-
838 5p inhibit Fusobacterium nucleatum-related colorectal cancer development. *Eur J*
839 *Pharmacol,* 888**,** 173459.doi: https://doi.org/ 10.1016/j.ejphar.2020.173459.
840 Zhou L.,Sonnenberg G. F. 2018. Essential immunologic orchestrators of intestinal homeostasis.
841 *Sci Immunol,* 3**,** eaao1605.doi: https://doi.org/ 10.1126/sciimmunol.aao1605.
842

Figure 1(on next page)

The role and mechanism of ETBF in the pathogenesis of CRC

(a)Activation of the Wnt/*β*-catenin pathway by BFT. When BFT-r on the surface of colonic epithelial cells (CECs) is exposed to (and binds to) BFT toxin, the extracellular structure of E-cadherin cleaves, falls off and is degraded completely. As the structure of E-cadherin changes, *β*-catenin, which is bound to its intracellular domain dissociates. The abnormally expressed *β*-catenin escapes the regulation of APC protein and enters the nucleus to form a complex with TCF4. This leads to c-Myc activation. Eventually, the CECs become cancerous. (b) Inflammatory cascade activation by BFT. Colonic epithelial cells (CECs), neutrophils, and Th17 cells interact during BFT-induced inflammation. Invasion of CECs by ETBF results in IL-8 release for the recruitment of neutrophils. IL-6 released from the neutrophils activates the JAK/STAT3 signaling pathway in Th17 cells and CECs, via binding to IL-6-r. IL-17 secreted from mobilised TH17 cells plays autocrine and paracrine roles by binding to IL-17-r, resultings in the activation of the NF-*κ*B pathway in CECs and IL-6 as well. (c)The role of BFT at the tumorigenesis stage. Following BFT-induced overexpression of lncRNA-BFAL1, the later binds to miR-*155-5p* and *miR-200a-3p* to activate the mTORC1 pathway, which promotes further tumor growth. Activation of TLR4 by BFT leads to NFAT5 activation, upregulation of JMJD2B and demethylation of H3K9me3. Upregulation of NANOG and stemness of CRC cells are finally enhanced.

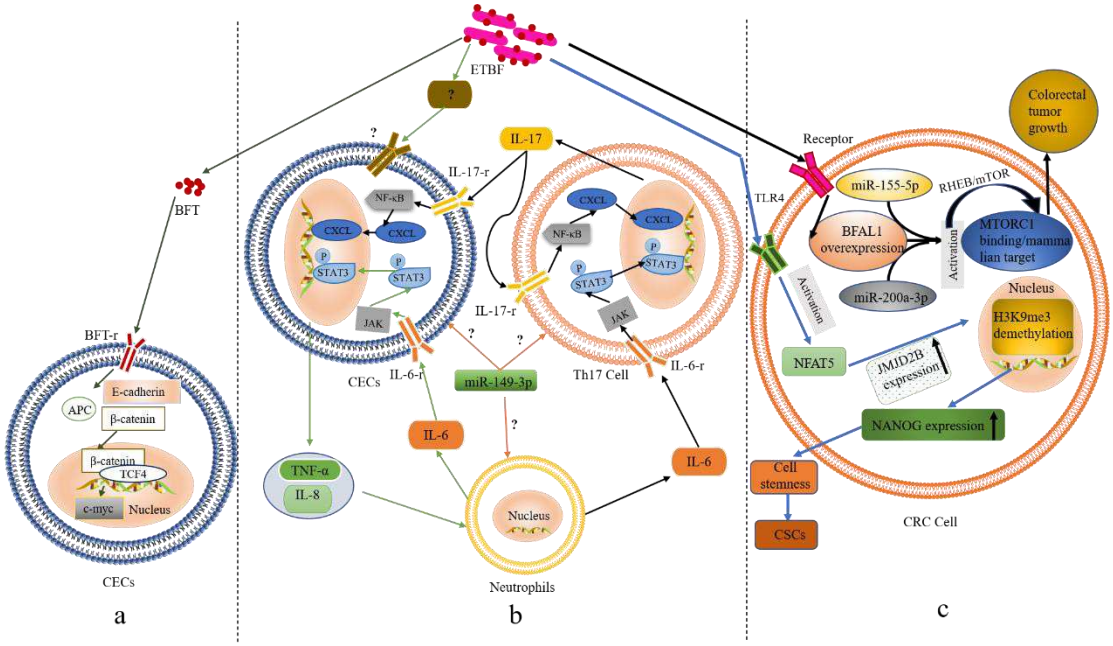

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Figure 2

The role and mechanism of *pks* + *E. coli* in the pathogenesis of CRC

Mutations in single bases and CIN are based on the "contribution" of *E. coli* toxins, which exhibit a "hit and run" mechanism. *E. coli* genotoxin induces *miR-20a-5p* expression via c-Myc (a transcription factor), and up-regulates the expression of *miR-20a-5p* (bound to SENP1) leading to the latter's translational silencing, and thus P53 SUMOylation. P53 SUMOylation leads to up-regulation of HGF phosphorylation of HGF-r and inactivation of *miR-34*, which promote tumor growth.

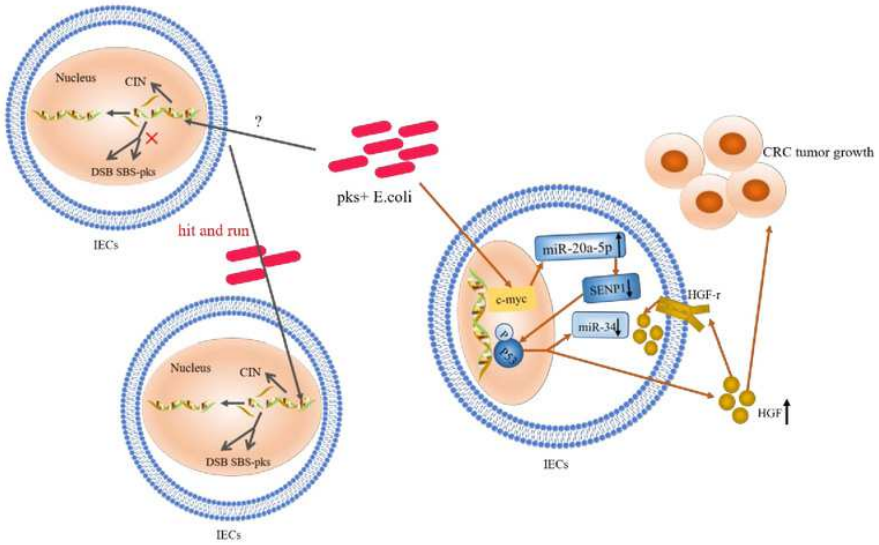

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Figure 3

The role and mechanism of *F.nucleatum* in the pathogenesis of CRC

F.nucleatum is recruited to colon tumor site by the binding of Fap2 to Gal-GalNAc, which is overexpressed in CRC. FadA (an *F.nucleatum* virulence factor) binds to E-cadherin to activate Wnt/ß-catenin signalling, leading to tumor development and CRC cell proliferation. Activation of immune cells such as NK and T cells is inhibited by specific binding of the adhesion protein Fap2 to hTIGIT. The expression of ENO1, via the transcription factor SP1 (regulated by *F. nucleatum*), leads to increased glycolysis.

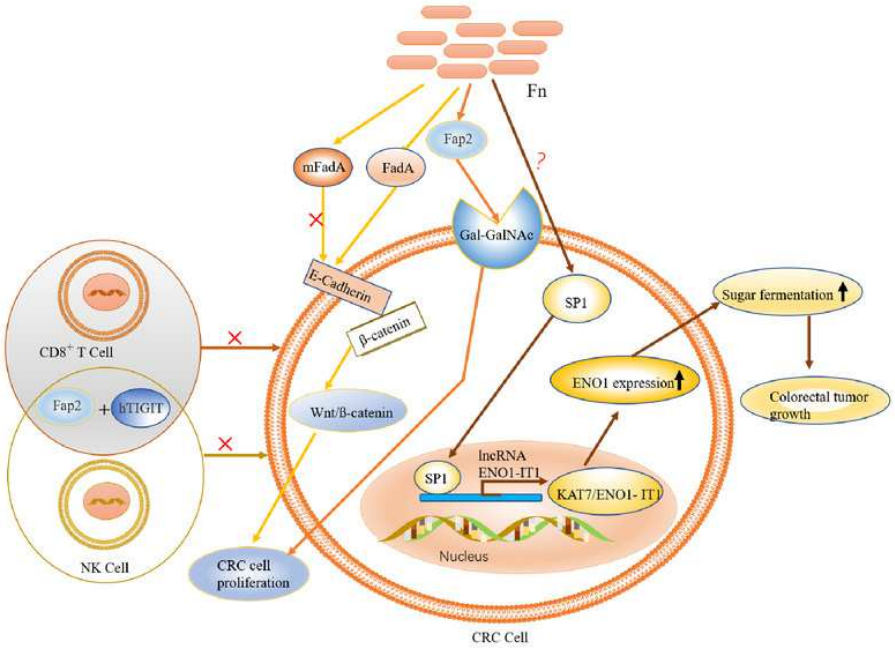

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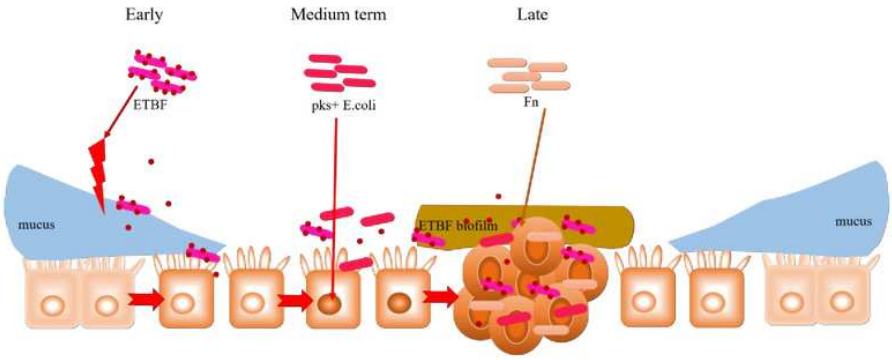
Figure 4

Hypothesised cooperative relationship between ETBF, *pks+ E. coli*, and *F. nucleatum*

During the precancerous stage of CRC, ETBF causes inflammation and this could lead to an imbalance in the ecological niche. This potential change in the intestinal ecology could provide the basic conditions for *pks+ E. coli* colonisation and the induction of genetic mutations in the carcinogenesis stage. Under the influence of *E. coli*, cancerous intestinal epithelial cells could further recruit *F.nucleatum* to colonise the lesion site. *F.nucleatum* may contribute to CRC advancement by primarily the development of cancer cells, stemization, and proliferation.



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