

***Fusobacterium's* link to colorectal neoplasia sequenced: A systematic review and future insights**

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Abstract

AIM

To critically evaluate previous scientific evidence on *Fusobacterium's* role in colorectal neoplasia development.

METHODS

Two independent investigators systematically reviewed all original scientific articles published between January, 2000, and July, 2017, using PubMed, EMBASE, and MEDLINE. A total of 355 articles were screened at the abstract level. Of these, only original scientific human, animal, and in vitro studies investigating *Fusobacterium* and its relationship with colorectal cancer (CRC) were included in the analysis. Abstracts, review articles, studies investigating other colonic diseases, and studies written in other languages than English were excluded from our analysis. Ninety articles were included after removing duplicates, resolving disagreements between the two reviewers, and applying the above criteria.

RESULTS

Studies have consistently identified positive associations between *Fusobacterium*, especially *Fusobacterium nucleatum* (*F. nucleatum*), and CRC. Stronger associations were seen in CRCs proximal to the splenic flexure and CpG island methylator phenotype (CIMP)-high CRCs. There was evidence of temporality and a biological gradient, with increased *F. nucleatum* DNA detection and quantity along the traditional adenoma-carcinoma sequence and in CIMP-high CRC precursors. Diet may have a differential impact on colonic *F. nucleatum* enrichment; evidence suggests that high fiber diet may reduce the risk of a subset of CRCs that are *F. nucleatum* DNA-positive. Data also suggest shorter CRC and disease-specific survival with increased amount of *F. nucleatum* DNA in CRC tissue. The pathophysiology of enrichment of *F. nucleatum* and other *Fusobacterium* species in colonic tissue is unclear; however, the virulence factors and changes to the local colonic environment with disruption of the protective mucus layer may contribute. The presence of a host lectin (Gal-GalNAc) in the colonic epithelium may also mediate *F. nucleatum* attachment to CRC and precursors through interaction with an *F. nucleatum* protein, fibroblast activation protein 2 (FAP2). The clinical significance of detection or enrichment of *Fusobacterium* in colorectal neoplasia is ambiguous, but data suggest a procarcinogenic effect of *F. nucleatum*, likely due to activation of oncogenic and inflammatory pathways and modulation of the tumor immune environment. This is hypothesized to be mediated by certain *F. nucleatum* strains carrying invasive properties and virulence factors such as FadA and FAP.

CONCLUSION

Evidence suggests a potential active role of *Fusobacterium*, specifically *F. nucleatum*, in CRC. Future prospective and experimental human studies would fill an important gap in this literature.

Key words: Colon microbiota; *Fusobacterium*; Systematic review; *Fusobacterium nucleatum*; Colorectal cancer; Colorectal polyps; Carcinogenesis

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Core tip: This is, to our knowledge, the first review to systematically examine the heterogeneous literature linking *Fusobacterium* to colorectal neoplasia. Accumulating evidence suggests that *Fusobacterium*, specifically *Fusobacterium nucleatum* (*F. nucleatum*), is more frequently detected in colorectal neoplasia, especially the pathway involving microsatellite instability. Multiple observational and animal experimental studies also suggest a procarcinogenic effect of *F. nucleatum*, likely due to activation of oncogenic and inflammatory pathways and modulation of the tumor immune environment. Virulence factors of *F. nucleatum* may contribute to its procarcinogenic effect. This information may be used to create novel strategies targeting colorectal cancer detection and chemoprevention.

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INTRODUCTION

Colorectal cancer (CRC) is the most common gastrointestinal cancer, as well as the third leading cancer by incidence and mortality, in the United States^[1]. The majority of CRC cases are sporadic, where a complex interaction among genetic and environmental factors impacts the carcinogenesis process. The underlying molecular changes follow at least two distinctive pathways of genomic dysfunction: the chromosomal instability (CIN) and CpG island methylator phenotype (CIMP)-high pathways. CIN is observed in 70%-85% of sporadic CRCs and describes aneuploidy due to gains or losses of whole or large portions of chromosomes^[2,3]. CIN is likely due to defects in chromosome segregation pathways and is likely initiated by the adenomatous polyposis coli (APC) mutation with subsequent β -catenin/Wnt-signaling pathway activation^[4]. APC mutation is followed by a cascade of molecular changes in a multistep fashion, as the flat mucosa evolves into a progressively larger adenoma that ultimately turns into cancer (adenoma-carcinoma sequence). CIMP-high CRCs include microsatellite instability (MSI)-high CRCs, with serrated polyps representing the main precursors^[3]. These account for 15% of all CRCs and are characterized by inactivation of mismatch repair enzymes and other tumor suppressor genes via mutations or hypermethylation^[5-8].

The human large intestine is a complex bacterial ecosystem that plays a significant role in health and disease. Increasing evidence suggests that a healthy symbiotic relationship between the host and microflora may be disrupted, leading to chronic metabolic and inflammatory changes promoting colorectal carcinogenesis^[9,10]. Although the technology to define the microbiome continues to evolve, the prevalence of some bacteria appears to be elevated in CRC. These include *Fusobacteria*, *Alistipes*, *Porphyromonadaceae*, *Coriobacteriaceae*, *Staphylococcaceae*, *Akkermansia*, and *Methanobacteriales*. Conversely, other bacteria exhibit reduced prevalence in CRC, including *Bifidobacterium*, *Lactobacillus*, *Ruminococcus*, *Faecalibacterium* spp., *Roseburia*, and *Treponema*^[11]. Although more research is warranted to establish firm causative links between CRC and flora diversity, patterns, specific microbial populations, and microbial functions, we are particularly intrigued by current data regarding *Fusobacterium*, a genus of the strictly anaerobic *Fusobacteria* phylum. Oral *Fusobacterium* consists mainly of the species *Fusobacterium nucleatum* (*F. nucleatum*), an

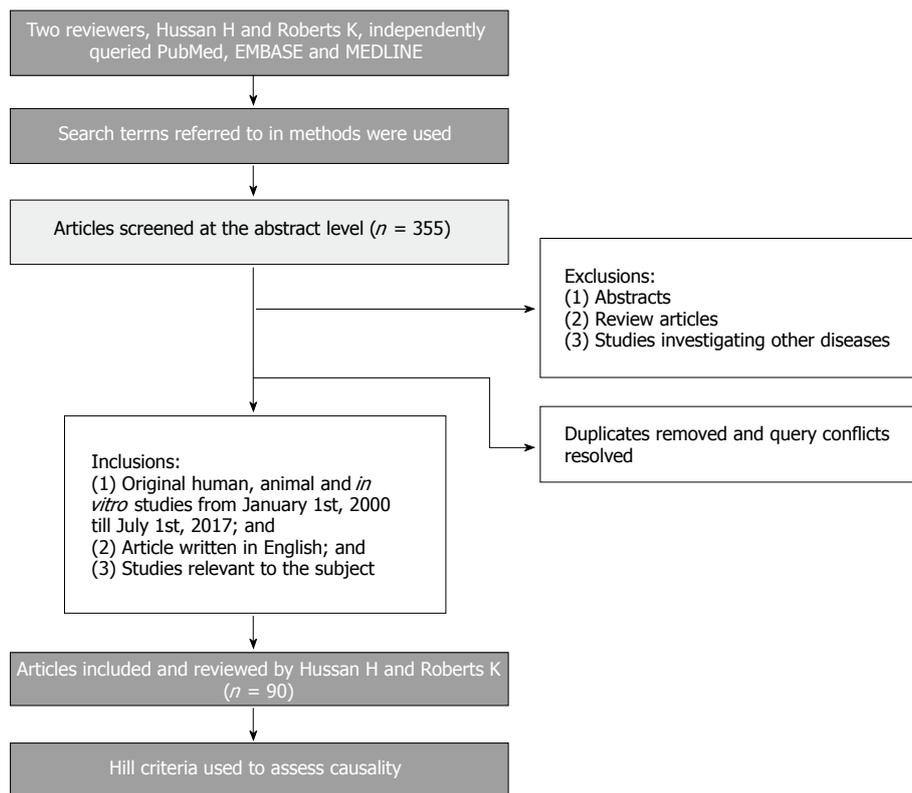


Figure 1 Diagram illustrating systematic review flow and methods.

adherent^[12], invasive^[13], and proinflammatory^[14,15] bacterium that is linked to periodontal disease^[16]. *F. nucleatum* is also the first anaerobic species to colonize the mouths of infants, indicating a potential prolonged exposure to *F. nucleatum* in adults who harbor it^[17-19]. *F. nucleatum* is classified into subspecies *animalis*, *fusiforme*, *nucleatum*, *polymorphum*, and *vincentii*^[20]. *F. varium* is another *Fusobacterium* species and has been associated with ulcerative colitis^[21, 22]. Other *Fusobacterium* species, such as *F. naviforme*, are mainly oral commensals and are associated with periodontal health^[19,23]. The presence of *Fusobacterium* in the colon, specifically *F. nucleatum*, is increasingly linked to CRC through a variety of recent studies, albeit with significant heterogeneity in study methods and findings. Thus, a critical evaluation of the scientific literature regarding the link between *Fusobacterium*/*F. nucleatum* and CRC may contribute to the development of more comprehensive and novel studies to better define this relationship.

MATERIALS AND METHODS

Two independent reviewers (HH and KR) systematically queried PubMed, Embase, and Medline using the following search terms: ("*Fusobacterium* " {All fields} OR "*Fusobacteria*" {All fields}) AND ("colon" {All fields}), "rectum" {All fields}, "colorectal" {All fields}, "colorectal cancer" {All fields}, "polyps" {All fields}, "adenomas" {All fields}, "serrated" {All fields}, "SSA" {All fields}, "SSP" {All fields}, "CIMP" {All fields},

"MSI" {All fields}, OR "microsatellite" {All fields}). On the basis of this search, 355 articles were screened at the abstract level. The following inclusion criteria were used: (1) Original human, animal, and in vitro studies investigating *Fusobacterium* and colorectal neoplasia that were published between January 1, 2000, and July 1st, 2017; (2) articles written in English; and (3) studies relevant to colorectal neoplasia. We excluded: (1) abstracts; (2) review articles; and (3) studies investigating other colonic diseases, such as ulcerative colitis. Ninety original articles were included after removing duplicates, resolving disagreements between the two reviewers, and applying the above criteria. The resulting 90 articles were then independently reviewed at the manuscript level by HH and KR. We used the Hill criteria to assess causality in the current evidence linking *F. nucleatum* and CRC^[24]. A brief illustration of our methods is shown in Figure 1.

RESULTS

Associations with CRC

Associations between *Fusobacterium* and CRC:

Consistent case-control studies using various samples including both stool and fresh and formalin-fixed paraffin-embedded (FFPE) colonic tissue demonstrated increased detection, quantity, and/or relative percentage of *Fusobacterium* rDNA copies in CRC tissue compared with matched adjacent noncancerous tissue and compared with healthy controls without colorectal neoplasia, as

summarized in Table 1^[25-40]. The histopathology of these findings is ambiguous, but some data suggest that *Fusobacteria* have been observed within the colonic bacterial biofilms, in the colonic mucus layer, within colonic crypts, and invading the colonic epithelium^[33,41-43]. *F. nucleatum* was the detected species of *Fusobacterium* in CRC tissue in 13 out of the 15 studies that presented species-level analysis^[41,44-55]. In two out of three studies that presented subspecies-level analysis, *F. nucleatum* subspecies *animalis* was the most frequent subspecies of *F. nucleatum* in CRC tissue^[40,54,56]. Other *Fusobacterium* species, such as *F. periodonticum*, *F. varium*, *F. ulcerans*, *F. necrophorum*, and *F. gonidiaformans*, were also identified in CRC tissue in the five remaining studies^[51,54,56-58]. *F. nucleatum*, *F. periodonticum*, *F. varium*, and *F. ulcerans* species can actively invade host cells, independently of mucosal compromise or presence of coinfection with other bacteria^[59,60]. Conversely, *F. necrophorum* and *F. gonidiaformans* are termed passive invaders, and their presence in CRC could be due to the disruption of the mucus layer seen with CRC or to coinfection with other invasive bacteria. In the largest study comparing genes of *Fusobacterium* species, active invaders such as *F. nucleatum* were found to harbor larger genomes encode adhesions, and contain twice as many genes encoding membrane-related proteins compared with other *Fusobacterial* species termed passive invaders^[59]. Thus, the presence of multiple *Fusobacterial* species could be due to their virulence and/or to early changes in the colonic environment that facilitate their presence in CRC tissue. Further studies are warranted to answer this question.

No major associations were found between *F. nucleatum* and characteristics of CRC patients such as age, gender, ethnicity, body mass index (BMI), smoking, or alcohol consumption, except in one South African study, where researchers found an association between *Fusobacterium* and both African race and age less than 60^[29,31,42,49,50,54,61,62]. In all studies, the prevalence of *F. nucleatum* rDNA in CRC tissue varied between 8.6% and 87.1%. This wide variability could be explained by heterogeneity in study design, sampling, analysis methodology, population, geographic location, or diet; these variations are summarized in Table 1^[38,46-48,52,63,64]. For instance, higher *F. nucleatum* detection is seen when using CRC tissue samples and whole-genome shotgun metagenomics sequencing methods, as compared with fecal samples or bacterial 16s sequencing^[10,26,50]. Furthermore, caution should be taken when interpreting studies of FFPE samples, because FFPE samples provide a less accurate assessment of the microbiome when compared with fresh-frozen samples^[65]. Finally, patients typically undergo bowel preparation when tissue samples are collected, which may affect *Fusobacterium* detection or abundance in tissue samples^[66]. We conclude that consistent associations are seen between *Fusobacterium*, mainly *F. nucleatum*, and CRCs, with variable prevalence of *F. nucleatum* in CRC subjects, which is likely due to heterogeneous methodologies. It would be of value

for future studies to utilize unprepared fresh-frozen colonic samples (for instance, unprepped flexible sigmoidoscopy with biopsies) when possible, combined with whole-genome shotgun metagenomic sequencing, in order to potentially yield more accurate detection and quantification of *Fusobacterium* and *F. nucleatum*.

Relation between *Fusobacterium* and dietary characteristics of CRC patients:

Low-fiber, high-fat Western diet administration over 2 wk to 20 Native Africans was associated with altered colonic microbiome and increased number of *F. nucleatum* rDNA copies in colonic tissue, in association with increases in early colonic biomarkers of CRC^[63]. It is interesting to note that colonic biopsies quantity of *F. nucleatum* rDNA copies did not decrease in 20 African Americans switched from a Western diet to a high-fiber, low-fat diet for 2 wk. This could be due to the small sample size, or it could take longer than 2 wk for dietary changes to reverse *F. nucleatum* rDNA abundance in colonic tissue^[63]. Recently, vegetable consumption was also inversely associated with relative concentration of *Fusobacterium* rDNA in stool of patients with advanced adenomas^[54]. However, the same study did not find associations between relative concentration of *Fusobacterium* rDNA in stools of 46 CRC patients and dietary habits such as consumption of red meat, processed meat, any meat, vegetables, or whole grains. This finding may be due to the cross-sectional nature of the study or to the researchers' superficial assessment of dietary habits and use of fecal samples as opposed to colonic tissue in their study^[54]. Mehta *et al.*^[64] prospectively investigated long-term dietary patterns in a cohort of 137217 patients using validated food frequency questionnaires. There were 1019 incidences of CRCs, which were classified into *F. nucleatum*-positive or *F. nucleatum*-negative CRCs based on presence or absence of *F. nucleatum* rDNA in CRC tissue respectively. They identified that, when compared with a Western diet, a diet rich in whole grains and dietary fiber (prudent diet) was associated with a lower risk of *F. nucleatum*-positive CRCs, with a hazard ratio (HR) of 0.43 (95%CI: 0.25-0.72; $P = 0.003$). No associations between prudent diet and *F. nucleatum*-negative CRC risk was identified, indicating a differential impact of prudent diet on CRC risk that are *F. nucleatum*-positive specifically^[64]. These inverse associations between prudent diet and *F. nucleatum*-positive CRCs were more pronounced when comparing high fiber intake (> 26 g/d for men and > 19 g/d for women) with the lowest fiber intake quartile (< 18 g/d for men and < 13 g/d for women; $P = 0.04$). Cereal-derived fiber had the strongest inverse association with *F. nucleatum*-positive CRCs (HR = 0.58; 95%CI: 0.34-0.99; $P = 0.03$)^[64]. Fruit consumption was also shown to reduce the risk of both *F. nucleatum*-positive and *F. nucleatum*-negative CRCs, with no specific relation to *F. nucleatum* status of the CRC^[64]. The researchers observed no impact of prudent diet subgroups (vegetables, legumes, or whole grains), on *F. nucleatum*-positive CRC risk, as also previously demonstrated^[29,54,64].

Table 1 Studies examining association between *Fusobacteria* and colorectal neoplasia

| Authors | Year | Cohort information | Specimen type | Detection method | Relation to CRC location | CRC features | <i>Fusobacteria</i> sequencing depth and associations with colorectal neoplasia |
|---------------------------------------|------|--|--|---|---|--------------|---|
| Ahn <i>et al</i> ^[25] | 2013 | United States cohort: 47 CRCs and 94 healthy controls. Matched for age, sex, BMI and hospital | Stool from cases and controls | 16S rRNA sequencing | - | - | Genus level: <i>Fusobacterium</i> rDNA was significantly more detected in of CRCs (31.9%) vs of controls (16%) |
| Vogtmann <i>et al</i> ^[26] | 2016 | United States cohort: 52 CRCs and 52 healthy controls, recruited between 1985-1987, matched by sex and BMI. Controls did not have a colonoscopy evaluation to rule out large polyps French validation cohort: 53 CRCs and 83 controls (61 healthy colons and 27 small adenomas) recruited from 2004-2006 | Stool from cases and controls | Whole-genome shotgun sequencing Compared to 16S rRNA sequencing from Ahn <i>et al</i> ^[25] study | - | - | Genus level: Whole genome analysis: <i>Fusobacterium</i> rDNA was significantly more detected in CRCs (76.9%) vs controls (48.1%). 16S rRNA sequencing: <i>Fusobacterium</i> rDNA was significantly more detected in CRCs (31.9%) vs controls (16%) Genus level: <i>Fusobacterium</i> rDNA was significantly more detected in CRCs (10.08%) vs controls (0.01%) |
| Gao <i>et al</i> ^[27] | 2015 | Chinese cohort: 31 CRCs and 30 healthy controls | Fresh-frozen tissue from cancer, adjacent non-cancerous tissue and normal mucosa samples at the time of surgery/ colonoscopy and after colonoscopy bowel preparation | 16S rRNA sequencing | Genus level: <i>Fusobacterium</i> rDNA was more detected in distal CRC compared to proximal CRC | - | Genus level: Higher relative percentage of <i>Fusobacterium</i> rDNA copies (relative to other bacterial rDNA) in CRCs (9.58%) vs adjacent non-cancerous tissues (0.57%) |
| Park <i>et al</i> ^[28] | 2016 | Korean cohort: 8 TAs, 10 SSA/Ps and 8 CRCs | Fresh-frozen tissue after colonoscopy bowel preparation | 16S rRNA sequencing | All CRCs were distal | - | Phylum level: <i>Fusobacterium</i> rDNA was detected in 37.5% of TAs, 50% of SSA/Ps and 100% of CRCs. Higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRC tissue (33.8%) vs TA (4.3%) and SSA (1.9%). No difference in relative concentration of <i>Fusobacterium</i> rDNA copies between TAs and SSA/Ps |
| Feng <i>et al</i> ^[29] | 2015 | Austrian cohort: 46 CRCs, 44 advanced adenomas and 57 healthy controls. Ages between 45-86 yr, both genders and White race | Stool from cases and controls | Metagenomics Whole-genome shotgun sequencing | - | - | Genus level: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRCs vs Carcinoma <i>in situ</i> . Higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRCs vs advanced adenomas vs controls Genus level: No association between relative percentage of <i>Fusobacterium</i> rDNA copies and anthropometric measures or diet (meat, fiber, vegetables and fruit intake) |

| | | | | | | | |
|--------------------------------------|------|---|--|-------------------------------------|--|---|---|
| Burns <i>et al</i> ^[30] | 2015 | United States cohort: 44 CRCs | Fresh-frozen tissue from cancer and adjacent non-cancerous tissue at the time of surgery and after bowel preparation | 16S rRNA sequencing | - | - | Species level: No specific species identification. Higher relative concentration of <i>Fusobacterium</i> rDNA copies in CRCs <i>vs</i> adjacent non-cancerous tissues. Correlated enrichment with <i>Providencia</i> Species level: No specific species identification. Tumor microbiome was enriched with genes encoding virulence and toxin proteins that were associated with and dependent on the presence of <i>Fusobacterium</i> and <i>Providencia</i> |
| Viljoen <i>et al</i> ^[31] | 2015 | South African cohort: 55 CRCs. 70.4% mixed ancestry, 14.8% White, 11.1% African, equal gender, 7 MSI-high (4 CRCs with Lynch syndrome), 3 MSI-low. 41.5% received chemo-radiation prior to sample collection. 18 FFPE CRCs that are MSI high (2 CRCs with Lynch syndrome) | Fresh-frozen tissue from cancer and adjacent non-cancerous tissue at the time of surgery and after bowel preparation. FFPE samples after bowel preparation | 16S rRNA sequencing Metagenomics | No association between number of <i>Fusobacterium</i> rDNA copies in CRC tissue and colon <i>vs</i> rectum location | Association between higher number of <i>Fusobacterium</i> rDNA copies in CRC tissue and MSI-H status | Species level: No specific species identification. <i>Fusobacterium</i> rDNA was detected in 82% of CRCs and 81% adjacent non-cancerous tissues with concurrent presence in 80% of paired CRC and adjacent tissue. Higher number of <i>Fusobacterium</i> rDNA copies in CRCs <i>vs</i> adjacent non-cancerous tissues. Species level: No specific species identification. Higher number of <i>Fusobacterium</i> rDNA copies in CRC tissue was associated with African race and age < 60. Species level: No specific species identification. Higher number of <i>Fusobacterium</i> rDNA copies in CRC tissue correlated with pks-positive <i>E. coli</i> in normal tissue; but no correlation with Enteropathogenic <i>Escherichia coli</i> , <i>Streptococcus gallolyticus</i> , <i>Enterococcus faecalis</i> , Enterotoxigenic <i>Bacteroides fragilis</i> or afaC-positive <i>E. coli</i> |
| Zhou <i>et al</i> ^[32] | 2016 | Chinese cohort: 97 CRCs and 48 healthy controls. Age and sex matched | Fresh-frozen tissue from cancer, adjacent non-cancerous tissue and normal mucosa samples at the time of surgery/ colonoscopy and after bowel preparation | 16S rRNA sequencing | No association between relative percentage of <i>Fusobacterium</i> rDNA copies in CRC tissue and colon <i>vs</i> rectum location | No association between relative percentage of <i>Fusobacterium</i> rDNA copies in CRC tissue and CEA, P53, EGFR, Ki67, CA199 or CRP | Species level: No specific species identification. Higher number of <i>Fusobacterium</i> rDNA copies correlated positively with presence of chronic inflammation in CRC tissue Species level: No specific species identification. <i>Fusobacterium</i> rDNA was detected in 72.16% in CRCs <i>vs</i> 67.01% of adjacent non-cancerous tissues, both higher than controls. Species level: Higher number of <i>Fusobacterium</i> rDNA copies correlated positively with that of Enterotoxigenic <i>Bacteroides fragilis</i> , <i>E. faecalis</i> in CRC tissue when compared to adjacent non-cancerous tissue |

| | | | | | | | |
|---------------------------------------|------|---|--|---------------------|--|---|--|
| Dejea <i>et al</i> ^[33] | 2014 | United States cohort: 30 CRCs, 6 adenomas and 22 healthy controls Malaysian cohort: 21 CRCs and 1 adenoma. | Fresh-frozen, formalin fixed tissue from tumors (adenomas and cancers), adjacent normal tissue and normal mucosa samples at the time of surgery/ colonoscopy after bowel preparation | 16S rRNA sequencing | Similar relative percentage of <i>Fusobacterium</i> rDNA copies ($\geq 25\%$) in CRC tissue proximal to hepatic flexure vs more distal CRC | - | Phylum level: <i>Fusobacterium</i> rDNA was detected in 32% of CRC, none of the matched normal tissue or healthy controls |
| Marchesi <i>et al</i> ^[34] | 2011 | Netherlands cohort: 6 CRCs | Fresh-frozen tissue from cancer and adjacent non-cancerous tissue at the time of surgery and after bowel preparation | 16S rRNA sequencing | - | - | Genus level: <i>Fusobacterium</i> rDNA was more detected with also higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRC compared to adjacent non-cancerous tissue |
| Thomas <i>et al</i> ^[35] | 2016 | Brazilian cohort: 18 rectal cancers (no prior neoadjuvant therapy) and 18 healthy controls | Fresh-frozen tissue from cancer and normal mucosa samples at the time of surgery/ colonoscopy and after bowel preparation | 16S rRNA sequencing | Rectal cancers only | - | Species level: No specific species identification. Higher number of <i>Fusobacterium</i> rDNA copies in rectal cancers compared to normal controls |
| Wang <i>et al</i> ^[36] | 2012 | Chinese cohort: 46 CRCs and 56 healthy controls | Stool, prior to bowel preparation | 16S rRNA sequencing | - | - | Genus level: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRC tissue compared to controls |
| Gao <i>et al</i> ^[37] | 2017 | Chinese cohort: 65 CRC patients | Fresh-frozen tissue from cancer and matched adjacent non-cancerous tissue at the time of surgery after bowel preparation | 16S rRNA sequencing | Genus level: <i>Fusobacterium</i> rDNA was more detected in distal CRC compared to proximal CRC | No association between relative percentage of <i>F. nucleatum</i> rDNA copies in CRC tissue and presence of K-ras mutation | Phylum level: <i>Fusobacterium</i> rDNA was significantly more detected in CRC (8.5%) compared to matched normal tissue (4.13%) |
| Allali <i>et al</i> ^[38] | 2015 | United States and Spanish cohorts: 90 CRC patients | Fresh-frozen tissue from cancer and adjacent non-cancerous tissue at the time of surgery after bowel preparation | 16S rRNA | - | United States cohort: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in both CRC and matched normal tissue in the right colon and splenic flexure vs left colon and sigmoid colon Spanish cohort: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRC tissue in the left colon | Phylum level: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRC vs matched normal tissue in Spanish cohort but not United States cohort. Higher relative percentage of <i>Fusobacterium</i> rDNA copies in matched adjacent non-cancerous tissue of the United States cohort compared to matched adjacent non-cancerous tissue of the Spanish cohort |
| Zackular <i>et al</i> ^[39] | 2014 | United States and Canadian cohort: 30 CRC, 30 TA and 30 healthy controls | Frozen fecal samples prior to colonoscopy and bowel preparation. | 16S rRNA | No relation between relative percentage of <i>Fusobacterium</i> rDNA copies in CRC tissue to CRC location | - | Genus level: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRC compared to both adenoma and healthy controls |

| | | | | | | | |
|--|------|--|--|--|---|---|---|
| Zeller <i>et al</i> ^[40] | 2014 | French cohort (population F): 53 CRC, 42 TAs, and 61 healthy control patients. German cohort (Population G): 38 CRC patients. German, Danish and Spanish cohorts (Population H): 297 IBD and healthy controls. German cohort (48 CRC patients at the time of CRC surgery) | Populations F and G: Stool prior to colonoscopy bowel preparation Population H: Stool German cohort: CRC and matched normal tissue | 16S rRNA | - | - | Species level: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRC compared to controls Subspecies level: <i>F. nucleatum</i> ssp. <i>vincentii</i> and <i>F. nucleatum</i> ssp. <i>animalis</i> are predominant in CRC tissue |
| Castellarin <i>et al</i> ^[41] | 2012 | Canadian cohort: 99 CRCs | Frozen tissue from cancer and adjacent non-cancerous tissue at the time of surgery after bowel preparation | Metagenomics <i>F. nucleatum</i> quantitative PCR | No association with proximal vs distal CRC location | Association between higher relative percentage of <i>F. nucleatum</i> rDNA copies in CRC and tumor involvement of more than 50% of the colon circumference | Subspecies level: Higher relative percentage of <i>F. nucleatum</i> subsp. <i>Nucleatum</i> rDNA copies in CRCs compared to matched normal tissues Species level: Confirmed <i>in vitro</i> invasion of <i>F. nucleatum</i> into human epithelial colonic cells Species level: No association between <i>F. nucleatum</i> and history of treatment or patient age |
| Chen <i>et al</i> ^[42] | 2017 | Chinese cohort: 14 CRCs 98 FFPE CRC | Frozen tissue at the time of surgery after bowel preparation FFPE CRC tissue after bowel preparation | 16S rRNA FISH <i>F. nucleatum</i> -targeted probe | Higher frequency of <i>F. nucleatum</i> rDNA detection in proximal CRC than distal location | - | 16s rRNA: Phylum level: <i>Fusobacterium</i> was a dominant phylum in CRC. FISH analysis. Species level: <i>F. nucleatum</i> rDNA was detected in 62.2% of FFPE CRC tissues No. difference in patients gender or age between CRCs that are <i>F. nucleatum</i> positive (detected) or absent |
| McCoy <i>et al</i> ^[43] | 2013 | United States Cohort: 10 CRCs, 48 adenomas and 67 healthy controls | Fresh-frozen normal rectal biopsies from adenoma and controls after bowel preparation Frozen tissue from CRC and adjacent non-cancerous tissue at the time of surgery after bowel preparation | <i>F. nucleatum</i> quantitative PCR 16S rRNA | - | Association between high number <i>F. nucleatum</i> rDNA copies in tissue and IL-6, IL-10, IL-17 and TNF- α in adenoma cases but no similar associations were seen in controls | Species level: Higher number of <i>F. nucleatum</i> rDNA copies seen in adenoma cases vs controls Species level: No association between <i>F. nucleatum</i> rDNA copy numbers and adenomas size/number Phylum level: Increased number of <i>Fusobacterium</i> rDNA copies in CRCs compared to matched normal tissues |
| Fukugaiti <i>et al</i> ^[44] | 2015 | Brazilian cohort: 7 CRCs and 10 healthy controls | Stool, prior to bowel preparation | 16S rRNA sequencing | - | - | Species level: Both <i>F. nucleatum</i> and <i>Clostridium difficile</i> had higher number of rDNA copies in stool of CRC patients when compared to controls |
| Warren <i>et al</i> ^[45] | 2013 | Canadian cohort: 65 CRCs | Frozen tissue from cancer and adjacent non-cancerous tissue at the time of surgery after bowel preparation | Metatranscriptomics | - | - | Species level: Higher relative percentage of <i>F. nucleatum</i> rDNA copies in CRC compared to matched normal tissue Genus level: Co-occurrence of <i>F. nucleatum</i> with <i>Campylobacter</i> (in vitro co-aggregation with <i>C. showae</i>) and <i>Leptotrichia</i> in CRC tissue Genus level: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in tumor tissue was correlated with host immune response genes and oncogenes |

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| Ito <i>et al</i> ^[46] 2015 | Japanese cohort: 138 Microvesicular HPs, 129 SSAs, 102 TSAs, 131 adenomas and 544 CRCs with matched adjacent non-cancerous tissue as well as 20 healthy controls | FFPE CRC tissue after bowel preparation | <i>F. nucleatum</i> quantitative PCR | No relation between <i>F. nucleatum</i> detection or higher number of rDNA copies in CRC and CRC location (Rectum vs Transverse colon to cecum) Gradual increase in percentage of SSAs that are <i>F. nucleatum</i> positive from sigmoid colon to cecum; no similar finding seen for TA, TSA and HP | High number of <i>F. nucleatum</i> rDNA copies in CRC was associated with MLH1 methylation, CMP-high status and MSI-high status. No association between detection or number of <i>F. nucleatum</i> rDNA copies in CRC to KRAS mutation, PIK3A mutation or miRNA 31 expression | Species level: <i>F. nucleatum</i> rDNA was detected in 56% of CRCs. Higher number of <i>F. nucleatum</i> rDNA copies in CRC tissue compared to matched normal tissue. <i>F. nucleatum</i> rDNA was not detected in 17/20 of healthy controls; no significant difference in number of <i>F. nucleatum</i> rDNA copies between matched normal tissue and healthy controls Species level: <i>F. nucleatum</i> rDNA detected in 24% of HPs, 35% of SSAs, 30% of TSAs and 33% of TAs. No difference in frequency of <i>F. nucleatum</i> rDNA detection between these groups Species level: <i>F. nucleatum</i> rDNA more frequently detected in CRCs compared to polyps after adjustment for confounders Species level: High number of <i>F. nucleatum</i> rDNA copies in CRC was positively associated with large tumor size |
| Nosho <i>et al</i> ^[47] 2016 | Japanese cohort: 511 CRCs | FFPE CRC tissue after bowel preparation | <i>F. nucleatum</i> quantitative PCR | - | <i>F. nucleatum</i> rDNA presence in CRC was associated with MSI high status | Species level: <i>F. nucleatum</i> rDNA was present in 8.6% of CRCs |
| Mima <i>et al</i> ^[48] 2015 | United States cohort: 598 CRCs from the Nurses' Health Study and Health Professionals Follow-up Study | FFPE CRC tissue after bowel preparation | <i>F. nucleatum</i> quantitative PCR | - | High number of <i>F. nucleatum</i> rDNA copies in CRC tissue was associated with lower CD3+ T-cells density. No association with CD8+, CD45RO+, or FOXP3+ T-cells density in CRC. No significant association with Crohn's-like histology, or tumor infiltrating lymphocytes | Species level: <i>F. nucleatum</i> rDNA was more frequently detected in CRCs (13%) compared to matched normal tissue (3.4%) |
| Kostic <i>et al</i> ^[49] 2012 | Spanish, United States and Vietnamese cohort: 95 CRCs | Frozen tissue from cancer and adjacent non-cancerous tissue at the time of surgery after bowel preparation | Whole genome sequencing 16S rRNA <i>F. nucleatum</i> quantitative, PCR | No association with CRC location | No association with CRC purity, inflammation, necrosis, and vascularization | Species level: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in CRC compared to matched normal tissue Detected species: <i>F. nucleatum</i> (most dominant species), <i>F. necrophorum</i> , <i>F. mortiferum</i> , and <i>F. perforans</i> . Species level: Higher relative percentage of <i>Fusobacterium</i> rDNA copies in Spanish vs US/Vietnamese cohorts Species level: No association with age, gender, ethnicity |
| Flanagan <i>et al</i> ^[50] 2014 | Czech cohort: 49 CRCs German cohort: 45 CRCs. Irish cohort: | Frozen tissue from cancer, adjacent non-cancerous tissue at the time of surgery after bowel preparation. Stool | <i>F. nucleatum</i> quantitative PCR | No association between relative percentage of <i>F. nucleatum</i> rDNA copies in CRC and colon vs | Association between higher relative percentage of <i>F. nucleatum</i> rDNA copies | Species level: Higher relative percentage of <i>F. nucleatum</i> rDNA copies in CRC and HGD compared to matched normal tissue. Similar relative |

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| | | 28 CRCs and 52 TAs Stool from 7 CRCs, 24 TAs patients (10 adenoma with HGDs, 12 TVAs and 2 adenomas) and 25 healthy controls | | | rectum location | in CRC tissue and TP53 mutation in the Irish cohort (Small sample size) Association between higher relative percentage of <i>F. nucleatum</i> rDNA copies in CRC tissue with KRAS mutation No association between <i>F. nucleatum</i> and BRAF mutation. No association between <i>F. nucleatum</i> and CRC grade | percentage of <i>F. nucleatum</i> rDNA copies in TA, TVA compared to their respective matched normal tissue Species level: Increased relative percentage of <i>F. nucleatum</i> rDNA copies during the adenoma-carcinoma progression in cancerous and matched normal tissue (TA to TVA to HGD to CRC) Species level: Increased relative percentage of <i>F. nucleatum</i> rDNA copies in stool of CRC patients compared to adenomas. Stool relative percentage of <i>F. nucleatum</i> rDNA copies is similar between adenoma and healthy controls patients. No significant correlation in relative percentage of <i>F. nucleatum</i> rDNA copies between disease tissue (CRC and adenomas) and stool samples from same patient |
| Wu <i>et al</i> ^[51] | 2013 | Chinese cohort: 19 CRCs and 20 healthy controls. Matched for age, sex and body mass index | Stool | 16S rRNA sequencing | | | Species level: Higher relative percentage of Fusobacterium rDNA copies compared to controls. Several species involved: <i>F. nucleatum</i> , <i>F. periodonticum</i> , <i>F. necrophorum</i> , <i>F. ulcerans</i> , <i>F. varium</i> , and <i>F. gonidiaformans</i> Species level: Increased relative percentage of <i>F. nucleatum</i> rDNA copies in 87.13% of CRCs compared to controls Higher relative percentage of <i>F. nucleatum</i> rDNA copies in CRC compared to controls Species level: <i>F. nucleatum</i> rDNA more frequently present in fecal and tissue samples of tumor group (CRCs and polyps) compared to controls |
| Li <i>et al</i> ^[52] | 2016 | Chinese cohort: 101 CRC patients | Fresh-frozen tissue from cancer and adjacent non-cancerous tissue at the time of surgery after bowel preparation | <i>F. nucleatum</i> quantitative PCR | - | - | Species level: Increased relative percentage of <i>F. nucleatum</i> rDNA copies in 87.13% of CRCs compared to controls Higher relative percentage of <i>F. nucleatum</i> rDNA copies in CRC compared to controls Species level: <i>F. nucleatum</i> rDNA more frequently present in fecal and tissue samples of tumor group (CRCs and polyps) compared to controls |
| Mira-Pascual <i>et al</i> ^[53] | 2015 | Spanish cohort: 7 CRC, 8 TA, 7 healthy controls | Frozen fecal samples prior to colonoscopy and bowel preparation Biopsies from normal rectal mucosa of controls and neoplasm of cases after bowel preparation | <i>F. nucleatum</i> quantitative PCR | - | - | Species level: <i>F. nucleatum</i> rDNA more frequently present in fecal and tissue samples of tumor group (CRCs and polyps) compared to controls |
| Amitay <i>et al</i> ^[54] | 2017 | German cohort of patients aged 50 years old and above: 46 CRC, 113 advanced adenomas (TA > 1 cm in size, TVA, or with HGD), 110 adenomas, and 231 healthy controls | Frozen fecal samples prior to colonoscopy and bowel preparation. Median time between collection and storage was 7 d | <i>F. nucleatum</i> quantitative PCR | - | - | Subspecies level: <i>Fusobacterium</i> rDNA was more frequently present in CRC (54.3%) than advanced adenoma (23.9%), TA (23.6%) and healthy controls (25.1%) ($P < 0.001$) rDNA sequence of <i>F. periodonticum</i> was more detected in CRC compared to controls ($P = 0.003$). No difference in detection of rDNA of <i>Fusobacterium simiae</i> , <i>F. nucleatum</i> ssp. <i>nucleatum</i> , <i>F. nucleatum</i> ssp. <i>animalis</i> , <i>F. nucleatum</i> ssp. <i>vincentii</i> and <i>F. nucleatum</i> ssp. <i>polymorphum</i> between CRC and controls No significant difference between relative concentration of <i>F. nucleatum</i> rDNA copies in advanced adenomas/TAs vs Controls |

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| Yu <i>et al</i> ^[55] | 2015 | Chinese cohort: 42 CRCs, 47 TAs and 52 healthy controls | Stool Left colonic biopsies | <i>F. nucleatum</i> quantitative PCR 16S rRNA | - | - | Species level: Relative percentage of <i>F. nucleatum</i> rDNA copies per sample gradually increased from healthy control to TAs and to CRC. Results seen in both stool and tissue samples |
| Ye <i>et al</i> ^[56] | 2017 | United States cohort: 25 CRC patients | Fresh-frozen tissue from CRC and adjacent non-cancerous tissue at the time of surgery after bowel preparation | <i>F. nucleatum</i> quantitative PCR 16S rRNA | - | Increased Chemokine (C-C motif) ligand 20 (CCL20) chemokine expression in all stages of CRC suggesting it is an early event in carcinogenesis. <i>F. nucleatum</i> ssp. <i>Animalis</i> induced CCL20 cytokine expression in CRC cell lines and monocytes. Monocytes are activated and migrate in the presence of <i>F. nucleatum</i> ssp. <i>Animalis</i> , this effect is inhibited by blocking CCL20 No control bacteria used in this experiment | Genus level: Increased relative percentage of <i>Fusobacterium</i> rDNA copies in CRC tissue <i>vs</i> normal matched tissue Species level: CRC samples contained <i>F. periodonticum</i> , <i>F. canifelinum</i> , <i>F. varium</i> , <i>F. simiae</i> , and <i>F. nucleatum</i> . <i>F. nucleatum</i> was the most frequently detected among <i>Fusobacterium</i> species Subspecies level: <i>F. nucleatum</i> ssp. <i>Animalis</i> most dominant among <i>F. nucleatum</i> subspecies in CRC samples |
| Chen <i>et al</i> ^[57] | 2012 | Chinese cohort: 46 CRCs and 56 healthy controls. BMI range 20-24, matched by sex | Stool and fecal swabs from cases and controls prior to bowel preparation Fresh-frozen tissue from cancer and adjacent non-cancerous tissue from cases at the time of surgery after bowel preparation | 16S rRNA sequencing | - | - | Species level: Increased relative concentration of <i>F. varium</i> rDNA copies in fecal swabs of CRCs compared to controls Genus level: Increased relative concentration of <i>Fusobacterium</i> rDNA copies in CRC tissues <i>vs</i> stool specimens (4.97% <i>vs</i> 0.47%, <i>P</i> < 0.001) Genus level: Unifrac PCA analysis found no difference in microbial composition of cancers and adjacent non-cancerous tissues |
| Kasai <i>et al</i> ^[58] | 2016 | Japanese cohort: 9 CRCs (3 invasive and 6 carcinoma in adenoma), 50 TAs and 49 healthy controls | Stool prior to colonoscopy bowel preparation | 16S rRNA sequencing | - | - | Species level: Increased relative percentage of <i>F. varium</i> rDNA copies in carcinoma in adenomas <i>vs</i> not detected in controls |
| Tahara <i>et al</i> ^[61] | 2014 | United States cohort: 149 CRCs and 89 adjacent tissues | Fresh-frozen CRC and adjacent non-cancerous tissue | Pan <i>Fusobacterium</i> and <i>F. nucleatum</i> quantitative PCR | - | Association with CIMP-high CRC, wild type TP53, MLH1 methylation, MSI-high and CHD7/8 mutation positivity | Genus and species level: <i>Fusobacterium</i> and <i>F. nucleatum</i> rDNA were more frequently detected in CRCs (74.3% and 52.3% respectively) compared to adjacent normal appearing mucosa (52.8% and 30.3% respectively). Higher relative percentage of <i>Fusobacterium</i> and <i>F. nucleatum</i> rDNA in CRC compared to normal appearing mucosa |

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| Yazici <i>et al</i> ^[62] | 2017 | United States cohort: CRC (97 African Americans and 56 Whites). Healthy controls (100 African Americans and 76 Whites) | Fresh-frozen CRC, adjacent non-cancerous tissue and normal mucosa samples at the time of surgery/ colonoscopy and after bowel preparation | <i>F. nucleatum</i> PCR 16S rRNA | - | - | Genera level: <i>Fusobacterium</i> was most abundant sulfidogenic bacteria identified in the study. No difference in relative concentrations of <i>Fusobacterium</i> rDNA copies between normal mucosa of cases and controls. Increased sulfidogenic bacteria in African Americans compared to non-Hispanic whites |
| Park <i>et al</i> ^[69] | 2017 | South Korean cohort: 160 MSI-high CRC. Excluded rectal carcinoma post neoadjuvant chemotherapy | FFPE CRC tissue after bowel preparation | <i>F. nucleatum</i> quantitative PCR | No association between relative concentrations of <i>F. nucleatum</i> rDNA copies in CRC and proximal versus distal/rectal CRC location | Association between high relative concentrations of <i>F. nucleatum</i> rDNA in CRC and BRAF mutation, CDKN2A (P16) promoter hypermethylation, tumor-infiltrating pan-macrophage density and CD68+ macrophages in tissue when compared with <i>F. nucleatum</i> -low/negative CRCs No association between high relative concentration of <i>F. nucleatum</i> rDNA in CRC tissue and CRC infiltrating CD3+ lymphocyte; PD-L1 expression status; Kras mutation; expression of MLH1, MSH2, MSH6 or PMS; CIMP status; or MLH1 methylation when compared to CRCs that were <i>F. nucleatum</i> -low/negative | Species level: Of the MSI-high CRCs, 9% had high <i>F. nucleatum</i> rDNA relative concentrations, 58% had low <i>F. nucleatum</i> rDNA relative concentrations, and 33% of CRCs had no detected <i>F. nucleatum</i> in the tissue |
| Wei <i>et al</i> ^[70] | 2016 | Chinese cohort: 180 CRCs, all stages, median follow up 47 months | Fresh-frozen tissue from cancer and adjacent non-cancerous tissue at the time of surgery after bowel preparation | <i>F. nucleatum</i> quantitative PCR | No association between relative concentration of <i>F. nucleatum</i> rDNA copies in CRC and CRC location in colon vs rectum | High relative concentration of <i>F. nucleatum</i> rDNA copies in CRC was associated with high TNF- α , MMP9, NF- κ B, β -catenin, CTNNB, KRAS and BRAF expression as well as lower MLH1 expression No association between relative concentration of <i>F. nucleatum</i> rDNA copies in CRC and COX1 or COX2 protein levels | Species level |
| Mima <i>et al</i> ^[72] | 2016 | United States cohort: 1,102 CRCs | FFPE CRC tissue after bowel preparation | <i>F. nucleatum</i> | Percentage of CRCs | - | Species level |

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| | | from the Nurses' Health Study and Health Professionals Follow-up Study. Median follow up of 10.7 years | | quantitative PCR | | with high number of <i>F. nucleatum</i> rDNA copies increased gradually from rectum (2.5%) to cecum (11%) with a linear trend ($P < 0.0001$) | | |
| Mima <i>et al.</i> ^[73] | 2015 | United States cohort: 1069 CRCs from the Nurses' Health Study and Health Professionals Follow-up Study. Median follow up of 10.7 years | FFPE CRC tissue after bowel preparation | <i>F. nucleatum</i> quantitative PCR | - | Association with proximal CRCs location | Association between high number of <i>F. nucleatum</i> rDNA copies in CRCs and poor tumor differentiation Association between high number of <i>F. nucleatum</i> rDNA copies in CRCs and MSI-high CRC independent of CIMP and BRAF status Association between high number of <i>F. nucleatum</i> rDNA copies in CRC and MLH1 methylation | Species level: |
| Yoon <i>et al.</i> ^[78] | 2017 | North Korean cohort: 6 CRCs, 6 TAs, 6 SSAs and 6 healthy controls. Equal male female distribution | Normal rectal mucosa after bowel preparation | 16S rRNA | - | - | - | Species level: <i>F. nucleatum</i> found in only one SSA patient rectal biopsy |
| Kostic <i>et al.</i> ^[79] | 2013 | United States and United Kingdom cohorts: 27 CRCs, 28 TAs and 31 healthy controls | Fresh-frozen tissue from adenomas and adjacent normal tissue at the time of colonoscopy after bowel preparation Stool | <i>Fusobacterium</i> quantitative PCR | - | - | - | Species level: No specific species identified <i>Fusobacterium</i> detected in 48% of adenomas. Increased number of <i>Fusobacterium</i> rDNA copies in adenomas compared to matched normal tissue Species level: No specific species identified Higher <i>Fusobacterium</i> detection and number of copies in stool from CRC and adenoma compared to controls |

CRC: Colorectal cancer; MSI: Microsatellite instability; FFPE: Formalin-fixed paraffin-embedded; BMI: Body mass index; PCR: Polymerase chain reaction; TAs: Tubular adenomas; SSA/Ps: Sessile serrated adenomas/polyps.

Limitations to the Mehta *et al.*^[64] study include the use of FFPE as opposed to fresh colonic samples and the study's observational design.

One explanation for the relationship between diet and colonic *F. nucleatum* is the potential impact of diet on oral *Fusobacterium* abundance. However, in a population-based case-control study, no associations were found between fiber intake and presence of oral *Fusobacteria*, and only modest positive correlations were found between consumption of saturated fatty acids, vitamin C, B vitamins, and vitamin E, on the one hand, and oral *Fusobacteria* abundance, on the other ($P < 0.01$)^[67]. Furthermore, no associations were observed

between oral *Fusobacterium* presence or abundance and CRC^[68]. However, that could be due to the study design, whereby patients' oral microbiomes were sampled after CRC resection and treatment; the study also lacks oral hygiene data^[68].

In summary, diet may have a differential impact on colonic *F. nucleatum* enrichment, with increased abundance of *F. nucleatum* in the colons of patients consuming a Western diet. Long-term consumption of a high fiber diet may reduce the risk of a subset of CRCs that are *F. nucleatum*-positive. Future studies investigating the mechanisms of the impact of diet on colonic *Fusobacterium* and, subsequently, CRC risk are

needed in order to determine dietary patterns targeting CRC chemoprevention and treatment. Furthermore, these data underline the importance of considering the impact of diet when investigating the links among *Fusobacterium*, other bacteria, and CRC.

***Fusobacterium* associations with CRC anatomic location:**

Many previous publications have reported no difference in presence or relative percentage of *Fusobacterium/F. nucleatum* rDNA copies in tissue or stool with respect to CRC location, as illustrated in Table 1^[31-33,39,43,46-48,69,70]. This could be due to varying definitions of high versus low *F. nucleatum* enrichment, unmeasured dietary confounders, and comparisons of colon versus rectum cancers, as opposed to proximal versus distal location. However, a few research teams have observed differences by CRC location. Yu *et al.*^[71] identified an increased *F. nucleatum* prevalence and relative concentrations in CRCs proximal to the splenic flexure as opposed to more distal CRCs^[42]. A recent report by Mima *et al.*^[72,73] looked primarily at *F. nucleatum* enrichment in relation to CRC location and found significant relationships between *F. nucleatum*-high CRC and location. The study used FFPE samples from a large United States CRC cohort and found a gradual linear increment in CRCs that had high number of *F. nucleatum* rDNA copies from rectum to cecum (2.5% vs 11%, respectively; $P < 0.0001$)^[72,73]. Contradictory findings were reported in two studies involving Chinese and Spanish cohorts with an increased detection and relative concentrations of *Fusobacterium* in CRCs distal to splenic flexure. These results could be due to small sample sizes, sampling bias, different geographic location and associated dietary patterns, or looking at *Fusobacterium* as opposed to *F. nucleatum* specifically^[27,37,38]. The increased prevalence of *F. nucleatum* in proximal CRC coincides with the presence of invasive bacterial biofilms in 89% of right-sided colonic cancers and their surrounding normal mucosa, which may suggest a more active bacterial role in right CRC carcinogenesis^[33]. Thus, current evidence is conflicting, but *F. nucleatum* may be more prevalent in CRC proximal to the splenic flexure, with a gradual increase in *F. nucleatum*-high CRCs from rectum to cecum. The increased *F. nucleatum* in proximal CRCs maybe due to *F. nucleatum* favoring anaerobic conditions, the presence of bacterial biofilms that facilitate its presence or to the differential impact of colonic lumen content on *F. nucleatum* abundance^[63,64]. These associations are summarized in Figure 2A.

***Fusobacterium* associations with CRC molecular features:**

Associations have been observed between *Fusobacterium/F. nucleatum* and certain subsets of CRC, such as MSI-high and CIMP-high phenotypes (Table 1)^[31,46,47,61,73]. *F. nucleatum* has also been associated with higher expression of *BRAF* and decreased *MLH1* expression, both of which are seen in MSI-high sporadic CRCs^[46,61,69,70,73]. *KRAS* mutations are usually associated with lower CRC methylation (CIMP negative), and

conflicting results were seen when the relation of *F. nucleatum* to *KRAS* mutation was evaluated^[37,46,50,69,70,74]. However, a large study by Ito *et al.*^[46] found no association between *KRAS* mutations and detection or number of *F. nucleatum* rDNA copies in CRC, which is consistent with an *F. nucleatum* predilection to CIMP-high CRCs. A recent, more in-depth investigation showed that presence of high number of *F. nucleatum* rDNA copies in CRC was associated with a 5-fold increased risk of having MSI-high CRCs, irrespective of CIMP-high status or *BRAF* mutation status^[73]. This suggests that CRCs with MSI-high status are linked to *F. nucleatum*, whether owing to inherited, somatic, or epigenetic inactivation of *MLH1*^[5,6]. Further testing that was restricted to MSI-high CRCs showed that high relative concentrations of *F. nucleatum* rDNA was associated with *CDKN2A* (P16) promoter hypermethylation, a tumor suppressor gene associated with CIMP-high CRC^[69]. Thus, there is increasing evidence linking MSI-high CRC to *Fusobacterium*, but ambiguity exists regarding whether the increased detection of *Fusobacterium* is a cause or consequence of MSI-high status and associated molecular findings in colorectal neoplasia.

***Fusobacterium* associations with CRC stage and prognosis:**

Previous investigation has assessed *Fusobacterium* in relation to CRC staging and patient survival with variable results, as summarized in Table 2. High percentage of *Fusobacterium* rDNA copies in CRC tissue was associated with worse depth of invasion in two large studies that looked specifically at CRC prognosis^[70,73]. Heterogeneity was seen when *F. nucleatum* abundance was evaluated in relation to lymph node metastasis^[32,41,42,70,71,73]. None of the aforementioned studies found any associations between *F. nucleatum* and distal metastatic disease. Lastly, higher *Fusobacterium* rDNA was associated with more advanced CRC stage in 2 out of 11 studies, suggesting a lack of correlation between *Fusobacterium* abundance and the Duke's or tumor, node, metastasis (TNM) staging classifications^[31,32,41,46,50,72,73]. Conflicting observations were made when *F. nucleatum* was investigated as a predictor of CRC-specific survival. However, in two large studies by Wei *et al.*^[70] and Mima *et al.*^[73] with 10-year follow up, high quantity of *F. nucleatum* rDNA copies in CRC samples was associated with shorter CRC-specific survival after adjustment for multiple confounders. CRC-specific survival was assessed only secondarily in the other studies, with negative results^[47,48,70,73]. Heterogeneous observations were made when *Fusobacterium* enrichment was assessed in relation to overall survival in CRC patients^[41,46,50,70,73]. A comprehensive evaluation by Mima *et al.*^[73] adjusted for many confounders and found no association between high, low, or negative *F. nucleatum* rDNA copies presence in CRC tissue and CRC patients' overall survival. The other two studies showing worsened overall survival had a shorter follow-up period and adjusted for fewer

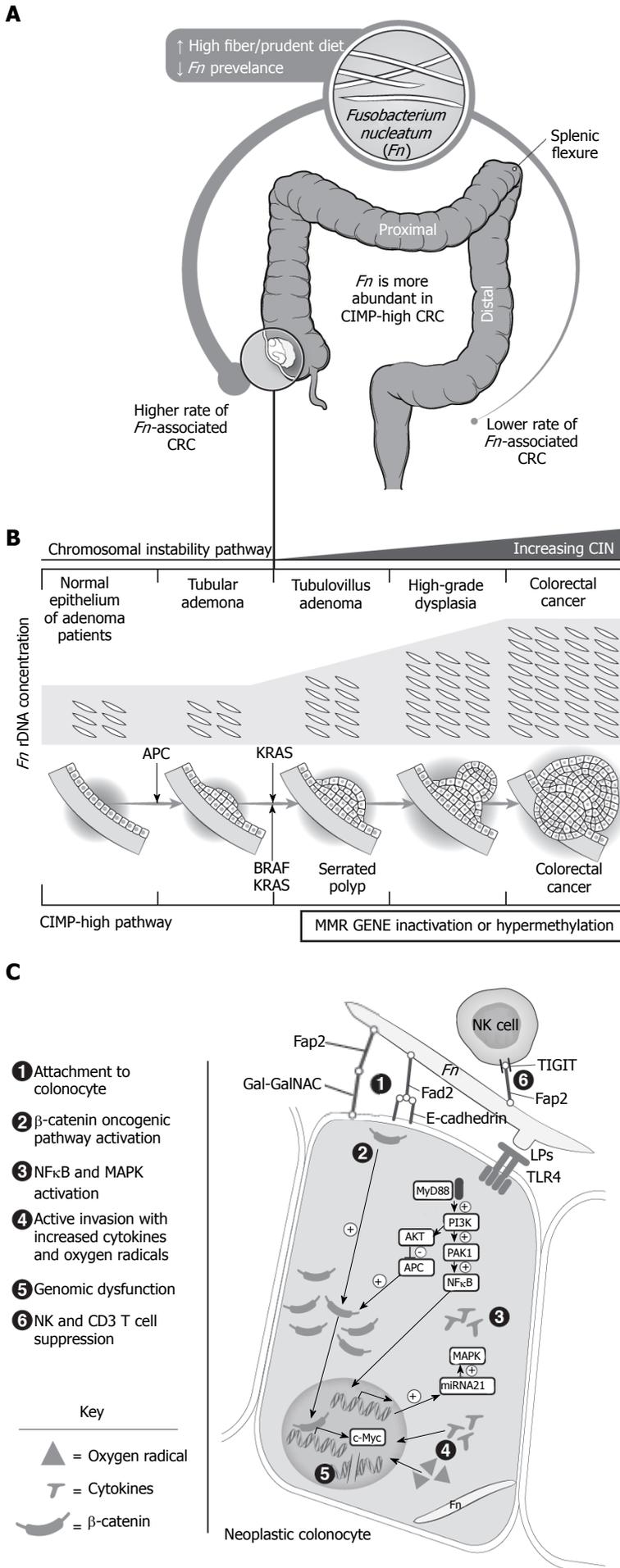


Figure 2 A simplified figure illustrating the link between *Fusobacterium nucleatum* and colorectal cancer. **A:** Association between *Fusobacterium nucleatum* (*F. nucleatum*) and colorectal cancer (CRC) pathways and location; **B:** *F. nucleatum* prevalence along chromosomal instability (CIN) and the CpG island methylator phenotype (CIMP)-high CRC pathways and their precursors; **C:** Postulated mechanisms of *F. nucleatum* induced colorectal carcinogenesis.

Table 2 Studies looking at *Fusobacterium* associations with colorectal cancer stage and prognosis

| Authors | Sample size | CRC depth of invasion | CRC lymph nodes metastasis | CRC metastatic disease | CRC stage | CRC prognosis |
|--|---|---|---|---|---|--|
| Viljoen <i>et al</i> ^[31] | South African cohort: 55 CRCs. | - | - | - | Association between higher number of <i>F. nucleatum</i> rDNA copies and late stage CRC (stage III and IV compared to stage I and II) | - |
| Zhou <i>et al</i> ^[32] | Chinese cohort: 97 CRCs | No association with relative percentage of <i>Fusobacterium</i> rDNA copies | No association with relative percentage of <i>Fusobacterium</i> rDNA copies | No association with relative percentage of <i>Fusobacterium</i> rDNA copies | No association with relative percentage of <i>Fusobacterium</i> rDNA copies | - |
| Zackular <i>et al</i> ^[39] | United States and Canadian cohort: 30 CRC, 30 TA, 30 healthy controls | - | - | - | No association with relative percentage of <i>F. nucleatum</i> rDNA copies in CRC | - |
| Castellarin <i>et al</i> ^[41] | Canadian cohort: 99 CRCs | - | Association between relative percentage of <i>F. nucleatum</i> rDNA copies in CRC and regional lymph nodes metastasis | - | No association with relative percentage of <i>F. nucleatum</i> rDNA copies in CRC | No association to between relative percentage of <i>F. nucleatum</i> rDNA copies in CRC and CRC overall survival |
| Chen <i>et al</i> ^[42] | Chinese cohort: 98 CRCs | - | No association with presence of <i>F. nucleatum</i> rDNA in CRC | - | - | - |
| Ito <i>et al</i> ^[46] | Japanese cohort: 544 CRCs | - | - | - | No association with detection or number of <i>F. nucleatum</i> rDNA copies | No association between detection or number of <i>F. nucleatum</i> rDNA copies and CRC overall survival-unknown follow up period |
| Nosho <i>et al</i> ^[47] | Japanese cohort: 511 CRCs | - | - | - | No association with detection of <i>F. nucleatum</i> rDNA in CRC | No association between <i>F. nucleatum</i> rDNA presence in CRC and CRC-specific survival-unknown follow up period |
| Mima <i>et al</i> ^[48] | United States cohort: 598 CRCs. | - | - | - | - | No relation between <i>F. nucleatum</i> rDNA copies in CRC and CRC-specific survival or CRC overall survival- unknown follow up period |
| Flanagan <i>et al</i> ^[50] | Czech, German and Irish cohorts: 122 CRCs | - | - | - | No association with relative percentage of <i>E. nucleatum</i> rDNA copies | Higher relative percentage of <i>F. nucleatum</i> rDNA copies was associated with shorter CRC overall survival within 3-5 years follow up (HR = 19.96, 95%CI: 1.42-281.42) (no adjustment for other confounders) |
| Li <i>et al</i> ^[52] | Chinese cohort: 101 CRC | No association | Association between relative percentage of <i>F. nucleatum</i> rDNA copies in CRC and lymph nodes metastasis | - | No association with relative percentage of <i>F. nucleatum</i> rDNA copies in CRC | - |
| Amitay <i>et al</i> ^[54] | German cohort: 46 CRC | - | - | - | Relative percentage of <i>F. nucleatum</i> rDNA copies in CRC was associated with advanced stage [stage I vs II ($P = 0.012$) and stage I vs III ($P = 0.042$)] | - |

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|-----------------------------------|--|--|--|--|--|--|
| Park <i>et al</i> ^[69] | South Korean cohort: 160 MSI-high CRC. | - | No association with <i>F. nucleatum</i> rDNA detection or number of copies | - | No association with <i>F. nucleatum</i> rDNA detection or number of copies | No association between <i>F. nucleatum</i> rDNA detection or number of copies and disease-free survival |
| Wei <i>et al</i> ^[70] | Chinese cohort: 180 CRCs | Association between high relative percentage of <i>F. nucleatum</i> rDNA copies in CRC and depth of invasion | Association between high relative percentage of <i>F. nucleatum</i> rDNA copies in CRC and lymph nodes metastasis | - | - | High relative percentage of <i>F. nucleatum</i> rDNA copies in CRC was associated shorter CRC overall survival within 3 years follow up [HR = 1.993 (1.024 to 3.879)] High relative percentage of <i>F. nucleatum</i> rDNA copies in CRC was associated with shorter CRC disease-free survival within 3 years follow up [HR = 1.829 (1.000 to 3.345)] |
| Yu <i>et al</i> ^[71] | Chinese cohort: 88 CRCs | - | <i>F. nucleatum</i> rDNA was more frequently detected in metastatic lymph nodes of proximal <i>vs</i> distal CRC <i>F. nucleatum</i> detected in 100% of metastatic lymph nodes compared to 40% of lymph nodes without metastasis ($P < 0.001$) | - | - | - |
| Mima <i>et al</i> ^[73] | United States cohort: 1069 CRCs | Association between number of <i>F. nucleatum</i> rDNA copies in CRC and higher pT of the TNM staging | No association with number of <i>F. nucleatum</i> rDNA copies in CRC | No association with number of <i>F. nucleatum</i> rDNA copies in CRC | No association with number of <i>F. nucleatum</i> rDNA copies in CRC | High number of <i>F. nucleatum</i> rDNA copies in CRC was associated with shorter CRC-specific survival within 10.7 years follow up [HR = 1.58 (1.04 to 2.39)] for <i>F. nucleatum</i> -high <i>vs</i> <i>F. nucleatum</i> -negative CRCs]. (Multivariable models included CRC stage, age, sex, year of diagnosis, family history of CRC, CRC location, MSI status, CIMP status, KRAS status, BRAF, PIK3CA and CRC LINE-1 methylation.) No association between <i>F. nucleatum</i> rDNA copies in CRC and CRC overall mortality |

CRC: Colorectal cancer; CIMP: CpG island methylator phenotype.

covariates than did Mima *et al*^[50,70]. In one study, *Fusobacterium* subspecies were predominantly present in the goblet-like transcriptional CRC subtype^[74-76]. This CRC subtype confers good prognosis in chemotherapy-untreated patients, but it has a detrimental effect on prognosis when adjuvant chemotherapy or chemoradiation are used^[77]. Thus, CRC treatments, as well as CRC molecular subtypes, need to be investigated when looking at the impact of *F. nucleatum* presence on CRC survival. The above evidence is conflicting but suggests a more aggressive CRC biology with shorter CRC disease-specific survival periods in the presence of *F. nucleatum*; however, there are no relations between *F. nucleatum* and CRC staging. Thus, clarification is warranted of whether *F.*

nucleatum modulation in CRC tissue is associated with better disease-free and CRC-specific survival rates after accounting for CRC molecular features and treatments.

Temporality and biological gradient

F. nucleatum prevalence and enrichment was evaluated in CRC precursors in order to assess temporality and an earlier role in CRC carcinogenesis (Figure 2B and Table 1). The number of rDNA copies of *F. nucleatum* was higher in normal rectal/left colonic biopsies of patients with tubular adenomas (TAs) compared with controls^[43,55]. The exception is a study that found no evidence of *F. nucleatum* in rectal biopsies of controls, TA patients, or CRC patients; this result is likely due to a small sample size^[78]. Higher presence

and relative percentage of *Fusobacterium*^[29] and *F. nucleatum*^[55,79] rDNA copies was seen in stools of patients with TAs compared with those of controls. On the contrary, two studies found no significant difference in relative percentage of *Fusobacterium*^[39] and *F. nucleatum*^[54] rDNA copies in fecal samples of patients with TAs, advanced TAs, and controls. Reasons for this discrepancy could include the use of fecal samples, which may represent transit from oral microbiome and may not necessarily correlate with true *Fusobacterium* abundance in colonic tissue, as well as absence of information on prior antibiotic use^[10,26,50]. Detection and relative percentage of *Fusobacterium*^[28] and specifically *F. nucleatum*^[46,50,55] rDNA copies were found to increase in colonic tissue as it progressed through the CIN pathway (healthy control vs TA vs tubulovillous adenoma [TVA] vs high grade dysplasia vs CRC). Interestingly, no difference in relative percentage of *F. nucleatum* DNA copies was observed between TA and TVA tissue when compared with surrounding normal tissues^[28,46,50]. This finding maybe be due to presence of bacterial biofilms or precancerous molecular changes in the surrounding normal mucosa, despite normal histological appearance, which may favor the attachment or invasion of *Fusobacterium*^[33,80]. Data are limited but suggest no relation between adenoma size or burden and *F. nucleatum* rDNA copy numbers in rectal tissue^[43]. Finally, *F. nucleatum* was associated with CIMP-high and right-sided sessile serrated adenomas/polyps (SSA/Ps) when SSA/Ps were compared with TAs^[28,46,71]. Limitations to the above studies include an absence of information on concomitant preneoplastic tissue in patients who had hyperplastic polyps and the simultaneous use of FFPE and colonic preparation in specimens collected, which could reduce *F. nucleatum* detection^[66,81]. All these data suggest that there may be a stepwise increase in *F. nucleatum* rDNA quantity and detection as colorectal neoplasms progress through the CIN pathway. Furthermore, *F. nucleatum* may play an earlier role in the CIMP-high CRC pathway. These results suggest temporality and a biological gradient of *F. nucleatum* in CRC development.

Plausible mechanisms and experimental evidence

Despite the accumulating associations between *Fusobacterium/F. nucleatum* and colorectal neoplasia, establishing direct causality is challenging with the absence of prospective human studies supported by correlative laboratory science. In brief, multiple observational and animal experimental studies suggest plausible mechanisms by which *F. nucleatum* may contribute to CRC development, and these warrant additional investigation (Figure 2C).

***F. nucleatum* transmission to colorectal neoplastic tissue:**

Rats treated with 1,2-dimethylhydrazine (DMH) had increased *Fusobacterium* detection in tumors, whereas it was absent in nontreated controls, indicating a predilection of *Fusobacterium* to tumor tissue^[82].

Oral administration of *F. nucleatum* into *APC*^{min/+} and DMH-treated mice led to colorectal colonization and promoted colorectal neoplasia development, suggesting an active role of *F. nucleatum* in CRC neoplasia^[55,79]. Similar findings were not seen in wild-type mice, suggesting that *F. nucleatum* can be contracted through oral ingestion if individuals are already predisposed to CRC. The mechanism by which *F. nucleatum* reaches the colonic epithelium are unclear. However, some *F. nucleatum* strains display the potential to disrupt the colonic mucosal barrier, suggesting that it can be transmitted from the colonic lumen to the epithelium, potentially causing colorectal disease^[60]. Other *Fusobacteria* may take advantage of coinfection with other invasive bacteria or of disruption of the mucosal layer, seen with CRC. Another mechanism by which *Fusobacteria* home and localize to dysplastic colorectal epithelium is the blood-borne route^[83]. In a novel study, a host lectin (Gal-GalNAc) was shown to mediate *F. nucleatum* attachment to CRC and precursor cells through interaction with an *F. nucleatum* protein, fibroblast activation protein 2 (FAP2)^[83]. The expression of Gal-GalNAc is increased in a stepwise fashion in colorectal adenoma and matched surrounding normal tissue to villous adenomas with highest levels seen in CRC^[83,84]. In a prior study, Gal-GalNAc was also more abundant in visually normal colonic epithelium of patients with CRC and its precursors, when compared to healthy controls^[85]. Gal-GalNAc is mainly expressed in embryonic colonic goblet cells, and, in parallel, *Fusobacterium* was predominantly present in the goblet-like transcriptional CRC subtype^[76,85-87]. The above data suggest that *F. nucleatum* can be localized through the lumen, or it can be blood borne. *F. nucleatum*'s preferential adherence to colorectal neoplasia maybe due to increased colonic epithelial Gal-GalNAc expression potentially due to goblet-like transformation of colorectal dysplastic epithelium. This increased Gal-GalNAc expression may explain *F. nucleatum*'s prevalence in visually normal colonic tissue of predisposed individuals, as well as *F. nucleatum*'s stepwise abundance through the adenoma-carcinoma sequence. Further evaluations confirming the goblet-like transformation of visually normal appearing colonic tissue of CRC patients in relation to *Fusobacterium* and bacterial biofilm formation are warranted.

***F. nucleatum* leads to increased expression of oncogenic and inflammatory factors early in CRC development:**

Stool metabolomics and CRC tissue inflammasome analysis supported associations between *Fusobacterium*^[31,88], specifically *F. nucleatum*^[45,89], and inflammatory metabolites as well as pathways implicated in colon carcinogenesis: Interleukin (IL) 6, IL8, IL10, IL17F, IL21, IL22, the Regenerating gene family, tumor necrosis factor (TNF), Matrix metalloproteinase 9 (MMP9) and Nuclear Factor kappa B (NF- κ B)^[70,76,90-92]. Quantity of *F. nucleatum* rDNA copies and inflammatory markers were both higher

in visually normal rectal mucosa of adenoma patients compared with healthy controls^[43,89]. Fluorescence in situ Hybridization (FISH) further confirmed the presence of *F. nucleatum* in the mucus layer and within colonic crypts of normal appearing colonic mucosa^[43]. Bacterial biofilms were also found to cover normal appearing colorectal mucosa adjacent to CRC; and this was associated with an increase in colonic epithelial proliferation, *IL6* and *STAT3* activity as well as decreased E-cadherin in the normal appearing colonic epithelium^[33]. All this suggests that *F. nucleatum* is associated with increased colorectal inflammation in CRC tissue. There is also an association between presence of *F. nucleatum* rDNA and inflammation in visually normal appearing colorectal epithelium. The presence of inflammation in normal appearing colonic epithelium could potentially be due to presence of bacterial biofilms. These findings are interesting since inflammation is considered to be a marker of carcinogenesis which suggest a potential early role for *F. nucleatum* in carcinogenesis even prior to adenoma formation^[93].

Indeed, data showed that incubation of *F. nucleatum* with CRC cell lines promotes proliferation and invasion of CRC cell *in vitro* and mice xenograft models^[91]. Experimental mouse data using *APC*^{Min/+} and DMH models are supportive showing that *F. nucleatum* administration increases the number and size of aberrant crypt foci and colorectal tumors, with activation of JAK/STAT and MAPK/ERK pathways critical for CRC development^[55,79,91]. The mechanism for MAPK activation is thought to be due to recognition of *F. nucleatum* lipopolysaccharide by toll-like receptor 4 (TLR4) surface protein present on CRC cells leading to initiation of the TLR4/MYD88/NF- κ B pathway, with subsequent binding of NF- κ B to the micro RNA (miRNA)-21 promoter site^[42,91]. This leads to increased expression of miRNA 21 which regulate RASA1 gene with subsequent activation of the MAPK pathway^[91]. Similarly, *F. nucleatum* lipopolysaccharide possibly activates the TLR4/p21-activated kinase 1 (PAK1) cascade with subsequent increased β -catenin expression^[42]. In parallel, it is proposed that *F. nucleatum*'s adhesion molecule, FadA, mediates induction of E-cadherin/ β -catenin with subsequent abundance of target genes, such as *C-myc* and *CCND1*^[89,91]. These proposed mechanisms are described in Figure 2C. In a recent study, *F. nucleatum* 2 equipped with FadA and FAP2 proteins did not increase inflammation or promote CRC in *APC*^{Min/+} nor in *IL10*-knockout mice, suggesting that FadA and FAP2 are necessary but not sufficient to promote CRC^[94]. This could be due to some *F. nucleatum* strains having distinct virulence factors and/ or distinct lipopolysaccharides that are associated with more invasive and inflammatory behavior^[60]. Functional pathway analysis supports this hypothesis, with increased bacterial virulence and motility protein pathways in *F. nucleatum*-invading CRC tumors^[30]. Finally, *F. nucleatum* invasion and survival inside colorectal cells may cause increased production of

reactive oxygen species^[92,95]. The resultant activation of inflammatory cascades is hypothesized to induce DNA damage and epigenetic silencing of key targets, such as the mismatch repair gene MLH1, potentially leading to MSI seen frequently with *F. nucleatum*^[46,61,89,90-92]. Additional investigation is warranted focusing upon the relationship between virulent *Fusobacterium* strains, specifically *F. nucleatum*, and induction of an inflammatory microenvironment that facilitates epigenetic and genetic alterations involved in early colorectal carcinogenesis.

***F. nucleatum* modulates the tumor immune microenvironment favorably towards carcinogenesis:**

Mounting evidence suggests that *F. nucleatum* modulates the microenvironment at the interface between the developing cancer and the host immune response. For instance, *F. nucleatum* rDNA abundance in tumor tissue was correlated with host immune response genes and oncogenes^[45]. *F. nucleatum* can impact tumor T-cell abundance by inducing T-cell apoptosis, as well as by reducing T-cell proliferation, activation and response to certain mitogens and antigens^[79,96-102]. This effect could be due to the FAP2 protein of *F. nucleatum* directly interacting with T-cell immunoreceptor with immunoglobulin (Ig) and ITIM domains (TIGIT), leading to the inhibition of natural killer (NK) cell-induced tumor cytotoxicity. Other tumor-infiltrating CD3+ T cells (CD4+ and CD8+) also have TIGIT and are possibly inhibited by FAP2^[103]. This is consistent with the observation that *Fusobacterium*-high CRC cases are inversely associated with the density of CD3+ T cells, a type of T cell that is usually associated with better patient survival^[48]. In parallel, Forkhead box P3 (FOXP3)-low T cells do not possess tumor suppressive activity and can secrete proinflammatory cytokines. FOXP3-low T-cell-infiltrated CRCs show increased expression of inflammation and immune-mediated genes such as *IL12A*, *IL12B*, transforming growth factor (TGF)- β 1, and TNF, and they are associated with *F. nucleatum* abundance, paradoxically conferring better CRC-free survival^[104]. *F. nucleatum* also recruits CD11b myeloid-derived immune cells, which are precursors to macrophages, consistent with the finding of increased tumor macrophages in the presence of *F. nucleatum*^[69,79,105]. Furthermore, *F. nucleatum* induces activation of the *CCL20/CCR6* axis in monocytes and CRC cells, potentially promoting monocyte migration and CRC development^[56]. Thus, *F. nucleatum* abundance is associated with increased CD68 tumor-infiltrating macrophages, monocytes, and FOXP3-low T cells, but lower infiltration of CD3 lymphocytes. These findings support the hypothesis that *F. nucleatum* may exert an immunosuppressive effect in the cancer microenvironment that promotes the sustained survival of CRC cells. It may also explain the mystery of why the high load of MSI-induced antigens does not lead to immune eradication of MSI-high CRCs; this could be due to infiltration by *F. nucleatum* and associated immunosuppression. The relation between the immune

microenvironment and prognosis is still controversial, and future studies linking bacteria such as *Fusobacterium* to survival through peripheral immune modulation are warranted.

Practical applications of *fn* in CRC prevention

The accumulating literature linking *F. nucleatum* to CRC led to efforts investigating the utility of *F. nucleatum* in CRC detection. Fecal-based *F. nucleatum* polymerase chain reaction (PCR) can serve as a noninvasive tool for CRC detection, with even better results when using digital PCR based on water-oil emulsion droplet technology^[39,54,106-109]. Compared with PCR, loop-mediated isothermal amplification (LAMP) is a simple, noncostly and accurate method for bacterial testing that was shown to be more sensitive than PCR for *F. nucleatum* detection^[110]. Two drawbacks of LAMP are the potential for false positivity and the complex design primer used. Metagenomic analysis of fecal microbiome across European and Chinese cohorts also showed that butyryl-CoA dehydrogenase gene *F. nucleatum* gene markers accurately distinguished CRC cases from controls, with area under the curve (AUC) = 0.84 and an odds ratio of 23^[111]. Finally, Wang *et al.*^[112] demonstrated that *F. nucleatum* can also induce a serological anti-*F. nucleatum*-IgA immune response that is higher in CRC patients compared with patients with benign colonic polyps, those with inflammatory bowel disease, and healthy controls. In that study, the combination of anti-*F. nucleatum*-IgA and carcinoembryonic antigen (CEA) was found to be better for diagnosing CRC compared with either one alone (sensitivity: 53.10%; specificity: 96.41%; AUC = 0.848).

The finding that diet can alter the microbiome and associated colonic carcinogenesis led to efforts investigating *F. nucleatum* modulation in CRC chemoprevention and therapeutics through the use of probiotics and herbals^[63]. Probiotics including *Bifidobacterium longum*, *Lactobacillus acidophilus* and *Enterococcus faecalis* significantly reduced *Fusobacterium* levels by nearly 5-fold in CRC surgery patients when compared with placebo probiotics (10.08% vs 1.91%, respectively; $P = 0.03$)^[113]. Limitations of that study include the variable length of probiotic treatment and the presurgery use of antibiotics and bowel preparation, which can alter the microbiome^[113]. Berberine (BBR) is an isoquinoline alkaloid and a component of the Chinese herb *Coptis chinensis*. BBR was shown to prevent insulin resistance and obesity in mice fed a high-fat diet, in association with an impact on the intestinal microbiome^[114]. Administration of BBR to *APC^{Min/+}* and DMH mice inoculated with *F. nucleatum* led to reduced tumorigenesis and *Fusobacterium*-induced activation of the JAK/STAT and MAPK/ERK pathways^[55]. Both probiotics and herbals may provide tactics for modulating *F. nucleatum*, but the implications are still under investigation.

Conclusions

Fusobacteria are significantly more abundant in colorectal tissues and stools of patients with CRC than in healthy controls. The histopathology of these findings is ambiguous, but the few available data suggest that *Fusobacteria* have been observed within the colonic biofilms, the colonic mucus layer, colonic crypts, and inside the colonic epithelium. *F. nucleatum* has been associated with proximal CRCs and CRCs with MSI-high features, a finding warranting additional investigation. Findings also suggest temporality and a biological gradient with presence of fusobacteria in CRC precursors. Further, researchers have observed increased detection and quantity of *F. nucleatum* rDNA in the visually normal mucosa of colorectal neoplasia patients when compared with healthy controls. The pathophysiology and significance of this finding is unclear, as is its relation to cancer progression. *Fusobacteria* are usually indigenous to healthy mouth microbiota, highly adherent to teeth and oropharyngeal epithelium in the presence of a low viscous saliva environment, and unspecialized for viscous environment. Therefore, they are normally only transient in the colon, which is protected by a mucus layer. Disruption of the colonic mucus layer or coinfection with other invasive bacteria may facilitate the presence of *Fusobacterial* species in CRC tissue. Furthermore, some *Fusobacterial* strains, specifically *F. nucleatum*, are considered active invaders, giving them the potential to disrupt an intact colonic mucosal barrier and potentiate colorectal disease. The presence of a host lectin (Gal-GalNAc) in the colon may also mediate *F. nucleatum* blood-borne transmission and attachment to CRC and precursors through interaction with an *F. nucleatum* protein, FAP2. *F. nucleatum* was demonstrated to have cancer-promoting properties in several rodent models, supporting its role in the human colon cancer cascade. This is thought to be due to its activation of inflammatory and oncogenic pathways associated with colon carcinogenesis, as well as its modulation of the immune microenvironment in a manner that favors cancer progression. The lack of prospective human studies is a large limitation of current literature regarding the temporality of *Fusobacterium* and cancer; most human studies to date were cross-sectional case-control studies. Thus, more evidence is needed to confirm causality and inform future detection and therapeutic efforts targeting *F. nucleatum* and other microbiota involved in CRC.

ARTICLE HIGHLIGHTS

Research background

The presence of *Fusobacterium*, specifically *Fusobacterium nucleatum* (*F. nucleatum*), in the colon is increasingly linked to colorectal cancer (CRC). However, significant heterogeneity in study methods and findings poses challenges to interpretation. An evaluation of this rapidly expanding literature will help direct future studies to answer unresolved questions and to avoid previous design pitfalls in order to further our knowledge in this exciting field.

Research motivation

A critical evaluation of the scientific literature regarding the link between *Fusobacterium/F. nucleatum* and CRC may contribute to the development of more comprehensive and novel studies to better define this relationship and its potential applications in CRC treatment and prevention.

Research objectives

This systematic review evaluated the clinical and experimental evidence linking *Fusobacterium* and CRC. The authors reviewed studies investigating the relationship between *Fusobacterium* and the following variables: CRC, CRC patients' characteristics and dietary patterns, CRC anatomic location, CRC molecular features, and CRC stage and prognosis. The authors also reviewed studies looking at presence of *Fusobacterium* in pre-neoplastic lesions, as well as experimental evidence testing the procarcinogenic potential of *Fusobacterium*. Finally, the authors looked at the implications of *Fusobacterium* for CRC detection and treatment. Elucidating these heterogeneous studies may impact our understanding of the relationship between *Fusobacterium* and CRC, as well as improve detection and chemoprevention tactics for CRC.

Research methods

This is, to our knowledge, the first systematic review of the scientific evidence surrounding the link between *Fusobacterium* and CRC. Using PubMed, Embase, and Medline, the authors systematically reviewed all original studies investigating *Fusobacterium/F. nucleatum* and CRC published between January 1st, 2000, and July 1st, 2017. All abstracts were screened to identify original human, animal, and in vitro research. Out of the 355 articles that were screened, 90 articles were included in this review. Articles were excluded if diseases other than CRC were included and if they were written in languages other than English. All review articles and citations including only an abstract were excluded from analysis.

Research results

An accumulating body of evidence supports the hypothesis that *Fusobacterium*, especially *F. nucleatum* is more frequently detected in colorectal neoplasia, especially the microsatellite instability neoplastic pathway and proximal CRC. Studies investigating *F. nucleatum* in colorectal precancerous tissue suggest temporality and a biological gradient; however, ambiguity still exists on whether this increased detection of *Fusobacterium* is a cause or consequence of colorectal neoplasia. Diet may have a differential impact on colonic *F. nucleatum* enrichment, high fiber diet potentially reducing the risk of a subset of CRCs that are *F. nucleatum*-positive. Evidence also suggests a shorter CRC disease-specific survival in the presence of *F. nucleatum*, albeit with no relations between *F. nucleatum* and CRC staging. The homing of *Fusobacteria* and *F. nucleatum* to the colonic epithelium maybe partly due to increased Gal-GalNAc expression on colonic cells, virulence factors of *F. nucleatum* and other *Fusobacteria*, and changes to the local colonic environment with disruption of the protective mucus layer. Experimental evidence suggests that *Fusobacterium nucleatum* has a procarcinogenic potential that is likely mediated by activation of oncogenic and inflammatory pathways, as well as modulation of the tumor immune environment. The lack of prospective human studies is a large limitation of current literature. Furthermore, it will be essential to further delineate mechanisms and timing of *Fusobacterium* homing to the colonic mucosa, as well as its relation to cancer progression. This review may be used to develop hypotheses for novel strategies targeting colorectal cancer detection and prevention. Future robust analysis would also benefit from adjusting for confounders, such as *Fusobacterial* strain virulence factors, colonic preparation, antibiotic use, and diet.

Research conclusions

Accumulating evidence supports the hypothesis that *F. nucleatum* may enhance colorectal carcinogenesis, especially the neoplastic pathway involving defects in microsatellite instability. Virulence factors of *F. nucleatum* may contribute to its procarcinogenic effect. The lack of prospective human studies is a large limitation of current literature regarding the link between *Fusobacterium* and CRC. This review may be used to guide novel strategies targeting colorectal cancer detection and prevention.

Research perspectives

This review gathers an ample evidence implicating *Fusobacterium* in CRC

etiology and highlights the promising global efforts aimed at testing the role of *Fusobacterium* in CRC detection, chemoprevention and outcomes. There are multiple gaps in knowledge and the current evidence lacks prospective human studies. To advance our knowledge, future prospective studies need to clarify the timing and mechanisms of *Fusobacterium* transmission to the colon in relation to colorectal carcinogenesis and histopathology of these findings. In order to potentially design future CRC therapies, additional investigations are also warranted to delineate the relationships between virulent *Fusobacterium* strains, specifically *F. nucleatum*, and induction of inflammatory, pro carcinogenic and immune mechanisms involved in early colorectal carcinogenesis. Furthermore, future efforts need to prospectively test the impact of diet, probiotics and other chemopreventative agents on colonic *Fusobacterium* and whether modulation of colonic presence/concentrations of *Fusobacterium* will alter the risk or outcomes of CRC. Finally the role of *Fusobacterium* in CRC screening is intriguing and studies combing *Fusobacterium* testing with other CRC screening methods such as stool DNA testing or even colonoscopy may potentially improve CRC detection and preventative efforts. This study outlines the significant heterogeneity in the methods used and the need for more consistent design. In order to attain more robust results, the authors suggest future studies to: (1) use a blinded prospective randomized controlled design and/or large sample size when possible; (2) perform better sampling by collecting unprepared colonic tissue stored as fresh frozen samples; (3) have more detailed microbiome sequencing using whole-genome shotgun metagenomic sequencing, FISH and other methods in order to assess *Fusobacterium* sub-species concentrations, the presence of virulence factors and location of *Fusobacterium* in relation to the colonic epithelium; and (4) adjust for potential dietary, geographic and racial variables that may have an impact on *Fusobacterium/F. nucleatum* presence or concentration within specimens.

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