Current Colorectal Cancer Reports (2019) 15:149–156
https://doi.org/10.1007/s11888-019-00442-2

NUTRITION AND NUTRITIONAL INTERVENTIONS IN COLORECTAL CANCER (K WU, SECTION EDITOR)

Fusobacterium nucleatum and the Immune System in Colorectal Cancer

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Published online: 6 September 2019

Abstract

Purpose of Review To summarize the relationship between colorectal cancer (CRC), immunity, and the gut microbiome, focusing on the population of *Fusobacterium*, particularly *Fusobacterium nucleatum*, which may mediate CRC initiation and progression by inhibiting host anti-tumor immunity.

Recent Findings The onset and advancement of CRC involves genetic and epigenetic alterations and are modified by dietary and environmental factors. There is increasing evidence suggesting that gut bacteria, such as *Fusobacterium nucleatum*, may promote CRC development. The mechanisms through which *Fusobacterium nucleatum* from the oral cavity colonizes the gut mucosa and affect CRC development and progression remain unclear. Data from metagenomics analyses have shown an enrichment of *Fusobacterium nucleatum* in CRC tissues, which has been confirmed by quantitative PCR for the 16S ribosomal RNA gene DNA sequence of *Fusobacterium nucleatum*. Recent studies also suggest that *Fusobacterium nucleatum* may preferentially bind to cancerous cells, aided by Annexin A1, specifically expressed in proliferating CRC cells. This is consistent with a previous report that although *Fusobacterium nucleatum* is detected in both colorectal adenoma and adenocarcinoma tissues, the fadA gene levels are significantly higher in the latter than in the former. Other potential mechanisms include the ability of Fusobacterium to produce cancer-associated metabolites or genotoxic factors and possibly a direct interaction with the host immune system. Supporting a possible interaction with the host immune system are recent data indicating that overload of *Fusobacterium nucleatum* elicits high levels of *Fusobacterium nucleatum*-specific antibodies in CRC patients, suggesting that *Fusobacterium nucleatum* may escape host humoral immune responses by evolving inside host cells. Additionally, it has been found that the interaction of *Fusobacterium nucleatum* with immune response to CRC differs by tumor microsatellite (MS) status, suggesting that *Fusobacterium nucleatum* and MS status interact to influence anti-tumor immune functions.

This article is part of the Topical Collection on *Nutrition and Nutritional Interventions in Colorectal Cancer*

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Summary The current literature suggests that *Fusobacterium nucleatum*, a Gram-negative oral anaerobe, may significantly contribute to CRC development. Furthermore, the presence of *Fusobacterium nucleatum* in CRCs has also been associated with MSI-high status, lower levels of infiltrating T-lymphocytes, and poor clinical outcomes. We believe that the integration of new technologies, including genomics, bioinformatics and systems medicine, may help to better understand how *Fusobacterium nucleatum*, immunity status, and environmental factors interact in the initiation and progression of CRCs and generate further information regarding prognostic and therapeutics options for this tumor.

Keywords Colorectal cancer · *Fusobacterium nucleatum* · Immunity · Microbiome, diet

Introduction

Globally, colorectal cancer (CRC) still represents the third most common neoplasia and the second most common cause of cancer death with over 1.8 million new CRC cases and 881,000 deaths estimated to occur in 2018 [1].

CRC is a multifarious disease, rising from precursor adenomas and serrated polyps [2, 3]. It has been demonstrated that the main genetic and epigenetic features of CRC, classified as microsatellite instability (MSI), chromosomal instability (CIN), and CpG island methylator phenotype (CIMP), are associated with different clinical phenotypes [4] .

For more information on potential genetic and epigenetic transformations involved in CRC onset and progression, please refer to a recent review in the *Topical Collection on Nutrition and Nutritional Interventions in Colorectal Cancer of this journal* entitled "Lifestyle, Diet, and Colorectal Cancer Risk According to (Epi)genetic Instability: Current Evidence and Future Directions of Molecular Pathological Epidemiology by Hughes et al. [5]. In addition to the more known molecular pathways underlying colorectal carcinogenesis, tumor cells may also invade and develop metastases in other organs through additional (and less researched) mechanisms. For example, it has been shown that TGF-β1 induces a mesenchymal phenotype consistent with epithelial-mesenchymal transition (EMT) in MSS CRC cell lines [6], and that the peritumoral stroma of CRC tissues contains TWIST1$^+$ cells with mesenchymal phenotype and neoplastic genotype [7]. Along with diet, lifestyle, and genetics, oncogenic infection affecting either individual bacteria strains or the entire microbiome has also been shown to be associated with CRC [8, 9].

Although the functions of the gut microbiome on CRC development and advancement still remains obscure [10–13], an increasing number of studies (Fig. 1) have shown that bacteria, including *Fusobacterium nucleatum*, may likely participate in colorectal carcinogenesis through interactions with the host immune system, generation of cancer-associated metabolites, and genotoxins (Fig. 2) [14–16].

In this review, we will summarize the literature on the relationship between CRC (incidence and survival), immunity, and gut bacteria, focusing on the population of *Fusobacterium*, particularly *Fusobacterium nucleatum*. While composition of gut microbiota including *Fusobacterium nucleatum* can be altered by dietary factors [17, 18], this review will focus on the role of *Fusobacterium nucleatum* and immunity in colorectal carcinogenesis. For more detail on the role of diet on the gut microbiota, refer to a previous review in this journal by Song and Chan [12].

Fusobacterium nucleatum and the Gut Microbiome

It has been estimated that the human gut hosts over 100 billion bacteria, with the main fraction of these bacteria stationed in the colon. It has also been reported that the bacterial amount in the large intestine is much higher than that in the small intestine and that cancer risk in the large intestine is approximately 12-fold greater than in the small intestine [15, 19]. *Fusobacterium nucleatum* is a gram-negative anaerobe, which belongs to the Bacteroidaceae family and is found commonly in the mouth in healthy or diseased humans [20, 21]. Several oral microbes are commensals but a few are opportunistic pathogens. *Fusobacterium nucleatum* can also be isolated from skin ulcers, septic arthritis, and endocarditis [20].

It has been shown that *Fusobacterium nucleatum* is different from other Bacteroidaceae because they produce *N*-butyrate without isobutyrate or isovalerate (3-methylbutanoate) [22]. Additionally, although *Fusobacterium nucleatum* is defined as anaerobe, it is able to grow in up to 6% oxygen [23].

Fusobacterium nucleatum and CRC

Potential Mechanisms Linking *Fusobacterium nucleatum* and CRC

Metagenomic has demonstrated an increase of presence of *Fusobacterium nucleatum* in CRC tissue. How *Fusobacterium nucleatum* is able to colonize the gut mucosa from the oral cavity is, however, still debated [24]. It has been reported that the microbial community observed in the oral cavity and the gut were

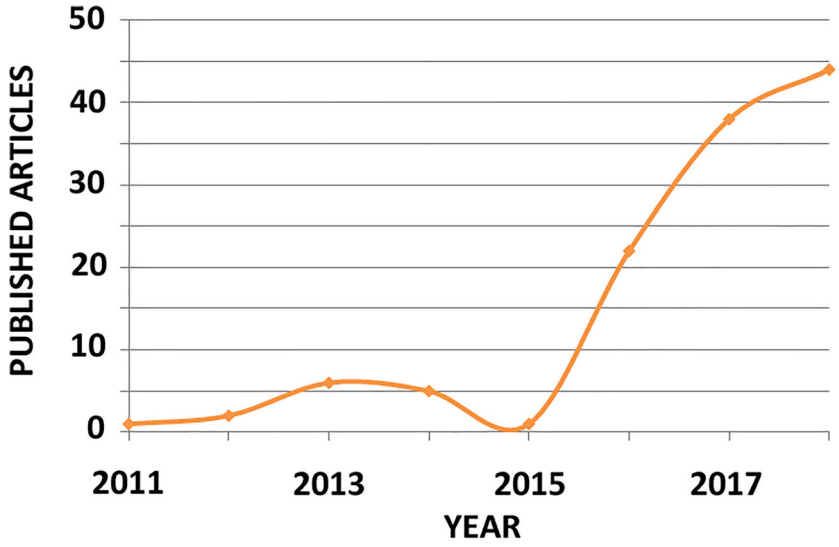

Fig. 1 The number of published articles that investigate "*Fusobacterium nucleatum*" and "colorectal cancer" indexed in the PubMed database has rapidly increased during the last years

predictive of each other [25], which may suggest an oral-gut translocation route for *Fusobacterium nucleatum*, via swallowing the bacteria, which may further lead to dysbiosis of the gut microbiota, disruption of gut homeostasis, and alteration of the microenvironment that favors the development of CRC [26]. However, it has been also shown that colonization of human CRC with *Fusobacterium nucleatum* and its associated microbiome is maintained at distance (i.e., metastases), suggesting "microbiome stability" in both primary and metastatic tumors [27••]. Furthermore, it has been shown that a greater density of *Fusobacterium nucleatum* in CRC tissue is correlated with high degrees of microsatellite instability (MSI-high) and CIMP [28••, 29]. Lee et al. have recently reported that in patients with metastatic CRCs, CRCs characterized by *Fusobacterium nucleatum* high state showed worse survival than those with *Fusobacterium nucleatum* low state [30].

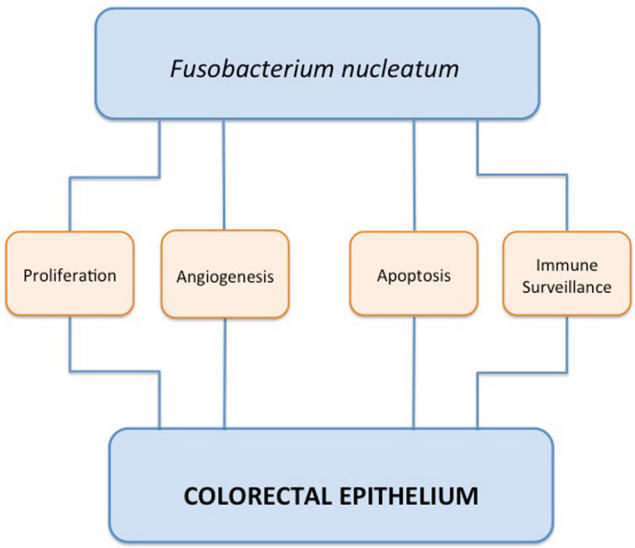

Fig. 2 Potential mechanisms of action of *Fusobacterium nucleatum* in colorectal cancer

Recently, it has been demonstrated that *Fusobacterium nucleatum* adheres to and invades epithelial cells mainly through virulence factors, including adhesin A (FadA), autotransporter protein 2, and outer membrane protein A [16, 31, 32]. To date, it remains still unclear whether *Fusobacterium nucleatum* can activate the colonic EMT process. Studies in vitro have shown that EMT is characterized by loss of epithelial E-cadherin and subsequent deregulation of the Wnt signaling pathway [33]. Ma et al. [34] demonstrated that *Fusobacterium nucleatum* increases the inflammatory responses when β-catenin, but not E-cadherin, expression is knocked down in NCM460 cells. An association between *Fusobacterium nucleatum* amount and lymphatic metastasis has also been observed in CRCs [35–37]. While these findings suggest that *Fusobacterium nucleatum*-high CRC may be a more biologically aggressive cancer subtype, other studies have not confirmed these findings [35, 38, 39].

Fusobacterium nucleatum and Innate Immunity in Colorectal Cancer

CRCs display different degrees of infiltrating immune cells, essential dynamic populations of the tumor microenvironment [40–42]. The host immune response is closely related with clinical disease behavior [43, 44]. Recently, it has been shown that the overload of *Fusobacterium nucleatum* elicits high levels of *Fusobacterium nucleatum*-specific antibodies in CRC patients, implying that *Fusobacterium nucleatum* may escape host humoral immune responses by developing inside host cells [45, 46]. Macrophages constitute the first line of defense against infecting pathogens. It has been reported that some aggressive intracellular bacteria can survive and propagate in the cytoplasm of colonized macrophages [47]. Chen et al. have found an immunosuppressive effect of *Fusobacterium nucleatum* by activating M2 polarization of

macrophages through a Toll-like receptor 4 (TLR4)-dependent mechanism [48]. Although it has been shown that *Fusobacterium nucleatum* infection rapidly induces inflammation and macrophage infiltration in gingival tissues, Park et al. [49] found that in CRC tissues, *Fusobacterium nucleatum*-high was not significantly associated with $CD163^+$ M2 macrophage density but significantly with $CD68^+$ tumor-infiltrating macrophages.

Considering differences in the tumor-immune microenvironment of carcinomas with high or low MSI, Hamada et al. hypothesized that the association of *Fusobacterium nucleatum* with immune response might differ by tumor MSI status. Using samples from rectal and colon cancer patients, Hamada et al. [28••] measured *Fusobacterium nucleatum* DNA in tumor tissue and examined the association between *Fusobacterium nucleatum* status and the density of $CD3^+$, $CD8^+$, $CD45RO^+$, and $FOXP3^+$ lymphocytes in strata of tumors by MS status. Considering differences in the tumor-immune microenvironment between MSI-high and non-MSI-high carcinomas, they concluded that in CRCs, *Fusobacterium nucleatum* and MS status may interact to mediate anti-tumor immune reactions [28••].

The role of Fap2 protein in the onset of CRC has been reported. The authors demonstrated that Fap2, when bound to the human inhibitory receptor, T cell immunoreceptor with Ig and ITIM domains (TIGIT), suppresses the cytotoxic role of natural killer (NK) cells [50]. Given the tumorigenic role of *Fusobacterium nucleatum* and its immune evasion properties, it has been suggested that *Fusobacterium nucleatum* elimination might improve treatment outcome of the above tumors. Interestingly, it has also been reported that TIGIT is an immunoreceptor inhibitory checkpoint, implicated in tumor immunosurveillance. Expression of TIGIT has been found in both NK cells and T-lymphocytes. The function of TGIT in tumor immunosurveillance has been found to be similar to the programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) axis in tumor immunosuppression [51].

Fusobacterium nucleatum and the Adaptive Immunity in Colorectal Cancer

The adaptive immune system primarily consists of tumor-infiltrating lymphocytes (TILs), comprising $CD8^+$ CTLs and $CD4^+$ T-helper lymphocytes [52]. It is known that $CD4^+$ T-lymphocytes release cytokines, which in turn activate and promote stimulation of CTLs. $CD8^+$ T cells synthetize and release perforin and granzyme B, which mediate their cytotoxic activity [53], and consequent anti-tumor actions [54, 55]. $CD4^+$ T cells are heterogeneous in function and their subsets can abolish antigen-specific T cell responses, alike the $Foxp3^+$ regulatory T-lymphocytes (Tregs) [56]. It is known that during tumor progression, Tregs concentrate in neoplastic areas and peripheral blood of cancer patients [57, 58]. The main function of Tregs is to suppress T cell activation to manage abnormal immune responses [59]. The transcription factor FOXP3 is a Treg cell marker together with $CD4^+$ $CD25^+$ [59, 60], although the specificity of these markers has been investigated since CD25 and FOXP3 might also be expressed by activated CTL [61]. In the tumor contexture, Tregs may exert different functions as they might explode anti-tumor immunity, as well as reduce pro-tumor inflammation [61]. The dense TIL aggregates observed in some CRC, particularly those with MSI phenotype, have also been referred to as Crohn's-like reaction [62, 63]. Moreover, peritumoral lymphocytes can organize in clusters, resembling anatomically and functionally secondary lymphoid organs [64, 65]. Such anatomical entities, called "tertiary lymphoid tissue" (TLT), are commonly observed during chronic inflammatory conditions and might be involved in the organization of the immune response [66].

Recently, Mima et al. [67] observed *Fusobacterium nucleatum* in 76 out of 598 (13%) CRCs investigated. Compared with *Fusobacterium nucleatum*-negative cases, *Fusobacterium nucleatum*-high cases were found inversely associated with the density of $CD3^+$ T cells. The amount of *Fusobacterium nucleatum* was not significantly associated with the density of $CD8^+$, $CD45RO^+$, or $FOXP3^+$ T cells. Regarding the association between the gut microbiome and immunity, several studies have shown that *Fusobacterium nucleatum* may exert immunosuppressive activities via inhibiting human T cell responses to mitogens and antigens [30–35]. Moreover, it has been reported that *Fusobacterium nucleatum* inhibitory protein can arrest human T-lymphocytes in the G1 phase of the cell cycle [33]. *Fusobacterium nucleatum* can also induce apoptotic cell death in peripheral blood mononuclear cells (PBMCs) and Jurkat T-lymphocytes [31]. It has been shown that *Fusobacterium nucleatum*-induced cell death is mediated through the clustering of the immune cells, which might have important implications for the pathogenesis of this bacterial species [35]. These findings suggest that *Fusobacterium nucleatum* has a suppressive modulation of the tumor-immune microenvironment. Recently, Chen et al. reported that *Fusobacterium nucleatum* may suppress anti-tumor immune responses by decreasing $CD4^+$ T cell density and TOX (thymocyte selection-associated high-mobility group box) expression in CRC [68].

Fusobacterium nucleatum and the Chemokine Network in CRC

The function of *Fusobacterium nucleatum* in periodontal disease still remains controversial. Some authors consider the *Fusobacterium nucleatum* as a commensal. In contrast, the prevalence of *Fusobacterium nucleatum* is significantly increased in subjects with periodontal or gastrointestinal diseases at diseased sites, and it possess putative virulence (pathogenic) factors, such as the ability to invade oral epithelial cells [69, 70]. The

interactions among the bacterium, intestinal epithelium, and host innate defense responses are critical for the consequences of *Fusobacterium nucleatum* infection. The inflammatory response to *Fusobacterium nucleatum* infection is the pivotal mediator of pathological changes in the intestinal mucosa, but the regulatory mechanisms of *Fusobacterium nucleatum*-induced inflammation are still not well understood.

Fusobacterium nucleatum may also contribute to aggressive tumor behavior through activation of chemokines (Fig. 3). CCL20 and its receptor CCR6 are known for their important roles in the recruitment of immune cells and their paradoxical functions in regulation of both immunological tolerance and inflammation [71]. It has been reported that tumor-associated macrophages recruit $CCR6^+$ regulatory T-lymphocytes to the tumor microenvironment throughout CCL20 signaling and thereby promote tumor growth [72]. Certain commensal bacteria, including *Fusobacterium nucleatum* [73], are excellent inducers of β-defensin, suggesting that the commensal bacterial community may act by priming innate immune readiness of the oral epithelium.

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway in *Fusobacterium nucleatum*-positive tissues was "cytokine-cytokine receptor interaction." Detailed analysis of these data identified CCL20 as the principal upregulated chemokine suggesting that *Fusobacterium nucleatum* contributes to the acquisition of aggressive tumor behavior by activating chemokines such as CCL20 [74]. It has been reported that CCL20 stimulation promoted cancer cell proliferation and migration [75]. Furthermore, CCL20 plays a crucial role in the migration of Tregs [76], and their accumulation has been associated with shorter survival in human cancers [77]. Recently, it has also been reported that CCL20 was related to tumor infiltration of Tregs in esophageal squamous cell carcinoma [78]. Further studies are needed to validate the current findings and to elucidate the mechanism(s) whereby *Fusobacterium nucleatum* affects tumor behavior. *Fusobacterium nucleatum* subspecies *animalis* induced CCL20 protein expression in CRC cells and monocytes [79]. It also stimulated the monocyte/macrophage activation and migration. The CCL20/CCR6 axis regulates recruitment of $CCR6^+$ immune cells, including subsets of interleukin-17 (IL17)-expressing T-helper cells (Th17), regulatory T cells, and dendritic cells, to neoplastic lesions. Interestingly, colorectal cancer can hijack CCL20/CCR6 function to promote hepatic metastasis of CRCs [80]. These observations suggested that infection with *Fusobacterium nucleatum* subspecies *animalis* in colorectal tissue could induce inflammatory response and promote CRC development. For the *Fusobacterium nucleatum* subspecies *animalis* interaction with CRC cells and monocytes, the response was stronger in the monocytes and showed a macrophage activation phenotype with increased migration and CCL20 protein expression, particularly under a hypoxic condition mimicking the tumor microenvironment. Further studies are warranted to determine if *Fusobacterium nucleatum* subspecies *animalis* could be a novel target for CRC prevention and treatment. *Fusobacterium nucleatum* subspecies *animalis* is an inducer of inflammatory responses via CXCL8, suggesting a role as a risk factor for tumorigenesis. Regardless of whether one defines it as a putative pathogen or as a commensal species, it has been shown that, in contrast to most oral streptococci, *Fusobacterium nucleatum* subspecies *animalis* is able to induce a wide range of pro-inflammatory responses in various types of host cells, including marked IL-8 induction in oral epithelial cells [81, 82] as well as ROS also in neutrophils [83, 84]. Upregulation of IL-8 mRNA by *Fusobacterium nucleatum* has been found to mainly involve NF-κB pathways and to some extent MAPK p38 and MAPK/ERK pathways. Tang et al. [85] firstly proposed that *Fusobacterium nucleatum*-induced impairment of autophagic flux enhances the expression of pro-inflammatory cytokines

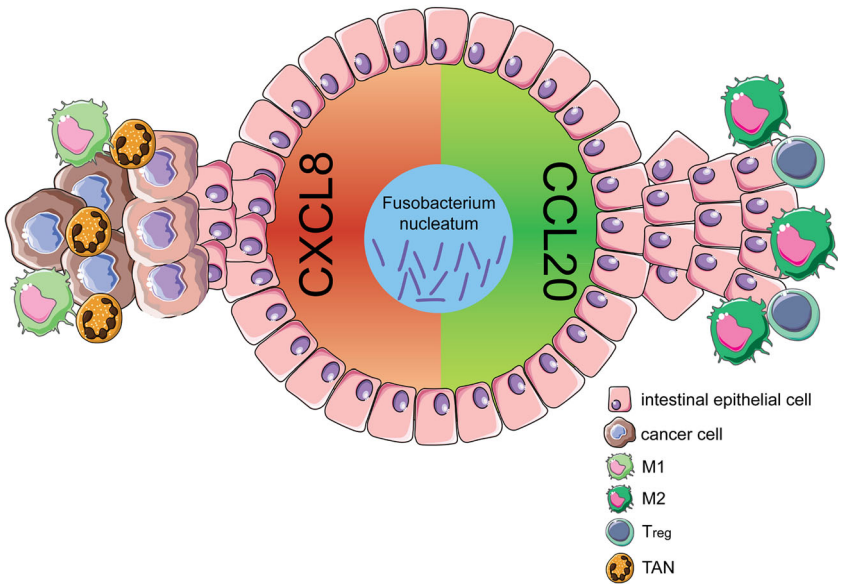

Fig. 3 Commensal or pathogenic nature of *Fusobacterium nucleatum*, the two sides of the same silver dollar

and chemokines (i.e., CXCL8) via reactive oxygen species (ROS) in intestine epithelial cells Caco-2 (Caucasian colon adenocarcinoma). However, it remains unclear whether disruption of autophagy or generation of ROS plays a critical role in the inflammation induced by *Fusobacterium nucleatum*.

Conclusions

The onset and advancement of CRC involves genetic and epigenetic alterations influenced by dietary and environmental factors. Increasing evidence has linked the intestinal microbiota and CRC. More recently, *Fusobacterium nucleatum*, an opportunistic commensal anaerobe in the oral cavity, has been repeatedly associated with CRC [14, 86].

Targeting *Fusobacterium nucleatum* in the oral cavity—as compared to feces—may provide insights for further studies in the field of human microbiome research and CRC. While the increased density of *Fusobacterium nucleatum* in CRC tissues compared to the level in healthy subjects has been confirmed by several studies, unanswered questions remain regarding mechanisms of pathogenesis. The integration of new technologies, including genomics, bioinformatics, and systems medicine may help to better understand how the colonic microbiota, immunity status, and environmental factors interact in the initiation and progression of CRCs.

Acknowledgments The authors are grateful to Teri Field for her editorial support.

Authors' Contributors Borroni EM, Qehajaj D, Farina FM, Yiu D, Bresalier RS, Chiriva-Internati M, Mirandola L, Stifter S, Laghi L, Grizzi F: drafted and discussed the manuscript and approved the final version.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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