Translational Oncology
www.transonc.com

Volume 12 Number 6 June 2019 pp. 846–851 846

Fusobacterium nucleatum Contributes to the Carcinogenesis of Colorectal Cancer by Inducing Inflammation and Suppressing Host Immunity

Jiao Wu[*], Qing Li[*] and Xiangsheng Fu[†]

[*]Department of Gastroenterology, the Affiliated Hospital of Southwest Medical University, Sichuan, China, 646000; [†]Department of Gastroenterology, the Affiliated Hospital of North Sichuan Medical College, Nanchong City, China, 637000

Abstract

The presence of *Fusobacterium nucleatum* (*F. nucleatum*) in the gut is associated with the development of colorectal cancer (CRC). *F. nucleatum* promotes tumor development by inducing inflammation and host immune response in the CRC microenvironment. Adhesion to the intestinal epithelium by the cell surface proteins FadA, Fap2 and RadD expressed by *F. nucleatum* can cause the host to produce inflammatory factors and recruit inflammatory cells, creating an environment which favors tumor growth. Furthermore, *F. nucleatum* can induce immune suppression of gut mucosa by suppressing the function of immune cells such as macrophages, T cells and natural killer cells, contributing the progression of CRC.

Translational Oncology (2019) 12, 846–851

Introduction

The human intestine is home to more than 100 trillion microbes, forming a unique genome that changes with human nutrition status, geographic location, and even age [1,2]. Gut microbiota is in harmony with the human body, affecting human health and shaping the immune system of the host [3]. Imbalance in gut microbiota can lead to a variety of diseases, such as colitis, colorectal cancer (CRC), infection, food allergies, obesity, diabetes, cardiovascular atherosclerosis, bone metabolic diseases, Parkinson's and neurodegenerative diseases [4].

The colon has been colonized by the largest density of microorganisms [5]. Recent studies have reported that certain pathogenic bacteria in the colon are associated with CRC [5,6]. It's possible to screen CRC by detecting tumor-associated microorganisms in the feces [7]. CRC is the third most common tumor in the world, causing significant morbidity and mortality [3]. Unfortunately, the mechanism of this malignancy has not been fully explained, but inflammation is a recognized risk factor [8]. CRC is a chronic disease which can arise from other intestinal inflammatory conditions [6].

In recent years, many studies have been conducted on the correlation between inflammatory microorganisms and CRC. The occurrence of intestinal inflammatory diseases such as colitis is related to the metastasis of intestinal microbes [2]. Colitis occurs when microbes turn from a 'eubiotic' to a 'dysbiotic' state [2]. Chronic bacterial infection of the colon is a driving factor in tissue inflammation, increasing the risk of developing CRC [9].

Gut Microbiota and Host Immunity

Human bodies are constantly exposed to a diverse array of microbes, as well as their metabolite byproducts [10]. The gut microbiome influences the development of the immune system of the host, and conversely the immune system regulates the microbe composition in the gut [5,11]. Microorganisms in the gut play a key role in the activation, training and regulation of the host immune system [12]. The communication between the intestinal microflora and the host's immune system begins at a very early stage of development [13]. Intestinal microbes can recruit immune cells and initiate inflammatory reactions, which play a direct role in promoting the maturation of the immune system [10,14]. The intestinal

Address all correspondence to: Dr. Xiangsheng Fu, Department of Gastroenterology, the Affiliated Hospital of North Sichuan Medical College, Road Wenhua 63#, Region Shunqing, Nanchong City, China, 637000. E-mail: drfuxs@gmail.com
Received 15 January 2019; Revised 22 March 2019; Accepted 26 March 2019

1936-5233/19
https://doi.org/10.1016/j.tranon.2019.03.003

mucosal immune system is a protective barrier system composed of cellular (i.e. epithelial- and mesoderm-derived immune cells) and non-cellular components (e.g. antimicrobial peptides, cytokines and antibodies), which can resist microbial attack [15].

On the one hand, intestinal microbes affect the host immune system through secretion of metabolites (e.g. butyrate, L-tryptophan, indole, bile acids and retinoic acid), signaling pathways (e.g. Toll-like receptors and Nod-like receptors), and small noncoding RNAs (e.g. miRNA) [9,16]. On the other hand, the intestinal immune system exposes bacteria to the host, reducing pathological outcomes, and modulating the stratification of bacteria in the epithelial barrier [17]. The intestinal immune system affects the composition of bacteria, whereas the bacteria promote the development of the intestinal immune system. Disruption of the relationship between intestinal bacteria and the host immune system will affect the overall health of the host [15,17]. Intestinal epithelial cells recognize pathogen-associated molecular structures (such as lipopolysaccharides and flagella) through their surface Toll-like receptors. This triggers the maturation of antigen-presenting cells (such as dendritic cells), the initiation of immune responses, and the release of inflammatory factors, which are associated with the development of CRC [18]. This dysregulation of the host immune system represents a potential mechanism for the effect of intestinal microbes on the development and progression of CRC [19].

Invasion of *Fusobacterium nucleatum* Contributes to the Carcinogenesis of CRC

Fusobacterium nucleatum (*F. nucleatum*) is a Gram-negative, anaerobic oral commensal bacterium that is associated with a variety of human diseases, including periodontal disease [20], Alzheimer's disease [21], brain abscess [22], cardiovascular disease [23], miscarriage [24] and inflammatory bowel disease [25]. Recently, *F. nucleatum* has been proposed to be associated with CRC [26]. *F. nucleatum* promotes the occurrence of CRC through several virulence mechanisms: colonization, invasion, and modulation of host immune response [27].

F. nucleatum bacteria interact with each other by expressing a variety of different virulence factors, and can adhere to many different mammalian cell types, including epithelial and endothelial cells, polymorph nuclear neutrophils, monocytes, erythrocytes, fibroblasts, and natural killer (NK) cells [28,29]. The cell surface protein FadA is a key virulence factor in *F. nucleatum* which regulates adhesion and invasion of the bacterium. The expression of FadA gene in human CRC specimens was significantly higher than that in adjacent normal tissues [30]. This protein enables *F. nucleatum* to bind E-cadherin in CRC and epithelial cells, activate the β-catenin pathway, and induce the expression of transcription factors lymphoid enhancer factor (LEF)/T cell factor (TCF) which promote tumor cell growth [30,31]. It's recently reported that FadA can up-regulate Wnt/β-catenin modulator Annexin A1 expression through E-cadherin [32]. FadA can also bind to endothelial cells VE-cadherin, which is a linker molecule on endothelial cells [33]. This combination alters the integrity of the endothelium, increases the permeability of the endothelium, and allows the bacteria to overcome the blood brain barriers, placental barriers, and colonize different parts of the body [34]. Outer membrane vesicles (OMVs) from *F. nucleatum* can degrade E-cadherin, thus promoting bacterial invasion and tumor metastasis [35].

In addition, *F. nucleatum* also has two other outer membrane proteins, Fap2 and RadD [36]. The lectin Fap2 can bind Gal-GalNAc, a polysaccharide overexpressed in CRC. This binding of Fap2 facilitates colonization of *F. nucleatum* and explicates fusobacteria abundance in CRC [37]. RadD can mediate communication between *F. nucleatum* and other bacterial species, contributing to the formation of multispecies biofilms [36,38], which has been shown to be associated with proximal colon cancer [39].

F. nucleatum-Induced Inflammation Contributes to CRC Development

There has been a growing body of literature suggesting a link between chronic inflammation and CRC, in which gastrointestinal inflammation may promote CRC development [30]. Increased evidence suggests that *F. nucleatum* can shape the inflammatory microenvironment in the CRC, promoting tumor growth and metastasis [40]. For example, *F. nucleatum* can stimulate reactive oxygen species (ROS) production, inducing inflammatory responses in CRC cells [41]. Infection of CRC cells with *F. nucleatum* increased the expression of miR21, a pathogenic role in chronic inflammation and colitis-associated colon cancer, therefore promoting tumor cell proliferation and invasive activity [8,42].

Adherence of *F. nucleatum* to CRC cells via FadA stimulates the release of inflammatory factors, such as NF-κB, IL-6, IL-8, IL-10 and IL-18, which promote cell proliferation in CRC [30]. In patients with *F. nucleatum* infection, strong humoral immunity is induced, and antibodies against *F. nucleatum*, IgA and IgG are present [43]. *F. nucleatum* infection increases the infiltration of inflammatory cells, such as macrophages, dendritic cells, and granulocytes, which create a pro-inflammatory microenvironment that is conducive to the occurrence of CRC [44]. Macrophages infected with *F. nucleatum* can induce the release of inflammatory cytokines [45,46]. Natural cytotoxic receptor NKp46 of NK cell can directly recognize *F. nucleatum* through its surface ligand, secreting TNF-α to aggravate inflammation [20]. Some inflammatory response signatures were specific to *F. nucleatum* but not to other bacteria founded in CRC tissues, such as IL1β, IL24, PTGS2 (COX-2), IL8, IL6 and TNF, which were enriched in *F. nucleatum*-infected CRCs [44].

F. nucleatum is prevalent in gastrointestinal inflammation diseases especially inflammatory bowel disease (IBD) [25]. *F. nucleatum* strains originating from IBD patients were significantly more invasive than strains isolated from healthy tissues [25]. Highly invasive *F. nucleatum* isolates derived from the inflamed area of human Crohn's disease triggered high expression of MUC2 and TNF-α in colon cancer cells [47]. In IBD patients, the release of IL-1β and TNF-α can damage colon cells and impair epithelial integrity, which increases the chance of contact between *F. nucleatum* and the colon epithelium [48]. This may partially explain why patients with IBD are susceptible to CRC. Mechanisms of adhesion, invasion and inflammation mediated by *F. nucleatum* in CRCs were summarized in Table 1.

F. nucleatum-Induced Immune Suppression Promotes CRC Development

Macrophages

F. nucleatum modulates the tumor immune environment by amplifying bone marrow-derived cells [49] such as tumor-associated macrophages, which play an important role in tumor invasion and metastasis [50]. Meanwhile, the tumor microenvironment can affect the heterogeneity of macrophages, which can differentiate from pro-inflammatory M1-phenotype to a tumor-promoting M2-phenotype [51,52].

Our recent study revealed that *F. nucleatum* displayed an immunosuppressive effect by promoting M2 polarization of macrophages in *F. nucleatum*-related CRCs, possibly through the TLR4/IL-6/p-STAT3/c-MYC signaling pathway [53]. *F. nucleatum*

Table 1. *Fusobacterium nucleatum* induced invasion and inflammation contributes to colorectal cancer

Virulence factor	Function	Mechanisms	References
Fn infection	Pro-inflammatory microenvironment	Inducing inflammatory cells infiltration	[49]
	Inflammatory cytokines production	Accumulation of reactive oxygen species	[41]
	Cell proliferation and invasion	Increasing the expression of miR21	[8,42]
FadA	Cell proliferation	Activating the β-catenin pathway	[30,31]
		Up-regulating Wnt/β-catenin modulator Annexin A1	[32]
	Bacterial colonization	Binding endothelial cell VE-cadherin	[33,34]
Fap2	Bacterial colonization	Binding Gal-GalNAc overexpressed in CRC	[37]
RadD	Biofilms formation	Mediating communication between Fn and other bacteria	[36,38]
LPS	Inflammatory cytokines production	Activating immune cells	[55]
OMVs	Bacterial invasion and tumor metastasis	Degrading E-cadherin	[35]

Fn, *Fusobacterium nucleatum*; CRC, colorectal cancer; OMV, outer membrane vesicles.

induces infiltration of M2 macrophages in the colorectal environment, thus forming a tumor-promoting microenvironment [54,55]. The metabolite of *F. nucleatum*, butyric acid, can induce apoptosis in monocytes/macrophages and lymphocytes by activating free fatty acid receptors [56]. In addition, *F. nucleatum* can invade macrophages and induce the expression of indoleamine2,3-dioxygenase on the cell surface, creating a toxic microenvironment which impairs the function of peripheral blood lymphocytes, thereby allowing macrophages to escape cytotoxic T lymphocyte attack [55].

T cells

T cell activity can be inhibited by the virulence factors of *F. nucleatum* [36,57]. *F. nucleatum* abundance is negatively correlated with $CD3^+$T cell density in CRC [1]. Additionally, we have shown that a high abundance of *F. nucleatum* in CRC is associated with lower numbers of $CD4^+$T cells [58]. Decreased T cell density in CRC could be explained by apoptotic cell death and arrested proliferation of T cells induced by *F. nucleatum* [59–61]. For example, *F.*

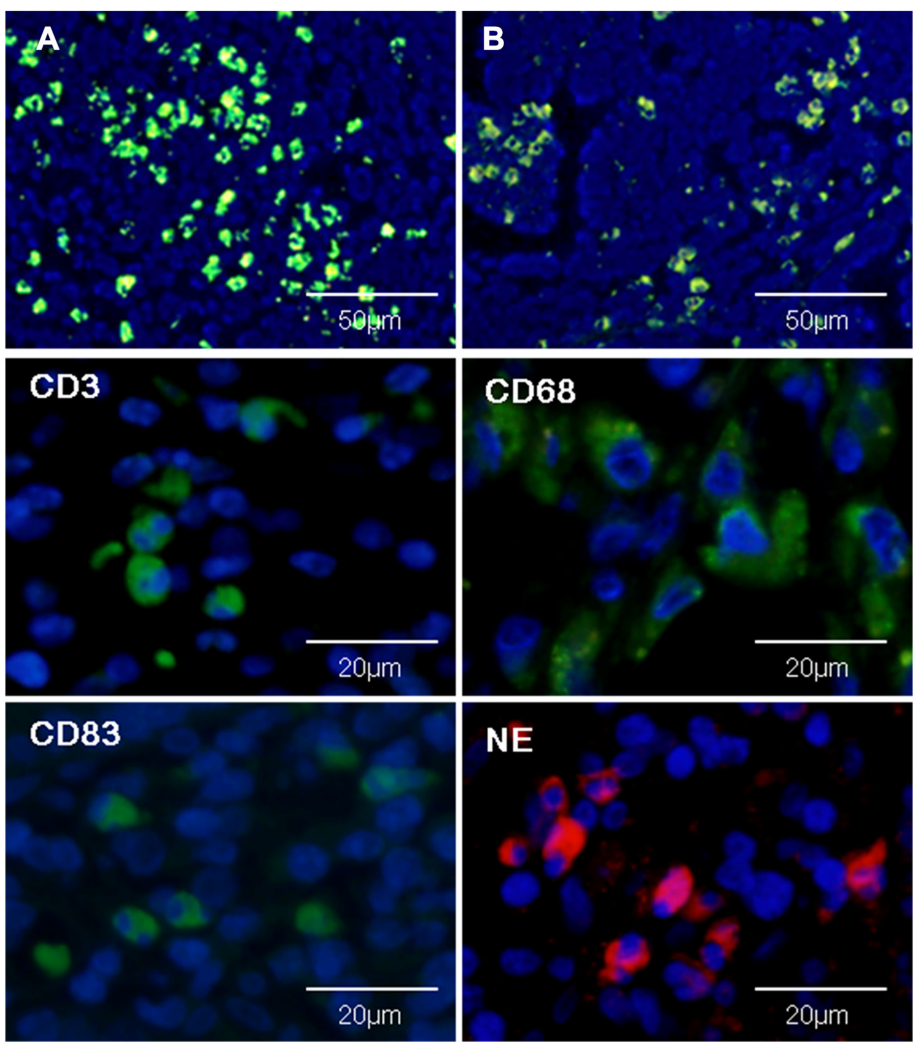

Figure 1. Infiltrating immune cell populations in human *F. nucleatum* related colorectal cancer. High abundance of *F. nucleatum* within colon cancer tissue (A) and matched metastatic lymph nodes (B) detected by immunofluorescence. High density of immune cells ($CD3^+$, $CD68^+$, $CD83^+$, and NE cells) within the environment of *F. nucleatum*-positive colon cancers (immunofluorescence). NE, neutrophils.

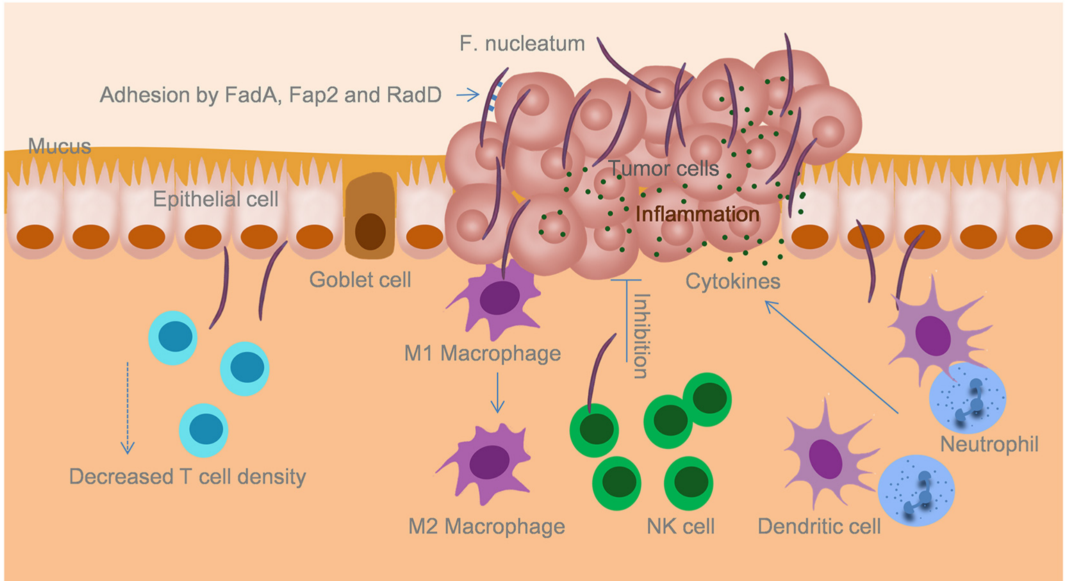

Figure 2. *F. nucleatum* induces a pro-inflammatory microenvironment and suppression of host immunity that favor tumor growth within the gut mucosa. *F. nucleatum* binds to colon epithelium through FadA, Fap2 and RadD, and invades the mucosa. This invasion by *F. nucleatum* increases the infiltration of inflammatory cells and the release of cytokines which stimulate cell proliferation. Moreover, invasive *F. nucleatum* interacts with the immune cells in the colon mucosa, resulting in the decrease of T cell density, increased M2 macrophage polarization, inhibition of NK cell activity, and the increase of dendritic cells and tumor-associated neutrophils that diminish anti-tumor immunity. The inhibition of mucosa immunity favors tumor progression.

nucleatum inhibitory protein (FIP) can inhibit human T cell activation by arresting cells in the G1 phase of the cell cycle [60]. A recent study revealed that the association of *F. nucleatum* with tumor-infiltrating lymphocytes (TIL) differed by MSI status of CRC. The presence of *F. nucleatum* was negatively associated with TIL in MSI-high tumors, but positively in non-MSI-high tumors [62].

In the colorectal tumor microenvironment, *F. nucleatum* can release short-peptides (formylmethionyl-leucyl-phenylalanine) and short-chain fatty acids (butyrate, propionate, and acetate) which lead to recruitment of myeloid-derived suppressor cells (MDSCs) [48]. MDSCs can regulate immune response by suppressing $CD4^+$ T helper cell function, inhibiting T cell proliferation, and inducing T cell apoptosis [48,63]. *F. nucleatum* can also induce human lymphocyte death through Fap2 and RadD [36]. Fap2 of *F. nucleatum* can inhibit human T cell activation by directly interacted with TIGIT, an inhibitory receptor present on various T cells [57]. Moreover, *F. nucleatum* can interact directly with monocytes, which may recruit T helper 17 cells and T regulatory cells through CCL20/CCR6 pathway, promoting CRC formation [64].

NK Cells

All human NK cells express the Fap2 receptor TIGIT, which recognizes poliovirus receptor (PVR) and nectin-2 as ligands [65]. This binding of NK cells to *F. nucleatum* inhibits the killing activity of NK cells, thereby promoting the formation of colorectal tumors [35,57,66].

Dendritic Cells

The infiltration of $CD103^+$ dendritic cells (DCs) was increased in tumors from *F. nucleatum*-fed mice compared with control group [44]. This population of DCs can promote the expansion of $Foxp3^+$ regulatory T cells, a $CD4^+$ T cell subset that inhibits cytotoxic and effector T cells, therefore diminishing anti-tumor immunity [67].

Tumor-Associated Neutrophils

Amount of tumor-associated neutrophils (TANs) in intestinal tumors of *F. nucleatum*-fed mice was significantly increased compared with controls [44]. It's recently reported that TANs play a role in tumor progression and in the regulation of anti-tumor immunity [68]. Increased TANs in CRC associate with malignant phenotype and predict poor prognosis of patients with CRC [69]. These findings suggest that *F. nucleatum* may suppress antitumor immunity through inducing the infiltration of TANs in CRC.

Representative images of infiltrating immune cells in *F. nucleatum*-related CRC were shown in Figure 1. Adhesion, invasion, inflammation and immune suppression mediated by *F. nucleatum* in CRC was sketched in Figure 2. Mechanisms of immune suppression induced by *F. nucleatum* in CRC were summarized in Table 2.

Table 2. *Fusobacterium nucleatum* induces immune suppression in colorectal cancer

Immune cells	Function	Mechanism	References
Macrophages	M2 polarization	Activating TLR4 signaling pathway	[53]
	Apoptosis	Butyric acid activating free fatty acid receptors	[56]
	Escape T lymphocyte attack	Impairing the function of peripheral blood lymphocytes	[55]
Lymphocytes	Reducing CD3 + T cells	Unknown	[1]
	Reducing CD4 + T cells	Correlated with TOX expression	[58]
	Inhibiting proliferation	Arresting cells in the G1 phase	[60]
	Inhibiting activation	Interaction with TIGIT	[57]
	Apoptosis	Recruitment of MDSCs	[48,63]
		Butyric acid	[56]
NK cells	Inhibition of NK cell cytotoxicity	Fap2 binding TIGIT molecule	[35,57]
DCs	Dampening anti-tumor immunity	Promoting the expansion of regulatory T cells	[67]
TANs	Dampening anti-tumor immunity	Increasing the number of TANs	[44]

TOX, thymocyte selection-associated high-mobility group box; MDSCs, myeloid-derived suppressor cells; NK, natural killer; DCs, dendritic cells; TANs, tumor-associated neutrophils.

Conclusion

The presence of *F. nucleatum* as symbiotic bacteria in the human intestinal tract has been confirmed to be related to the development of CRC. *F. nucleatum* promotes CRC through different virulence mechanisms, such as adhesion to the intestinal epithelium and inducing inflammatory and immune responses in the host. The resistance reactions induced in the host by *F. nucleatum* induce an inflammatory environment in the host, and promote the recruitment of inflammatory cells as well as the secretion of inflammatory factors. This response to *F. nucleatum* creates a microenvironment which favors tumor growth. Furthermore, *F. nucleatum* can induce immune suppression of gut mucosa by suppressing the function of immune cells such as macrophages, T cells and NK cells, contributing to the progression of CRC.

Conflicts of Interest

Authors declare no Conflict of Interests for this article.

Funding

This work was supported by Natural Science Foundation of Sichuan Science and Technology Agency under Award No. 2018JY0167.

References

[1] Mima K, Sukawa Y, Nishihara R, Qian ZR, Yamauchi M, Inamura K, Kim SA, Masuda A, Nowak JA, and Nosho K, et al (2015). *Fusobacterium nucleatum* and T cells in colorectal carcinoma. *JAMA Oncol* **1**(5), 653–661.

[2] Sethi V, Kurtom S, Tarique M, Lavania S, Malchiodi Z, Hellmund L, Zhang L, Sharma U, Giri B, and Garg B, et al (2018). Gut microbiota promotes tumor growth in mice by modulating immune response. *Gastroenterology* **155**(1), 33–37.e6.

[3] Louis P, Hold GL, and Flint HJ (2014). The gut microbiota, bacterial metabolites and colorectal cancer. *Nat Rev Microbiol* **12**(10), 661–672.

[4] Hall AB, Tolonen AC, and Xavier RJ (2017). Human genetic variation and the gut microbiome in disease. *Nat Rev Genet* **18**(11), 690–699.

[5] Tilg H, Adolph TE, Gerner RR, and Moschen AR (2018). The intestinal microbiota in colorectal cancer. *Cancer Cell* **33**(6), 954–964.

[6] Flemer B, Lynch DB, Brown JM, Jeffery IB, Ryan FJ, Claesson MJ, O'Riordain M, Shanahan F, and O'Toole PW, et al (2017). Tumour-associated and non-tumour-associated microbiota in colorectal cancer. *Gut* **66**(4), 633–643.

[7] Eklof V, Lofgren-Burstrom A, Zingmark C, Edin S, Larsson P, Karling P, Alexeyev O, Rutegard J, Wikberg ML, and Palmqvist R (2017). Cancer-associated fecal microbial markers in colorectal cancer detection. *Int J Cancer* **141**(12), 2528–2536.

[8] Yang Y, Weng W, Peng J, Hong L, Yang L, Toiyama Y, Gao R, Liu M, Yin M, and Pan C, et al (2017). *Fusobacterium nucleatum* increases proliferation of colorectal cancer cells and tumor development in mice by activating toll-like receptor 4 signaling to nuclear factor-kappaB, and up-regulating expression of microRNA-21. *Gastroenterology* **152**(4), 851–866.e24.

[9] Wang X, Yang Y, and Huycke MM (2017). Microbiome-driven carcinogenesis in colorectal cancer: models and mechanisms. *Free Radic Biol Med* **105**, 3–15.

[10] Kurilshikov A, Wijmenga C, Fu J, and Zhernakova A (2017). Host genetics and gut microbiome: challenges and perspectives. *Trends Immunol* **38**(9), 633–647.

[11] Zeng MY, Inohara N, and Nunez G (2017). Mechanisms of inflammation-driven bacterial dysbiosis in the gut. *Mucosal Immunol* **10**(1), 18–26.

[12] Belkaid Y and Hand TW (2014). Role of the microbiota in immunity and inflammation. *Cell* **157**(1), 121–141.

[13] Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, Kurilshikov A, Bonder MJ, Valles-Colomer M, and Vandeputte D, et al (2016). Population-level analysis of gut microbiome variation. *Science* **352**(6285), 560–564.

[14] Ost KS and Round JL (2017). A few good commensals: gut microbes use ifn-gamma to fight salmonella. *Immunity* **46**(6), 977–979.

[15] Sina C, Kemper C, and Derer S (2018). The intestinal complement system in inflammatory bowel disease: Shaping intestinal barrier function. *Semin Immunol* **37**, 66–73.

[16] Postler TS and Ghosh S (2017). Understanding the holobiont: how microbial metabolites affect human health and shape the immune system. *Cell Metab* **26**(1), 110–130.

[17] Gao J, Xu K, Liu H, Liu G, Bai M, Peng C, Li T, and Yin Y (2018). Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. *Front Cell Infect Microbiol* **8**, 13.

[18] Cremonesi E, Governa V, Garzon JFG, Mele V, Amicarella F, Muraro MG, Trella E, Galati-Fournier V, Oertli D, and Daster SR, et al (2018). Gut microbiota modulate T cell trafficking into human colorectal cancer. *Gut* **67**(11), 1984–1994.

[19] De Arcangelis A, Hamade H, Alpy F, Normand S, Bruyere E, Lefebvre O, Mechine-Neuville A, Siebert S, Pfister V, and Lepage P, et al (2017). Hemidesmosome integrity protects the colon against colitis and colorectal cancer. *Gut* **66**(10), 1748–1760.

[20] Chaushu S, Wilensky A, Gur C, Shapira L, Elboim M, Halftek G, Polak D, Achdout H, Bachrach G, and Mandelboim O (2012). Direct recognition of *Fusobacterium nucleatum* by the NK cell natural cytotoxicity receptor NKp46 aggravates periodontal disease. *PLoS Pathog* **8**(3)e1002601.

[21] Sparks Stein P, Steffen MJ, Smith C, Jicha G, Ebersole JL, Abner E, and Dawson D (2012). Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease. *Alzheimers Dement* **8**(3), 196–203.

[22] Kai A, Cooke F, Antoun N, Siddharthan C, and Sule O (2008). A rare presentation of ventriculitis and brain abscess caused by *Fusobacterium nucleatum*. *J Med Microbiol* **57**, 668–671 Pt 5.

[23] Genco R, Offenbacher S, and Beck J (2002). Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. *J Am Dent Assoc* (133 Suppl), 14S–22S.

[24] Chanomethaporn A, Chayasadom A, Wara-Aswapati N, Kongwattanakul K, Suwannarong W, Tangwanichgapong K, Sumanonta G, Matangkasombut O, Dasanayake AP, and Pitiphat W (2018). Association between periodontitis and spontaneous abortion: A case-control study. *J Periodontol*. https://doi.org/10.1002/JPER.18-0174.

[25] Strauss J, Kaplan GG, Beck PL, Rioux K, Panaccione R, Devinney R, Lynch T, and Allen-Vercoe E (2011). Invasive potential of gut mucosa-derived *Fusobacterium nucleatum* positively correlates with IBD status of the host. *Inflamm Bowel Dis* **17**(9), 1971–1978.

[26] Sears CL (2018). The who, where and how of fusobacteria and colon cancer. *Elife*. https://doi.org/10.7554/eLife.28434.

[27] Bullman S, Pedamallu CS, Sicinska E, Clancy TE, Zhang X, Cai D, Neuberg D, Huang K, Guevara F, and Nelson T, et al (2017). Analysis of Fusobacterium persistence and antibiotic response in colorectal cancer. *Science* **358**(6369), 1443–1448.

[28] Han YW (2015). *Fusobacterium nucleatum*: a commensal-turned pathogen. *Curr Opin Microbiol* **23**, 141–147.

[29] Liu Y, Baba Y, Ishimoto T, Iwatsuki M, Hiyoshi Y, Miyamoto Y, Yoshida N, Wu R, and Baba H (2019). Progress in characterizing the linkage between *Fusobacterium nucleatum* and gastrointestinal cancer. *J Gastroenterol* **54**(1), 33–41.

[30] Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, and Han YW (2013). *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. *Cell Host Microbe* **14**(2), 195–206.

[31] Chen Y, Peng Y, Yu J, Chen T, Wu Y, Shi L, Li Q, Wu J, and Fu X (2017). Invasive *Fusobacterium nucleatum* activates beta-catenin signaling in colorectal cancer via a TLR4/P-PAK1 cascade. *Oncotarget* **8**(19), 31802–31814.

[32] Rubinstein MR, Baik JE, Lagana SM, Han RP, Raab WJ, Sahoo D, Dalerba P, Wang TC, and Han YW (2019). *Fusobacterium nucleatum* promotes colorectal cancer by inducing Wnt/beta-catenin modulator Annexin A1. *EMBO Rep*. https://doi.org/10.15252/embr.201847638.

[33] Vander Haar EL, So J, Gyamfi-Bannerman C, and Han YW (2018). *Fusobacterium nucleatum* and adverse pregnancy outcomes: Epidemiological and mechanistic evidence. *Anaerobe* **50**, 55–59.

[34] Fardini Y, Wang X, Temoin S, Nithianantham S, Lee D, Shoham M, and Han YW (2011). *Fusobacterium nucleatum* adhesin FadA binds vascular endothelial cadherin and alters endothelial integrity. *Mol Microbiol* **82**(6), 1468–1480.

[35] Hashemi Goradel N, Heidarzadeh S, Jahangiri S, Farhood B, Mortezaee K, Khanlarkhani N, and Negahdari B (2019). *Fusobacterium nucleatum* and colorectal cancer: A mechanistic overview. *J Cell Physiol* **234**(3), 2337–2344.

[36] Kaplan CW, Ma X, Paranjpe A, Jewett A, Lux R, Kinder-Haake S, and Shi W (2010). *Fusobacterium nucleatum* outer membrane proteins Fap2 and RadD induce cell death in human lymphocytes. *Infect Immun* **78**(11), 4773–4778.

[37] Abed J, Emgard JE, Zamir G, Faroja M, Almogy G, Grenov A, Sol A, Naor R, Pikarsky E, and Atlan KA, et al (2016). Fap2 mediates *Fusobacterium nucleatum* colorectal adenocarcinoma enrichment by binding to tumor-expressed Gal-GalNAc. *Cell Host Microbe* **20**(2), 215–225.

[38] Kaplan CW, Lux R, Haake SK, and Shi W (2009). The *Fusobacterium nucleatum* outer membrane protein RadD is an arginine-inhibitable adhesin required for inter-species adherence and the structured architecture of multispecies biofilm. *Mol Microbiol* **71**(1), 35–47.

[39] Yu J, Chen Y, Fu X, Zhou X, Peng Y, Shi L, Chen T, and Wu Y (2016). Invasive *Fusobacterium nucleatum* may play a role in the carcinogenesis of proximal colon cancer through the serrated neoplasia pathway. *Int J Cancer* **139**(6), 1318–1326.

[40] Brennan CA and Garrett WS (2016). Gut microbiota, inflammation, and colorectal cancer. *Annu Rev Microbiol* **70**, 395–411.

[41] Tang B, Wang K, Jia YP, Zhu P, Fang Y, Zhang ZJ, Mao XH, Li Q, and Zeng DZ (2016). *Fusobacterium nucleatum*-induced impairment of autophagic flux enhances the expression of proinflammatory cytokines via ROS in Caco-2 cells. *PLoS One* **11**(11)e0165701.

[42] Shi C, Yang Y, Xia Y, Okugawa Y, Yang J, Liang Y, Chen H, Zhang P, Wang F, and Han H (2016). Novel evidence for an oncogenic role of microRNA-21 in colitis-associated colorectal cancer. *Gut* **65**(9), 1470–1481.

[43] Wang HF, Li LF, Guo SH, Zeng QY, Ning F, Liu WL, and Zhang G (2016). Evaluation of antibody level against *Fusobacterium nucleatum* in the serological diagnosis of colorectal cancer. *Sci Rep* **6**33440.

[44] Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, Clancy TE, Chung DC, Lochhead P, and Hold GL, et al (2013). *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* **14**(2), 207–215.

[45] Park SR, Kim DJ, Han SH, Kang MJ, Lee JY, Jeong YJ, Lee SJ, Kim TH, Ahn SG, and Yoon JH, et al (2014). Diverse Toll-like receptors mediate cytokine production by *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* in macrophages. *Infect Immun* **82**(5), 1914–1920.

[46] Noh EJ, Kang MJ, Jeong YJ, Lee JY, Park JH, Choi HJ, Oh SM, Lee KB, Kim DJ, and Shin JA, et al (2016). Withaferin A inhibits inflammatory responses induced by *Fusobacterium nucleatum* and *Aggregatibacter actinomycetemcomitans* in macrophages. *Mol Med Rep* **14**(1), 983–988.

[47] Dharmani P, Strauss J, Ambrose C, Allen-Vercoe E, and Chadee K (2011). *Fusobacterium nucleatum* infection of colonic cells stimulates MUC2 mucin and tumor necrosis factor alpha. *Infect Immun* **79**(7), 2597–2607.

[48] Bashir A, Miskeen AY, Hazari YM, Asrafuzzaman S, and Fazili KM (2016). *Fusobacterium nucleatum*, inflammation, and immunity: the fire within human gut. *Tumour Biol* **37**(3), 2805–2810.

[49] Keku TO, McCoy AN, and Azcarate-Peril AM (2013). Fusobacterium spp. and colorectal cancer: cause or consequence? *Trends Microbiol* **21**(10), 506–508.

[50] Edin S, Wikberg ML, Dahlin AM, Rutegard J, Oberg A, Oldenborg PA, and Palmqvist R (2012). The distribution of macrophages with a M1 or M2 phenotype in relation to prognosis and the molecular characteristics of colorectal cancer. *PLoS One* **7**(10)e47045.

[51] Edin S, Wikberg ML, Oldenborg PA, and Palmqvist R (2013). Macrophages: Good guys in colorectal cancer. *Oncoimmunology* **2**(2)e23038.

[52] Chen W, Xu Y, Zhong J, Wang H, Weng M, Cheng Q, Wu Q, Sun Z, Jiang H, and Zhu M, et al (2016). MFHAS1 promotes colorectal cancer progress by regulating polarization of tumor-associated macrophages via STAT6 signaling pathway. *Oncotarget* **7**(48), 78726–78735.

[53] Chen T, Li Q, Wu J, Wu Y, Peng W, Li H, Wang J, Tang X, Peng Y, and Fu X (2018). *Fusobacterium nucleatum* promotes M2 polarization of macrophages in the microenvironment of colorectal tumours via a TLR4-dependent mechanism. *Cancer Immunol Immunother* **67**(10), 1635–1646.

[54] Park HE, Kim JH, Cho NY, Lee HS, and Kang GH (2017). Intratumoral *Fusobacterium nucleatum* abundance correlates with macrophage infiltration and CDKN2A methylation in microsatellite-unstable colorectal carcinoma. *Virchows Arch* **471**(3), 329–336.

[55] Xue Y, Xiao H, Guo S, Xu B, Liao Y, Wu Y, and Zhang G (2018). Indoleamine 2,3-dioxygenase expression regulates the survival and proliferation of *Fusobacterium nucleatum* in THP-1-derived macrophages. *Cell Death Dis* **9**(3), 355.

[56] Abe K (2012). Butyric acid induces apoptosis in both human monocytes and lymphocytes equivalently. *J Oral Sci* **54**(1), 7–14.

[57] Gur C, Ibrahim Y, Isaacson B, Yamin R, Abed J, Gamliel M, Enk J, Bar-On Y, Stanietsky-Kaynan N, and Coppenhagen-Glazer S, et al (2015). Binding of the Fap2 protein of *Fusobacterium nucleatum* to human inhibitory receptor TIGIT protects tumors from immune cell attack. *Immunity* **42**(2), 344–355.

[58] Chen T, Li Q, Zhang X, Long R, Wu Y, Wu J, and Fu X (2018). TOX expression decreases with progression of colorectal cancers and is associated with CD4 T-cell density and *Fusobacterium nucleatum* infection. *Hum Pathol* **79**, 93–101.

[59] Huynh T, Kapur RV, Kaplan CW, Cacalano N, Kinder Haake S, Shi W, Sieling P, and Jewett A (2011). The role of aggregation in *Fusobacterium nucleatum*-induced immune cell death. *J Endod* **37**(11), 1531–1535.

[60] Shenker BJ and Datar S (1995). *Fusobacterium nucleatum* inhibits human T-cell activation by arresting cells in the mid-G1 phase of the cell cycle. *Infect Immun* **63**(12), 4830–4836.

[61] Shenker BJ and DiRienzo JM (1984). Suppression of human peripheral blood lymphocytes by *Fusobacterium nucleatum*. *J Immunol* **132**(5), 2357–2362.

[62] Hamada T, Zhang X, Mima K, Bullman S, Sukawa Y, Nowak JA, Kosumi K, Masugi Y, Twombly TS, and Cao Y, et al (2018). *Fusobacterium nucleatum* in colorectal cancer relates to immune response differentially by tumor microsatellite instability status. *Cancer Immunol Res* **6**(11), 1327–1336.

[63] Gabrilovich DI, Ostrand-Rosenberg S, and Bronte V (2012). Coordinated regulation of myeloid cells by tumours. *Nat Rev Immunol* **12**(4), 253–268.64.

[64] Ye X, Wang R, Bhattacharya R, Boulbes DR, Fan F, Xia L, Adoni H, Ajami NJ, Wong MC, and Smith DP, et al (2017). *Fusobacterium nucleatum* subspecies animalis influences proinflammatory cytokine expression and monocyte activation in human colorectal tumors. *Cancer Prev Res (Phila)* **10**(7), 398–409.65.

[65] Stanietsky N, Simic H, Arapovic J, Toporik A, Levy O, Novik A, Levine Z, Beiman M, Dassa L, and Achdout H, et al (2009). The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc Natl Acad Sci U S A* **106**(42), 17858–17863.

[66] Guevarra Jr LA, Afable ACF, Belza PJO, Dy KJS, Lee SJQ, Sy-Ortin TT, and Albano P (2018). Immunogenicity of a Fap2 peptide mimotope of *Fusobacterium nucleatum* and its potential use in the diagnosis of colorectal cancer. *Infect Agent Cancer* **13**, 11.

[67] Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, and Powrie F (2007). A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* **204**(8), 1757–1764.

[68] Mantovani A, Cassatella MA, Costantini C, and Jaillon S (2011). Neutrophils in the activation and regulation of innate and adaptive immunity. *Nat Rev Immunol* **11**(8), 519–531.

[69] Rao HL, Chen JW, Li M, Xiao YB, Fu J, Zeng YX, Cai MY, and Xie D (2012). Increased intratumoral neutrophil in colorectal carcinomas correlates closely with malignant phenotype and predicts patients' adverse prognosis. *PLoS One* **7**(1)e30806.