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Research Progress of Fusobacterium Nucleatum and Colorectal Cancer

Yuyuan Ma[1,a], Haijuan Xiao[2,b,*]

[1]The First Clinical Medical College of Shanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi, 712000, China
[2]The Third ward of Cancer Hospital of Shanxi University of Traditional Chinese Medicine, Xianyang, Shaanxi, 712000, China
[a]1123265857@qq.com, [b]daisytcm@hotmail.com
**Corresponding author*

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Abstract: Colorectalcancer (CRC) is one of the common malignancies of the gastrointestinal tract, multiple factors influence its pathogenesis. However, numerous studies have found that Fusobacterium nucleatum(Fn) is highly enriched in tumor microenvironment and fecal samples of CRC patients. Fn participates in and promotes the development of CRC by invading virulence factors, producing proinflammatory microenvironment, and inhibiting immune cell activity. This review provides an overview of the relationship between Fn and CRC by summarizing the existing literature, and provides new ideas for the treatment of CRC in the future.

1. Introduction

CRC is the highest incidence of cancer in the world. In 2020, the total number of CRC cases (1.932 million) ranked third among all cancer species, and the total number of deaths (935000) ranked second [1]. With the continuous development of CRC in western countries, the global incidence of CRC is projected to increase to 2.2 million cases and 1.1 million deaths by 2030[2]. Therefore, there is an urgent need to find and prevent new strategies for CRC. To a large extent, CRC is a disease caused by genetic and environmental factors, but more and more evidence indicates that gut microbiota plays a crucial role in the development and metastasis of CRC [3].

There are a large number of microorganisms in human intestines, including more than 1,000 different species and 1,014 microorganisms [4]. Healthy intestinal microflora is extremely important to keep the environment of epithelial cells stable and resistant to potential pathogens.[5]. Disturbances in intestinal flora balance can cause a variety of intestinal diseases, including CRC, inflammatory bowel disease (IBD), and obesity [6]. Fn is an oral microorganism, but can be abundantly enriched and colonized in the intestine, participating in intestinal mucosal barrier damage and immune response activation, thus promoting tumor cell proliferation, immune escape, and influencing CRC development. Therefore, determining the relationship between Fn and CRC is more helpful for the prevention and early diagnosis of CRC. This paper summarizes the research of Fn and CRC in recent years, in order to provide new ideas for the diagnosis, treatment and

prevention of CRC.

2. Overview of Fusobacterium Nucleatum

Fn belongs to the genus Fusobacterium, which is a Gram-negative, non-blastocytic, non-motile obligate anaerobe widely found in the oral cavity and other mucous membranes of humans and animals. The typical Fn has a spindle-shaped appearance with a sharp tip [7]. According to the RNA sequence differences between RNA polymerase subunit and zinc protease, Kim divided Fn into five subtypes: nucleated subspecies, polymorphic subspecies, spindle subspecies, Venn subspecies and animal subspecies [8]. It mainly encodes adhesion protein A (Fusobacterium AdhesinA, FadA), transporter 2 (Fusobacterium autotransporterprotein2, Fap2), RadD, and other adhesion proteins, which mediate important biofilm tissue behavior and interaction with host cells through the expression of a large number of adhesion proteins.

3. The Transport Pathway and Localization of Fn in Vivo

Exactly how Fn colonizes the intestinal mucosa from the oral cavity is unknown, but a recent study found that the microbial community types in the oral cavity and intestine are similar [9], indicating that Fn is likely to transfer and colonize through the oral-intestinal tract. Among them, there are two possible pathways: one is that Fn swallows directly from the mouth to the intestine, which causes dysbiosis of the intestinal microflora and creates a tumor microenvironment conducive to CRC development by disrupting the intestinal homeostasis [10] .As in the APCmin+ mouse model, by feeding Fn to mice every day, it was found that the tumor burden of CRC was significantly increased[11]; secondly, Fn was also detected in liver metastatic tissues [12], suggesting that Fn may act as part of the tumor metastasis colonizing tissues through the hematogenous pathway or the lymphatic metastasis pathway.

Through RNA sequence analysis of CRC tissue, Castellarin found that Fn in CRC tissue increased significantly [13]. And compared with the adjacent normal tissues, the Fn in CRC was abnormally enriched. More importantly, Fn was also isolated from gastrointestinal infections including appendicitis [14], inflammatory bowel disease [15], and was overrepresented especially in CRC, which shows that Fn has affected the development of cancer from the early stage. Notably, Fn is mainly detected in proximal CRC, and its expression is gradually increased in CRC tissues from rectum to cecum [16]. The relationship between the prevalence of Fn and the location of the tumor is in agreement with the observation that microbial tissue is a distinct characteristic of the proximal CRC [17]. However, the exact mechanism of transmission by mouth and colon, and the possible relationship between Fn abundance and tumor site, has yet to be elucidated.

4. Pathogenesis of Fn in CRC

4.1 Fn virulence Proteins Directly Invades CRC and Promotes the Proliferation of Tumor Cells

Fn is mainly enriched in the microbiota of mucosal adhesion, and several studies have shown that its virulence factors Fap2 and FadA are closely related to CRC lesions. Fap2 is a galactose sensitive hemagglutinin and adhesion protein, which helps to enhance Fn invasion [18]. TIGIT is an inhibitory receptor in the body, expressed on T cells and natural killer (NK) cells. Gur found that Fap2 protein of Fn directly interacts with TIGIT expressed on NK cells and tumor-infiltrating lymphocytes to inhibit NK cell cytotoxicity and lymphocyte activity, while FN-infected tumors are protected from NK cell-mediated killing and immune cell attack[19]. Abed also found that Fap2

could identify Gal-Galnac overexpressed in CRC, and the natural deficiency or inactivation of Fap2 reduced the binding of Fap2 to CRC cells expressing GalGalNAc, leading to a large enrichment of Fn in CRC [20]. The second virulence factor, namely FadA, FadA protein is FN-expressed surface adhesion protein, which plays a crucial role in the process of cell attachment. The FadA protein exists in two major forms. The first is complete preFadA, which consists of 129 amino acids fixed on the membrane. The second form is secreted mature FadA (mFadA), which consists of 111 amino acids and is secreted outside Fn [21]. When mFadA binds to pre-FADA, pre-FADAMFada is internalized and FadAc is activated [21]. Internalization of the pre-Fada and mFadA complex ensures Fn binding to host epithelial cells and invasion [21]. Rubinstein confirmed that FadA binds Wnt7be-cadherin on CRC cells, promotes the adhesion and invasion of Fn to host epithelial cells, activates β-catenin signal, leads to the increased expression of Wnt gene, oncogene, transcription factor and inflammatory gene, and promotes the proliferation of tumor cells [22].

4.2 Fn Produces a Proinflammatory Tumor Microenvironment

It has been confirmed that chronic inflammation caused by bacterial infections increases the risk of cancer [23]. In the ApcMin/+ mouse model, Kostic found that FN-fed mice developed more colorectal and small intestinal tumors than control mice, and Fn activated the NF-κB pathway and induced the expression of genes encoding several proinflammatory cytokines, including TNF-α, IL-6, IL-8, and IL-1β. This indicates that Fn infection can induce local inflammation and increase the expression of inflammatory cytokines in the tumor microenvironment, accelerating the progression of CRC in the proinflammatory microenvironment[11]. XiangcangYe also found that Fn could induce CRC cells to express chemokine CCL20 when co-cultured with Fn [24]. In addition, Fn can stimulate monocyte/macrophage activation and migration, thereby contributing to the development of CRC [24, 25].

4.3 Fn generates Tumor Immunosuppressive Microenvironment

A previous study showed that FDC364, an extract of Fn, inhibited human T cell responses to antigens and mitogens [26]. However, Fn suppressor protein inhibits T cell activity in CRC patients by blocking the mid-G1 phase of the cell cycle, thereby inhibiting the immune microenvironment and accelerating the growth of tumor cells [27]. In addition, Fn selectively attracts bone marrow-derived suppressor cells (MDSCs) [11]. MDSCs are a group of heterogeneous cells, which show strong T cell inhibitory activity in immune response[28]. MDSCs and their effectors are key components of tumor growth and jointly promote tumor progression. Fn infected tumors increased the infiltration of myeloid cells, including CD11b+, tumor-associated macrophages (TAMs), M2-TAMs, tumor-associated neutrophils, conventional myeloid dendritic cells (DCs) and CD103+ regulatory DCs[11]. These cells weaken anti-tumor immunity by enhancing the expression of Foxp3+ regulatory T cells [29], playing an important role in inhibiting anti-tumor immune activity and promoting tumor development.

Another mechanism by which Fn induces immune evasion of tumor cells is to inhibit the cytotoxic effect of natural killer (NK) cells [19]. NK cells belonging to the innate immune system have two types of receptors: activating and inhibitory receptors [30]. Activated receptors can detect tumor proteins, viral components, self-molecules, and stress-induced molecules [30,31]. TIGIT(T-cell immunoglobulin and immunodominant tyrosine inhibitory motif [ITIM] domain) is an inhibitory receptor for NK cells and other immune cells. Studies have shown that Fn interacts with the human TIGIT receptor (HTIGIT) of NK cells and initiates an inhibitory cascade. Furthermore, Fn cells isolated from CRC patients confirmed the inhibitory effect of Fn on NK cells by activating hTIGIT. Collectively, these studies suggest that Fn generates the tumor

immunosuppressive microenvironment and contributes to the development and progression of CRC.

5. Chemotherapy Resistance of CRC Induced by Fn

At present, the treatment of CRC is still a comprehensive treatment based on surgical resection and supplemented by chemotherapy. Commonly used chemotherapeutic drugs for CRC include 5-fluorouracil (5-FU) or capecitabine combined with oxaliplatin (LOHP). However, CRC patients often face chemotherapy resistance, resulting in a high tumor recurrence rate and a 5-year survival rate of less than 10%[32]. Therefore, predicting the efficacy of chemotherapy is the key to develop personalized treatment strategies for CRC patients. TaChungYu et al found a large amount of Fn in the tissues of CRC patients who relapsed after chemotherapy[33]. Further experiments show that Fn up-regulates autophagy-related genes ULK1 and ATG7 by inhibiting the expression of miR-18a and miR4802, thus activating the autophagy pathway of CRC cells to promote chemotherapy resistance, which is closely related to the recurrence of CRC. It was also found that M2 macrophages may induce drug resistance of CRC cells through IL-6[34]. Further detection by Fang Jingyuan et al found that under the biofilm condition, the biofilm formability of Fn increased, the drug resistance of the bacteria itself increased, and the expression of population-related genes changed, which directly led to a higher level of IL-6 induction of macrophages by Fn bacterial biofilm. It can also reduce the toxicity of chemotherapeutic drugs (LOHP/5-FU) to CRC cells[35]. It is suggested that the presence of Fn bacterial biofilm in CRC tissue and the M2 polarization of tumor-associated macrophages are related to drug resistance and recurrence in postoperative chemotherapy patients.

6. Potential Clinical Value of Fn in CRC

Traditional colonoscopy, as the main screening method for CRC, has certain heterogeneity in different patients due to its invasive operation, so simple and easy stool or blood examination has broad application prospects in CRC [36]. WongSH reported that the combination of fecal Fn quantification and fecal immunochemical tests could improve the diagnostic rate of advanced colorectal adenoma and carcinoma [37]. In this study, the relative fecal abundance of Fn was 132 times higher in the CRC group and 3.8 times higher in the advanced adenoma group than in the control group. LiangQ et al. found that when fecal Fn was used alone, the sensitivity and specificity of CRC diagnosis were 80.2% and 80.7%, respectively, while when fecal Fn was used in combination with FIT and three other fecal bacteria, the sensitivity increased to 92.8%[38]. In addition, several studies have shown that Fn infection leads to a high level of serum FN-IgA antibody in CRC patients, and the sensitivity of serum anti-FN- IgA combined with CA19-9 and CEA in screening early CRC is higher than that of CA19-9 and CEA alone. It is suggested that serum FN- IgA antibody may be regarded as a potential diagnostic biomarker for early CRC[39]. In conclusion, along with the detection of Fn, fecal and serum microbiome based examination may be a practical tool for the early diagnosis and treatment of colorectal adenoma and CRC.

7. Conclusions

This review focuses on the potential link between Fn and CRC, considers the main mechanism of Fn promoting the development of CRC, including Fn virulence proteins FadA and Fap2 directly invading CRC, promoting the proliferation of tumor cells, and anti-tumor by producing proinflammatory tumor microenvironment and inhibiting immune cell activity. Meanwhile, Fn can also promote CRC chemoresistance and cause tumor recurrence. These findings will help us to further understand the relationship between gut microbiota and CRC, and further improve the treatment and prevention strategies for CRC. Despite the prevalence of Fn infection in CRC

patients, the specific underlying molecular mechanisms are still unknown and deserve further investigation. Secondly, the quantification of Fn in feces and serum may be used as a non-invasive and simple screening for CRC detection. Although the prognostic value of Fn is controversial, different sample types and different methods affect the accuracy of Fn abundance detection. It is believed that in the near future, tumor microbes may be as influential as tumor host genetics in guiding prognostic and therapeutic decisions, and microbial profiling may soon become as routine as testing genetic tumor profiles.

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