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Progress of "Yiqi and Spleen Enhancement Method" in the Treatment of Colorectal Cancer based on Intestinal Flora

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Abstract: ***The intestinal microbiota is located close to the colorectal epithelium and consists of a large microbiota that interacts with host cells to regulate many physiological processes such as energy harvesting, metabolism and immune responses [1]. It corresponds to the functions of the "spleen" in Chinese medicine, which is responsible for the transportation of water and grains and the resistance to evil. The balance of intestinal flora directly reflects the functional status of the "spleen" in TCM. Sequencing studies have revealed the microbial composition and ecological changes in colorectal cancer (CRC) patients, and functional animal model studies have clarified the role of several bacteria in colorectal carcinogenesis, including Clostridium nucleatum, Escherichia coli, and Pseudomonas fragilis strains, which are closely related to CRC [2]. Based on this, this paper reviews the research progress on the mechanism of intestinal flora affecting CRC and the intervention of intestinal flora against CRC by Yiqi and Spleen Method, which provides ideas and references for the prevention of colorectal cancer by traditional Chinese medicine.***

Keywords: Intestinal flora, Colorectal cancer, Yi qi, Spleen strengthening.

1. Introduction

Colorectal tumor formation is multifactorial, and various genetic and environmental factors contribute to the development of the disease. Although genetic susceptibility syndromes for CRC are well described, such as Lynch syndrome, familial adenomatous polyposis. Based on evidence from twin and family studies, the heritability of CRC is estimated to be only 12-35% [3] This relatively low heritability of CRC reflects the importance of the environment, which plays a greater role in causing sporadic CRC. Among the environmental factors, the role of microorganisms in cancer biology has been increasingly recognized [3]. One study showed that infectious agents accounted for 13% of all new cancer cases in 2018, including the vast majority of gastric cancers (Helicobacter pylori), hepatocellular cancers (Hepatitis B virus and Hepatitis C virus), and cervical cancers (human papillomavirus) [4]. While the tumorigenic mechanisms of specific infectious agents have been investigated, researchers have also begun to study the collective microbial community in the tumor environment. This microbiota, also known as the microbiota, has emerged as an important environmental factor in a number of cancers, including cancers of the colorectum, liver, biliary tract, and even the breast [5]. The human gut is approximately 3×10^{13} bacteria and the colorectum interacts with a large number of microorganisms with which the intestinal epithelium has continuous crosstalk These microorganisms are important for gastrointestinal physiology, such as energy harvesting as well as immune maturation, and changes in their relative abundance alter the balance, leading to both intestinal and parenteral diseases [6].

1.1 CRC-associated Enterobacteriaceae

In recent years, researchers have conducted 16S ribosomal RNA sequencing studies to characterize the CRC microbiota and mucosal microbes in the feces, and the results have shown that overall the CRC microbiota shifts in overall composition compared to the microbiota of healthy individuals. This responds to the different gut microecology of CRC patients, and in addition to specific bacteria (Anaplasma fragilis, Escherichiaceae, Enterococcus faecalis, and Streptococcus lysimachiae) associated with being individually linked to CRC in various association mechanism studies, associations with other bacteria have been found in human macrogenome studies that have included Clostridium as well as bacteria of the genera Clostridium finely-formed monoclasts, Streptococcus gastricus, Porphyromonas, and Prevotella [7], a microbial core of seven enriched bacteria has been identified in CRC This core group includes: Bacillus fragilis (a bacterium with enterotoxin-producing ability associated with CRC); four oral bacteria: Nucleococcus, Microcystis microsporus, Porphyromonas desaccharomycetes, and Prevotella intermedia; and two other bacteria, Vibrio finelyani and Vibrio acidamidothermophilus [8]. These enriched bacteria are negatively correlated with a mutually beneficial symbiotic network of CRC-depleted bacteria [8], including several species that have been developed as probiotics, such as the butyrate producer Clostridium butyricum [9] and the lactobacillus Streptococcus thermophilus [10], which have been used for the prevention of diarrhea-associated diarrhea in infants [9][10] These two probiotics can be studied for their potential benefits in CRC independently or by antagonizing pathogenic bacteria.

1.2 Involvement of Intestinal Flora in Colorectal Cancer Carcinogenesis Mechanisms

1.2.1 Inflammation and immune regulation

The gastrointestinal tract provides an important interface for crosstalk between the gut microbiota and the host immune system [11]. Chronic inflammation is a hallmark and established risk factor for CRC, as patients with inflammatory bowel disease consistently have a higher risk of developing

cancer than the general population. Previous meta-analyses summarized a 30-year cumulative CRC risk of 13.91% in patients with ulcerative colitis [12] and 2% in patients with Crohn's disease [13] Reported risk levels varied by study population (e.g., high-risk cases versus population-based cohorts), hospital setting (e.g., tertiary versus general hospitals), and clinical practice (e.g., rectal colectomy rates). Intestinal flora interacts closely with the host immune system and can influence the course of the inflammatory response in the gastrointestinal tract. Gavage of feces from CRC patients to germ-free and carcinogen-fed mice resulted in increased tissue inflammation and expression of inflammatory gene markers [14], as microbes in the gut can induce chemokines. (e.g. CXCL9 and CXCL10 for cytotoxic T-lymphocytes and type 1 T helper (TH1) cells, and CCL17 and CCL20 for IL-17-producing TH cells) recruit T-cells into tumors [15] In terms of individual bacterial species, Clostridium perfringens (F. nucleatum) has been reported to activate the nuclear transcription factor, NF-κB, pathway that drives myeloid cell infiltration in tumors [16] thereby generating a pro-inflammatory environment conducive to colorectal tumor progression in ApcMin mice, a common CRC mouse model[17]. Enterotoxin-producing Bacillus fragilis, a strain enriched in CRC patients [18] can trigger an inflammatory cascade involving IL-17, transcription 3 signaling factors, and nuclear factor-κB signaling in colonic epithelial cells through its virulence factor, B. fragilis toxin [19]. These signaling pathways in turn induce CXC chemokine mice in Apc to recruit polymorphic nuclear immature myeloid cells, creating a pro-inflammatory environment, especially in the distal colon [20]. Inflammation is also important for the pro-cancer activity of other microorganisms.

Pattern recognition receptors (PRRs) act as an interface between the gut microbiota and the host immune system. In recognizing microbial antigens, PRRs activate the gut immune system through a cascade of downstream signaling molecules. In animal models, several of these PRRs have been associated with colitis-associated carcinogenesis, including toll-like receptors (TLRs) [21]. Nucleotide-binding oligomerization-like receptors [22], RIG-I-like receptors [23] and receptors missing from melanoma 2-like receptors [24]. Specifically, C. nucleatum activates TLR4 signaling and promotes tumor development in mice [25][26]. And another CRC-rich bacterium, Pseudomonas aeruginosa, can promote carcinogenesis in mice by activating TLR2 and/or TLR4 pathways [27].

1.2.2 Genotoxin production

Another carcinogenic mechanism associated with microbiota is the production of toxins with DNA damaging (genotoxic) effects. Cytolethal distending toxin (CDT) and E. coli bacteriocins are two well-characterized bacterial-produced genotoxins. CDT is produced by intestinal pathogens such as Escherichia coli and Campylobacter, and is carcinogenic by inducing double-stranded DNA breaks through its deoxyribonuclease activity [28][29]. CDT deficiency has shown a weak carcinogenic potential in a mouse model of CRC [30]. E. coli toxins are produced by members of the Enterobacteriaceae family and can also induce DNA strand breaks [31]. Moreover, both B. fragilis toxins [32] and reactive oxygen species produced by E. faecalis have been associated with DNA damage and genome destabilization in vitro [33][34]. Binding or inactivation of these toxins may have a therapeutic or preventive effect on CRC, as small molecule inhibitors targeting the production of E. coli proteins have been shown to reduce tumor load in mouse models [35].

2. Spleen and Intestinal Flora

It has been recognized in previous studies that there is a close relationship between the spleen and intestinal flora in TCM, and that intestinal flora may be an important biological basis for the physiological function of the spleen in TCM. Spleen deficiency, as a syndrome reflecting the insufficiency of the physiological functions of the spleen and stomach, prevents the normal transportation of water and grains, and impairs digestion and absorption, which may cause dysfunction of the intestinal flora structure and function, resulting in symptoms such as loose stools, poor appetite, and lethargy. The dysfunction of intestinal flora can aggravate the symptoms of “spleen deficiency”, and there is an interdependence and mutual root and use relationship between the two [36]. Intestinal flora homeostasis has important physiological significance in regulating body immunity, maintaining normal physiological functions of the intestinal tract and antagonizing pathogenic microorganisms. Spleen deficiency, as a common clinical syndrome in Chinese medicine, involves a variety of diseases, has a wide range of effects and is very harmful. Spleen deficiency is usually categorized into spleen qi deficiency, spleen yang deficiency and spleen yin deficiency, and the clinical manifestations of the three syndromes are different, but all of them are based on the pathogenesis of spleen qi deficiency, and they have the general symptoms of spleen qi deficiency, so the diagnosis of spleen deficiency is the most important in identifying the spleen qi deficiency [37]. Animal models of different types of spleen qi deficiency can be created by intervening with surgery, drugs, diet, and other methods. When the organism has a spleen deficiency syndrome, the species richness of the intestinal flora and the number of significantly different species are affected, the intestinal probiotic taxa (e.g., Bifidobacterium, Lactobacillus, and Anaplasma) are reduced, and the number of pathogenic bacteria (e.g., enterotoxin-producing Anaplasma, Escherichia coli, and Clostridium difficile) is reduced and increased [38]. When the intestinal flora is dysregulated, it in turn affects the body's absorption of nutrients, which leads to disorders in intestinal fatty acid and bile salt metabolism, weakening the intestinal barrier function, promoting diarrhea, and further aggravating the evidence of splenic deficiency, forming a vicious cycle [39]. A study found that using multifactorial composite modeling method to establish spleen qi deficiency model rats, at the portal level, ginseng and sour jujube nut extract significantly increased the relative abundance of thick-walled bacillus phylum and anaplastic bacillus phylum, and lowered the relative abundance of deformed bacillus phylum and actinomycetes phylum of the spleen qi deficiency model rats; at the genera level, it could significantly increase the relative abundance of Lactobacillus et al. and decrease the relative abundance of Streptococcus et al. And it affects the body sugar metabolism and amino acid metabolism, etc [40]. Some scholars [41] found that after the intestinal microbial balance of mice with spleen deficiency and constipation was disrupted, the intestinal enzyme activity was changed and the

intestinal flora was abnormal. Other scholars [42] detected alterations in the contents of the cecum of experimental dogs with spleen deficiency using high-throughput sequencing technology: the diversity and abundance of the flora were reduced. In rats with spleen deficiency model, Clostridia, as the dominant genus at the level of the phylum, showed a decreasing trend, the levels of α-Aspergillus and Actinobacteria decreased significantly, and the levels of Bacillus showed an increasing trend [43]. Spleen-deficient mouse model was obtained by feeding rhubarb decoction, and it was found that the intestinal flora of spleen-deficient mice was altered, and the values of Bifidobacterium and Lactobacillus were decreased [44].

3. Colorectal Cancer is based on Spleen Deficiency

"Spleen deficiency plays an important role in the development of colorectal cancer, as Zhang Jingyue said, "People with spleen and kidney deficiencies and weakness and disorders often have the disease of accumulation. Covering the spleen deficiency, the middle of body is not transported, kidney deficiency, the lower is not transformed, and if the Anti-pathogenic qi does not work, the evil stagnation is able to reside in it." In a study of TCM patterns involving 760 patients with CRC, patterns related to spleen deficiency accounted for 48% of the cases [45]. In a study on the distribution of TCM syndromes in 83 postoperative CRC patients, the syndromes related to spleen yang deficiency accounted for 26.5% [46], and in a [47] study on the distribution of TCM syndromes in 430 patients with early stage colorectal cancer and precancerous lesions showed that 242 (56.28%) of the syndromes were spleen deficiency syndromes, and in a [48] case analysis involving 780 patients with intestinal cancer, it was shown that the highest frequency of spleen deficiency syndromes accounted for 67.9% of the total. In a case analysis involving 780 patients with intestinal cancer in [48], the highest frequency of spleen deficiency was 67.9%. Hou Fenggang et al. found that among the various TCM symptoms of colorectal cancer, spleen and stomach weakness and spleen qi deficiency accounted for 38.6% and 76.8%, respectively, and had the highest proportion among the various symptoms. In the analysis of the evidence data of colorectal cancer patients involving 8,147 patients, it was shown that among the top five types of evidence with the highest proportion, spleen deficiency had the most [49]. The literature on the treatment of colorectal cancer with Chinese medicine is extensive, involving no less than a thousand prescriptions, and after further analysis, among the drugs with high frequency of clinical use, the spleen-enhancing and qi-benefiting drugs accounted for the majority [49][50].

4. Regulation of Intestinal Flora to Prevent CRC based on the Method of Strengthening Qi and Spleen

According to the theory of "sweetness can tonify", most of the medicines to tonify qi deficiency are sweet and mainly enter the spleen, lung, and heart meridians, and according to Chinese medicine theory, qi can regulate the blood, and tonifying qi can produce blood and fluids, so when qi tonifying Chinese medicines are used clinically for the treatment of spleen qi deficiency, most of them are used with medicines to tonify the blood, astringent sweating, hemostatic, urinary tract shrinkage, and fluids production. Commonly used qi tonifying herbs to strengthen the spleen include ginseng, codonopsis, astragalus, atractylodes macrocephala, and yam, etc. Representative formulas include Sijunzi Tang, Zhenzhong Yiqi Tang, and Lizhong Pill. The main active ingredients in the treatment of spleen deficiency are saponins, sugars, volatile oils, flavonoids, etc. The active ingredients of qi and spleen-healthy herbs are inseparable from the role of intestinal flora in treating spleen deficiency syndrome and exerting medicinal effects.

4.1 Single Drugs

4.1.1 Ginseng

Some scholars found that the discovery of diol-type ginsenosides in the human intestinal flora in the role of deglycosylation of ginsenosides gradually metabolized into secondary glycosides absorbed into the blood; can increase the relative abundance of the healthy human intestinal phylum Thick-walled Bacteria and Aspergillus; reduce the relative abundance of the phylum Anaplasma, Dolichosporium spp. and Megalobacterium spp. [51]. Protopanaxatriol-type saponin group can significantly increase the relative abundance of E. faecalis spp. and B. dyeri spp. and decrease the relative abundance of B. dorferi spp. in the intestinal tract of healthy people, which can improve the structure of intestinal flora [52].

4.1.2 Rhizoma Atractylodis Macrocephalae

As a typical traditional Chinese medicine for regulating qi and strengthening the spleen, Atractylodes macrocephala polysaccharide in Atractylodes macrocephala was metabolized in artificial intestinal fluids with a significant increase in the metabolic rate within 4h, and then was in the plateau period after 4h, basically no longer changing [53]. Atractylodes macrocephala was able to foster the beneficial flora in the intestinal tract, change the structure of intestinal flora in animals with liver and spleen deficiency, and make the distribution of intestinal flora have a tendency to converge to the intestinal flora in the normal organism [54]. Atractylodes macrocephala decoction was found to be able to maintain the homeostasis of intestinal flora, increase the adhesion of intestinal mucosa to bifidobacteria, inhibit the proliferation of methicillin-resistant Staphylococcus aureus, Staphylococcus aureus, Escherichia coli, and Enterococcus faecalis [55][56], and increase the content of propionic acid and butyric acid. Based on clinical observations, animal experiments were conducted to show that Atractylodes macrocephala interfered with metabolic pathways such as phenylalanine metabolism, niacin and nicotinamide metabolism, and pantothenic acid and coenzyme A biosynthesis in mice with irritable bowel syndrome [57].

4.1.3 Yam.

The aqueous extract of yam helps to promote intestinal development and the integrity of intestinal morphology and structure in chickens, and significantly enhances the body's immunity [58]. Administration of yam polysaccharides to

obese diabetic nephropathic rats resulted in an increase in the relative abundance of Thick-walled Bacteria, Trichoderma spp, Weilonella spp, Bacillus spp, Bacillus-like Bacteria, and Clostridium acidophilus, and a decrease in the relative abundance of Anaplasma, Aspergillus, Mycobacterium, Ehrlichia, Shigella, and Salmonella [59]. Yam oligosaccharides can be utilized as a carbon source by intestinal probiotics such as Lactobacillus acidophilus, Streptococcus thermophilus, Lactobacillus bulgaricus and Lactobacillus plantarum, Bifidobacterium adolescentis, and Bifidobacterium animalis as a carbon source in a simulated colonic environment to produce lactic acid, acetic acid, and propionic acid, which are known to have a positive effect on human health [60].

4.2 Traditional Chinese Medicine Compound Formulas

4.2.1 Sijunzi Tang

Wang Shiqi et al [61] found that Sijunzi Tang, composed of ginseng, atractylodes, poria, and licorice, could regulate the intestinal microecology and improve the imbalance of intestinal flora in peptic ulcer patients with spleen and stomach weakness syndrome, which manifested itself as an increase in the predominant flora and a decrease in the nondominant flora. Huang Wenwu et al [62] found that Sijunzi Tang could induce the values of Escherichia coli, Lactobacillus, Bifidobacterium, and Synechococcus to return to normal in the intestinal flora of patients with spleen weakness, and elevate the content of total short-chain fatty acids in the feces.

4.2.2 tonifying the middle and benefiting the qi soup

Studies have shown that tonifying Zhong Yi Qi Tang can regulate the α-diversity index of intestinal flora, tonifying Zhong Yi Qi Tang can effectively increase the abundance and diversity of intestinal flora in rats with CFS, increase the relative abundance of Thick-walled bacillus, Lactobacillus spp., Trichosporon, Ruminal coccus, Enterococci, and Salmonellae in rats with CFS, decrease the relative abundance of Anabaena phylum, Prevotella, Bacteroides, Helicobacter, and Streptococcus [63], and its mechanism of action may be related to the intestinal immune status [64].

4.2.3 Lizhong Wan

Lizhong Wan, composed of epiphyllum, dried ginger, licorice, fried atractylodes, and codonopsis, can promote the growth of probiotics such as Lactobacillus spp. in the intestines of spleen-yang-deficient IBS-D rats, and call back intestinal bacterial indices such as Bacillus thuringiensis, Bacillus anisopliae, and Bacillus brevetus, and regulate the bacterial flora that is closely related to inflammation/immune response [65]. Lizhongwan can increase the number of Bifidobacterium bifidum flora and decrease the number of Enterococcus faecalis flora in patients with type 2 diabetes mellitus with spleen deficiency [66].

5. Conclusion

Although the efficacy of Yiqi and Spleen Method in the treatment of colorectal cancer by regulating intestinal flora is accurate, there are still many issues to be explored: at present, domestic and international guidelines have introduced a number of screening methods for colorectal cancer such as: immunoassay and chemical assay fecal occult blood test, sigmoidoscopy, colonoscopy, etc. The clinical application has found that there are low sensitivity, low cost, low cost, and low sensitivity. Clinical application found to have low sensitivity, poor patient compliance, combined with the idea of Chinese medicine to treat the disease before it occurs, can start from the intestinal microecology to study a low-cost, simple and non-invasive, high specificity early diagnostic techniques; in recent years, it was found that the method of yi qi and spleen can be used to inhibit the development of tumors through the interleukin, vascular endothelial growth factor, macrophage colony-stimulating factor, sex hormones, the researchers can Considering the application of advanced microecological biological assay to study the mechanism of spleenic method in regulating intestinal flora, and to find out the new targets for the treatment of colorectal cancer; chemotherapy occupies an important position in the treatment of colorectal cancer, and the occurrence of chemoresistance is one of the main factors leading to the poor prognosis of colorectal cancer patients, therefore, it is necessary to combine with the mechanism of Chinese medicine to regulate the intestinal flora, and to study the mechanism of decreasing the resistance to chemotherapy in certain bacteria, such as Clostridium difficile, to reduce the risk of chemoresistance. Therefore, it is necessary to combine with Chinese medicine spleen method to regulate the intestinal flora to study the mechanism of reducing certain bacteria: such as Clostridium difficile induced chemoresistance of colorectal cancer cells, so as to improve clinical efficacy. We hope that we can emphasize the relationship between spleen, colorectal cancer and intestinal flora in Chinese medicine, and through in-depth research, we can open up new ways for the prevention and treatment of colorectal cancer.

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