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Research Progress in Intestinal Flora and Hepatocellular Carcinoma

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Abstract

Intestinal flora are involved in environmental homeostasis and the development of many diseases within the human body. The liver, as one of the most important digestive organs in human beings, communicates with the intestinal flora and their metabolites in the intestine through the biliary system, the hepatic portal system, and the corpuscular circulation, an interrelated system known as the intestinal-hepatic axis. Hepatocellular carcinoma is the most common malignant tumor of the liver and one of the leading causes of cancer-related deaths worldwide. There is increasing evidence that intestinal flora plays an important role in the evolution of hepatocellular carcinoma. Besides, intestinal flora has great potential in the treatment of liver cancer. On this basis, this paper summarizes the relevant studies on the role of intestinal flora in the development of hepatocellular carcinoma and discusses its potential value in the treatment of hepatocellular carcinoma.

Keywords

Hepatocellular Carcinoma, Intestinal Flora, Gut-Liver Axis

1. Introduction

The flora in the human body is most concentrated in the gastrointestinal system [1]. These flora influence many physiological processes, including digestion, metabolism, cognitive development, and functioning [2]. They form a symbiotic relationship with the host [3]. In addition, intestinal flora interacts closely with the immune system in the host's defense mechanisms [4]. The intestinal flora plays an important role in many aspects of human life and health. However, immune system deficiencies, disturbances in gut flora and environmental changes can disrupt symbiotic relationships and may lead to the development of disease,

including cancer [5].

The liver is widely thought to be the first organ exposed to gut-derived harmful substances, including bacteria and bacterial metabolites [6]. The composition of the intestinal flora, its own components or metabolites interact with the liver through a variety of mechanisms, and a growing body of research suggests that the intestinal flora may play an important role in the cancerous process of the liver. Primary liver cancer is currently the sixth most common malignancy and the third leading cause of cancer death worldwide, with hepatocellular carcinoma being the most common tumor of the liver. Interventional therapies such as arterial chemoembolization and arterial chemoinfusion are widely used in mid-stage hepatocellular carcinoma; targeted and immunotherapies are mainly applied to advanced hepatocellular carcinoma, but with limited effects [2]. In recent years, the incidence and mortality rates of primary liver cancer in China have shown a significant downward trend. Despite the decline in incidence rates, population growth and aging have led to a significant increase in the number of new cases, posing a serious threat to people's lives and health [7] [8]. So the development of new and effective therapies and the snatch of multidisciplinary treatments are still the contents of today's science that need to be discussed. It has been shown that novel therapeutic strategies such as probiotics, prebiotics, antibiotic therapy, fecal microbiota transplantation (FMT) therapy and dietary interventions have shown early promise as modulators of gut flora in the treatment of hepatocellular carcinoma. Therefore, an in-depth study of the effects of gut flora on hepatocellular carcinoma development is of great clinical value for the discovery of novel and actionable intervention targets and clinical evaluation. In this paper, we will elaborate on the study of the correlation between intestinal flora and hepatocellular carcinoma and summarize the current research status and application prospect of intestinal flora in hepatocellular carcinoma treatment.

2. Dysbiosis of Intestinal Flora and Liver Cancer

It is now widely recognized that gut flora characterizes cancer and has a complex interaction with the development and progression of hepatocellular carcinoma [9]. The association between the composition of specific intestinal flora and the development of hepatocellular carcinoma has progressed in many studies of patients with hepatocellular carcinoma of different etiologic origins. Numerous animal studies have shown a strong association between intestinal flora and hepatocellular carcinoma, and here we focus on relevant clinical trial studies.

Several studies have shown differences in the ecological composition of the corresponding gut flora at various stages of liver cancer development. It has been found that the intestinal flora profile associated with hepatocellular carcinoma in patients with cirrhosis is characterized by increased *E. coli* fecal counts and higher levels of *E. coli* in fecal samples than in non-tumorigenic patients, suggesting that an overgrowth of intestinal *E. coli* may contribute to hepatocellular carcinoma [10]. In order to study the alteration of intestinal flora in different

stages of hepatocellular carcinoma, Zhang determined the differences in the diversity of intestinal flora between groups from fecal specimens from hepatocellular carcinoma, cirrhosis, and healthy populations, respectively. At the portal level, the diversity of *Enterobacter ludwigii* tended to decrease from the healthy population group to the cirrhosis and hepatocellular carcinoma groups. At the species level, *Enterobacter ludwigii* tended to increase in the hepatocellular carcinoma group, where the relative abundance of *Enterobacter ludwigii* in the hepatocellular carcinoma group was 100 times higher than in the other groups, whereas the ratio of thick-walled bacteria/anaplasmodia decreased significantly with disease progression [11]. In addition, the researchers found that *Clostridium difficile* dominated the intestinal flora of a healthy population group, while Enterococcaceae, Lactobacillus, Bacillus, and Gammaproteobacteria could be used as diagnostic markers for hepatocellular carcinoma. Intestinal flora analysis using 16SrRNA sequencing by Ponziani *et al.* showed that Bacteroides, Klebsiella, Blautia, Enterobacteraceae, and Streptococcus, Ruminococcus were increased in abundance throughout the cirrhotic patient group [12], Enterobacteraceae and Streptococcus were the core intestinal flora in cirrhotic patients, and mucinophilic Akkermansia (Akkermansia) was decreased; the abundance of Bacteroides spp. and Ruminococcus spp. was increased in the intestinal tracts of hepatocellular carcinoma patients, whereas Bifidobacterium spp. abundance was decreased, compared with cirrhosis. Although the differences between different hepatocellular carcinoma stages were not significant, they suggest to some extent that the composition of the intestinal flora varies in hepatocellular carcinomas with different degrees of progression.

In addition, some scholars have studied the intestinal flora of hepatocellular carcinoma patients with and without HBV infection [13]. In patients with HBV-associated hepatocellular carcinoma, the species richness of their fecal flora was much higher than in healthy individuals as well as in non-HBV-associated hepatocellular carcinoma and had more anti-inflammatory bacteria (e.g., Prevotella, *E. faecalis*) as well as fewer pro-inflammatory bacteria (e.g., Shigella, Enterococci), and, at the portal level, a decrease in Aspergillus spp. in hepatocellular carcinoma patients; on the contrary, patients with non-HBV-associated hepatocellular carcinoma carried fewer potentially anti-inflammatory bacteria and more pro-inflammatory bacteria, as well as fewer *B. thickeniensis* and an increase in Aspergillus at the portal level. This difference may be due to differences in intestinal flora in response to HBV infection, suggesting that the composition of intestinal flora may also be different for patients with hepatocellular carcinoma of different etiologies. An increase in Aspergillus spp. in patients with advanced hepatocellular carcinoma was also found in another study [14]. Consistent with these results, this suggests that long-term enrichment of Aspergillus in the gut may represent an imbalance in the microbial community structure instability or the disease state of the host, and may be a potential diagnostic marker. This implies the potential value of changes in intestinal flora as an early diagnosis of hepatocellular carcinoma. Therefore, some scholars prospectively controlled the

feces of early-stage liver cancer patients, cirrhosis patients, and healthy people from three major regions in East China, Central China, and Northwest China, and successfully constructed a diagnostic model for early-stage liver cancer by using intestinal flora analysis and cross-regional validation, and showed that intestinal flora-targeted biomarkers may become a potential non-invasive tool for early-stage diagnosis of liver cancer [15]. This provides an important basis for the development of non-invasive biomarkers for the development of intestinal flora-related biomarkers, which would help clinicians to better identify and diagnose liver cancer, and to provide more effective treatment for the patients.

The above studies indicate that the occurrence and progression of hepatocellular carcinoma are closely related to the ecological dysregulation of intestinal flora. With the deepening of the understanding of the role of intestinal flora in hepatocellular carcinoma, it suggests that the therapeutic modality based on intestinal flora has a great potential for treating hepatocellular carcinoma, and provides a theoretical basis for the development of the therapeutic method related to intestinal flora.

3. Regulation of Intestinal Microecology in the Treatment of Hepatocellular Carcinoma

Gut flora therapy has become a new direction in liver cancer treatment, which improves the therapeutic effect and survival rate of liver cancer patients by changing the composition and function of intestinal flora. Currently, intestinal flora therapy mainly includes probiotics, prebiotics, antibiotic therapy and fecal microbiota transplantation (FMT) therapy.

3.1. Probiotics

Probiotics improve the balance of intestinal flora as well as stimulate bacterial products and metabolites, and may promote human health as well as preventive treatment of different diseases. In animal models of acute liver injury and cirrhosis, the use of probiotics has been reported to improve survival by correcting bacterial overgrowth, stabilizing mucosal barrier function, and reducing bacterial translocation and endotoxemia [16]. In patients with hepatic encephalopathy, the application of probiotics may delay the progression of hepatic encephalopathy, reduce plasma ammonia concentrations, and improve quality of life [17]. Currently, probiotic therapy for liver cancer is only based on animal models of liver cancer, and there are no specific data from clinical studies. Zhang *et al.* in a rat model of DEN-induced hepatocellular carcinoma found that administration of VSL#3 (including four species of Lactobacillus, three species of Bifidobacterium, and one species of Streptococcus thermophilus salivarius subgenera) reduced the incidence of hepatocellular carcinoma in rats by restoring intestinal homeostasis and attenuating hepatic and intestinal inflammation and prevented progression of cirrhosis to hepatocellular carcinoma, and that a high dose of

VSL#3 reduced the mortality rate of DEN-induced hepatocellular carcinoma in rats [18]. In addition, Li *et al.* used a novel probiotic, Prohep (composed of *Lactobacillus rhamnosus* (LGG), *Escherichia coli* Nissle1917 (EcN), and heat-inactivated VSL#3 (1:1:1)), for the treatment of a mouse model of hepatocellular carcinoma, and found that the Prohep group reduces the growth of hepatic tumors by inhibiting angiogenesis through decreasing the production of IL-17 and other angiogenic factors. And limit excessive Th17 cell production in the intestine and reduce the migration of Th17 from the intestine to the tumor site case reduce inflammation occurs, and by increasing the production of SCFAs by bacteria to down-regulate pro-inflammatory cytokines, inducing the differentiation of regulatory T-cells and inhibit the Th17 polarization, through the above mechanisms effectively and significantly reduce the growth of subcutaneous hepatocellular carcinoma in mice [19].

3.2. Prebiotics

Prebiotics are oligosaccharides that cannot be digested and absorbed by the body but can be used as a source of nutrients for the intestinal flora. Prebiotics can promote the growth of intestinal flora, improve intestinal dysbiosis, and play a key role in inducing anti-tumor effects [20]. Based on chemical structure, inulin-type fructans (ITFs), one of the prebiotics, are indigestible carbohydrates. In a study of BALB/c mice transplanted with BaF3 cells, ITF treatment reduced BaF3 cell infiltration and attenuated hepatic inflammation, while increasing propionate levels in the mice's portal veins. Propionate can inhibit the growth of BaF3 cells in vitro via a cAMP-dependent pathway. In addition, activation of free fatty acid receptor 2 (FFA2) upon activation of Gi/Gq-protein-coupled receptors bound to jasmonates reduces the proliferation of BaF3 and other human cancer cell lines [21]. It was shown that the fermentation of nutrients such as ITF into propionate counteracted the proliferation of malignant cells in liver tissue. In cirrhotic patients, anti-tumor immunity was significantly enhanced in patients given oral lactulose. In addition, oral lactulose can regulate the imbalance between oxidative and antioxidant systems in patients with hepatocellular carcinoma cirrhosis with hypersplenism after interventional therapy, attenuate liver injury, and improve anti-tumor immunity and prognosis. Therefore, given the antitumor properties of prebiotics, it is suggested that the modulation of gut flora by prebiotics may serve as a preventive measure against the progression of hepatocellular carcinoma and the potential of using mixtures of prebiotics and the corresponding prebiotics to be manufactured into synbiotics to enhance the efficacy of the probiotics and to selectively stimulate the growth of beneficial intestinal microorganisms is a novel strategy for further research to reduce the risk of cancer.

3.3. Antibiotics

The available evidence for the effectiveness of antibiotics in the treatment of he-

patocellular carcinoma is limited to animal studies. The number and size of DMBA-HFD or DEN-CCL4-induced hepatocellular carcinoma tumors in mice were effectively reduced by oral administration of a mixture of antibiotics, including neomycin, ampicillin, vancomycin, and metronidazole [22]. In MYC transgenic mice with spontaneous hepatocellular carcinoma, the addition of an antibiotic mixture (ABX, consisting of vancomycin, neomycin, and imipenem) to the drinking water in order to deplete the intestinal commensal bacteria induced liver-selective anticancer effects, an increase in hepatic CXCR6 NKT cells, and an enhancement of interferon gamma synthesis, while the NKT cells mediated selective tumor suppression in the liver [23]. Despite the demonstrated antitumor efficacy of antibiotics in animal studies, it has been argued that the use of antibiotics in the early stages of treatment of malignancy is detrimental because antibiotics reduce the abundance of Bifidobacteria, Mucinophilic Acromobacteria, and Ruminalcoccus in the intestinal flora and increase the abundance of Mycobacterium phylum, and predispose to intestinal dysbiosis, which can affect the response to systemic therapy [24]. In addition, antibiotic therapy is not entirely appropriate for clinical management, as the potential harmful effects of long-term antibiotic use must be considered, including gastrointestinal dysfunction, immunocompromise, nephrotoxicity, and other antibiotic-related side effects. In addition, in hepatocellular carcinoma patients treated with immune checkpoint blockade (ICB), Fessas *et al.* [25] and Cheung *et al.* [26] reached opposite conclusions in their analysis of the correlation between antibiotics and patients' survival, which may be due to the fact that the use of different antibiotic types increases or decreases the abundance and number of key strains of bacteria as well as the diversity of microbial functioning, causing alterations in the intestinal ecology, which, in turn, affects systemic or local metabolism, with significant impact on the immune system. Further exploration and development of low-risk antibiotics with clinical therapeutic potential for hepatocellular carcinoma is still needed.

3.4. FMT

FMT is the transplantation of intestinal flora from healthy people to the intestines of hepatocellular carcinoma patients, which can reduce the proliferation of hepatocellular carcinoma cells, improve liver function and alleviate the symptoms of hepatocellular carcinoma patients from promoting the repair of the intestinal mucosal barrier, altering the composition and function of the patient's intestinal flora and improving the function of the immune system. A phase 1 randomized, placebo-controlled trial has now shown that by oral administration of FMT capsules in recurrent hepatic encephalopathy, an increase in the abundance of Ruminalococcaceae and Bifidobacteriaceae and a decrease in the abundance of Weillonella and the Aspergillus constituent, Sutterellaceae, in the microbiota after FMT is associated with an improvement in duodenal mucosal diversity and hepatic encephalopathy, and that oral administration of FMT cap-

sules may be a safe and effective therapeutic option [27]. In addition, FMT has been shown to enhance the ability of immune checkpoint inhibitors to improve the efficacy of certain cancers, such as non-small cell lung cancer, colorectal cancer and melanoma [27] [28]. Enhancing the antitumor effect of ICB by FMT is therefore a potential strategy for hepatocellular carcinoma treatment. There is a lack of clinical research data on the treatment of hepatocellular carcinoma through FMT, and further studies are still needed. Although FMT as an emerging therapeutic approach shows great potential in the treatment of hepatocellular carcinoma, FMT may cause adverse reactions such as intestinal inflammation, infection, and allergic reactions, and further studies are needed to evaluate its safety and efficacy with the aim of providing better treatment options for hepatocellular carcinoma patients.

3.5. Influence of Intestinal Flora on the Efficacy of ICB Therapy

The importance of intestinal flora on the efficacy of ICB therapy has been well documented in preclinical models and patients. Intestinal flora modulate innate and adaptive immunity and influence anti-tumor immune responses in the tumor microenvironment [29]. It has been shown that some bacteria such as Ackermann's slime mold, *Bacteroidetes fragilis*, Bifidobacterium spp. and *E. faecalis* spp. enhance the antitumor efficacy of ICB in animal models and cancer patients [30]. Zheng *et al.* found that among patients with sorafenib-treated refractory hepatocellular carcinoma, Aspergillus spp. predominated in fecal samples from treatment non-responders, whereas fecal samples from treatment responders were enriched in mucinophilic Acinetobacter spp. and Acomyclobacter spp. and the diversity of the intestinal flora in the body showed a close relationship with the favorable response to anti-PD-1 immunotherapy [31]. In a prospective study by Mao et al, fecal samples from patients with advanced unresectable hepatocellular carcinoma treated with PD-1 monotherapy after progression to first-line therapy (gemcitabine + cisplatin) were analyzed by macro-genomic sequencing, and differential enrichment of intestinal flora taxa was found between patients with and without clinical benefit, with energy metabolism-associated pathways being enriched in the intestinal flora of patients with clinical benefit, and those of patients with non-clinical benefit. Patients' intestinal flora were enriched in amino acid metabolism-related pathways, with higher abundance enrichment of Trichoderma sp. GAM79 and Alistipes sp. Marseille-P147 being significantly associated with prolonged survival with immunotherapy, whereas the family of *E. glomerulosum* was associated with shorter PFS and OS, and the diversity of intestinal flora was found to correlate with irAE profiles [32]. These studies suggest that the intestinal microbiome modulates the response to cancer immunotherapy, and that interventions in the intestinal flora may be a novel therapeutic target and suggest that the intestinal flora of patients with hepatocellular carcinoma may be a potential biomarker for predicting a favorable response to ICI therapy and survival benefit.

In conclusion, current research suggests that gut flora therapy is potentially important for the treatment of hepatocellular carcinoma. However, further larger as well as robust clinical studies and explorations of the safety and limited availability of gut flora therapy are needed.

4. Conclusion

Gut flora is an important component of human health and exists in a delicate balance with the host. In recent years, more and more studies have shown that there is an important and complex relationship between gut flora and hepatocellular carcinoma, and that gut flora can act directly or indirectly in the process of hepatocellular carcinoma development, making it a potential new therapeutic target. However, our understanding of gut flora is still limited, and there are still many questions about the function of gut flora in liver cancer development that remain unclear, and there is a lack of a large number of prospective as well as large-scale clinical trials on the study of gut flora. Therefore, further research is needed to investigate which factors contribute to the long-term stability of gut flora in health and disease and to elucidate their potential pathogenic mechanisms. In addition to this, modulation of gut flora for the treatment of hepatocellular carcinoma includes probiotics, prebiotics, and FMT, etc. A large number of studies are still needed to validate these new therapeutic approaches and to provide a theoretical basis for the clinical development of new therapeutic strategies for hepatocellular carcinoma.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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