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Study of Immunotherapy and Intestinal Flora Changes in Patients with Liver Cancer

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Abstract

Hepatocellular carcinoma (HCC) is one of the common major malignancies worldwide and has the third highest mortality rate of any malignancy. The current field of treatment for hepatocellular carcinoma is multidisciplinary in involvement and a combination of therapeutic approaches, among which immunotherapy is the treatment modality for advanced hepatocellular carcinoma. This review provides new ideas for developing immunotherapy regimens and improving the efficacy of immunotherapy for patients with advanced liver cancer by summarising the progress of intestinal flora in the immunotherapy of patients with liver cancer.

Keywords

Hepatocellular Carcinoma, Intestinal Flora, Immunotherapy, Overview

1. Introduction

Hepatocellular carcinoma (HCC) is the most common type of hepatocellular carcinoma, with the sixth highest incidence and third highest mortality rate among malignant tumors [1]. The main risk factors for HCC include chronic hepatitis B virus (HBV) and chronic hepatitis C virus (HCV) infection, alcohol abuse, autoimmune hepatitis, diabetes, obesity, and some metabolic diseases [2]. Due to the diversity of HCC pathogenic factors, complexity of pathogenesis, heterogeneity of HCC among different countries, regions and populations, and complexity of molecular mechanisms, it poses a great challenge for effective treatment of HCC. And many patients come to the clinic only in the middle and late stages, and the prognosis is not ideal [3] [4]. Hepatic arterial chemoembolization (TACE) combined with targeted and immunotherapy has become the first-line treatment option for patients with intermediate to advanced HCC, but there are still many patients with poor outcomes, suggesting the need to find new treat-

ment options. Microorganisms in the human gut are involved in the life activities of individuals and play an important role in the immune system and metabolic function. Due to the special anatomical relationship between the intestine and the liver, the portal system of the liver is involved in the transport of intestinal metabolites, which exposes the liver directly to bacteria and endotoxins, thus promoting liver damage and hepatocarcinogenesis. More and more studies have found that changes in intestinal flora can influence the effect of immunotherapy for liver cancer [5] [6]. Transplantation of intestinal flora can assist tumor immunotherapy and reduce the side effects of immunotherapy [7] [8], to provide new ideas for the treatment of liver cancer patients.

2. Intestinal Flora Affects the Development of Liver Cancer

2.1. The Role of Intestinal Flora

The human gut contains a large number of microorganisms, and a healthy adult has about 1014 bacteria, 10 times the number of human cells, which are vital to human nutrition and metabolism as well as life and health [9]. According to the results of 16S rRNA sequence analysis, more than 1000 species of microorganisms routinely survive in the intestine, most of which belong to the phylum Bacteroides and Thick-walled Bacteroides [10]. These florae form a microecological balance system in the gastrointestinal tract of a healthy human body. When the balance of the number or types of flora is disrupted, resulting in dysbiosis, damage to intestinal mucosal cells and destruction of the intestinal barrier, it will lead to inflammatory diseases of the intestine itself, as well as diseases of other sites, such as hepatitis, pneumonia, cardiovascular disease, obesity, diabetes, tumors, and autism, depression, etc [11].

2.2. The Relationship between Intestinal Flora and Liver Cancer

The intestine and the liver are closely linked in both directions through the bile duct, portal vein and the body circulation, the "intestine-liver axis". The liver communicates with the intestine by releasing bile acids and many biologically active mediators into the biliary tract and the body circulation. In the intestine, hosts and microorganisms metabolize endogenous (bile acids and amino acids) and exogenous (from dietary and environmental exposure) substrates, the products of which are transported to the liver via the portal vein and affect liver function. When the intestinal flora is dysregulated, the barrier function is impaired and the permeability of the intestinal mucosa increases, triggering the colonization and invasion of other potential pathogens (including conditionally pathogenic bacteria) in the intestine with bacterial translocation and accumulation of lipopolysaccharide (LPS), causes the development of liver disease. The intestinal-liver axis has implications for the pathogenesis of many chronic liver diseases, including chronic hepatitis B (CHB), chronic hepatitis C (CHC), alcoholic liver disease (ALD), non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC).

3. Impact of Intestinal Flora on Tumor Immunotherapy

3.1. The Role of Immunotherapy

Immune checkpoint inhibitors (ICI) have made revolutionary advances in recent years as a novel and effective therapeutic approach to tumor immunotherapy, activating T cells and killing tumor cells through an active immune approach. (CTLA-4) and programmed cell death 1 (PD-1) and its ligand programmed death receptor ligand-1 (PD-1) [12]. Scientists have explored the relationship between intestinal flora and immune checkpoint inhibitors and found that intestinal flora can influence antitumor immunotherapy and predict the efficacy of immunotherapy.

3.2. The Link between Immunotherapy and Intestinal Flora

Multiple studies show that enhancement of endogenous T-cell function by CTLA-4 blockade prolongs survival in patients with advanced metastatic melanoma [13], Experiments with sterile or antibiotic (ATB) treated mice reveal that the antitumor activity of ICI requires the presence of intestinal microorganisms, that CTLA-4 antibody efficacy is compromised by the lack of intestinal commensal flora, and that Bacteroides phylum such as Bacteroides fragilis (Bf) and Burkholderia cepacia (Bc) can enhance interleukin 12 (IL-12)-dependent Th1 immune response and promote the maturation of dendritic cells (DC) in tumors, contributing to tumor control [14]. Another study found that CTLA-4 expression on breast cancer cells was functional and inhibited the maturation and function of DCs, and that CTLA-4 blockade not only restored the antigen-presenting function of DCs and T cell activation, but also inhibited the biological activity of the breast cancer cells themselves [15].

Excellent results have also been achieved with immune checkpoint inhibitors (ICI) targeting the intestinal flora affecting the PD-1/PD-L1 axis. Lisa [16] *et al*. performed a bird shot macrogenomics-based microbiome analysis of advanced non-small cell lung cancer (NSCLC) patients treated with PD-1 blockade and showed that the relative abundance of the fecal mucinophilic Akkermans (Akk) was significantly associated with the efficacy of ICI treatment and confirmed that ATB use was associated with poor clinical outcomes [17]. In addition to consuming favorable genera (e.g., rumen cocci) [18], it also biases the composition of the gut microbiome toward harmful bacteria (e.g., *E. coli* and *Clostridium borreliae*) [19]. By integrating three DNA sequence-based bacterial identification methods, including 16S ribosomal RNA gene sequencing, macrogenomic birdshot sequencing, and quantitative polymerase chain reaction of selected bacteria, Vyara *et al*. [20] scientists found that the commensal microbiome may have a mechanistic impact on antitumor immunity, and that beneficial species such as *Bifidobacterium longum*, *Corynebacterium aerogenes*, and *Enterococcus faecalis* are enriched in responding patients, and that fecal transplants from responders may improve tumor control, enhance T-cell responses, and improve the efficacy of PD-1 blockers. Routy *et al*. [21] Study of the efficacy of "fecal microbiota transplantation" or oral supplementation with the mucormycete Acineto-

bacter in restoring the efficacy of PD-1 blockade in a mouse epithelial tumor model with anti-pd-1 agents, where primary resistance to ICI can be attributed to an abnormal gut microbiome composition, was restored in an interleukin 12-dependent manner by increasing the recruitment of CCR9 + CXCR3 + CD4 + T lymphocytes to the tumor bed of mice.

4. Gut Flora Aids in Immunotherapy of Liver Cancer

In recent years, Nivolumab, Pembrolizumab, Durvalumab and Tremelimumab monoclonal antibodies have been developed and validated for the treatment of HCC in response to the immune escape mechanism of liver tumor cells, providing a powerful weapon to improve the current treatment status of intermediate to advanced HCC [22]. Species diversity and abundance of intestinal flora as biomarkers of liver cancer immunotherapy can influence the response to liver cancer immunotherapy and the severity of adverse effects [23]. Another study characterized the composition of the gut flora of patients with advanced hepatocellular carcinoma treated with navulizumab by macrogenomic sequencing of 16S ribosomal RNA, revealing that the composition and diversity of the gut microbiota of responders to navulizumab treatment were significantly different from that of non-responders, with bacterial species such as *Citrobacter freundii*, *Azospirillum* sp. and *Enterococcus durans* being specific for responders were specific to the responders [24]. In addition, the higher average proportion of *P. pruriens*/*mimosas* genus in responders and Akkermansia species may both be useful predictive markers for response to nabumetinumab therapy in patients with advanced HCC. Rational use of antibiotics also presents some antitumor synergism in cellular peripatetic immunotherapy of hepatocellular carcinoma. An experiment to study the tumor immune response of microbiota in $\gamma\delta$ T cells following tumor immunotherapy in mice grown with human HepG2-fluorophore enzyme hepatocellular carcinoma cells showed that antibiotic treatment enhanced the immunotherapeutic efficacy of $\gamma\delta$ T cells and that intestinal microbes and their associated metabolites were the key factors responsible for this phenomenon [25].

In addition to the role of intestinal flora in the immune system checkpoint blockade response, there is an association with immunotherapy-related toxicities [26]. Recent studies have shown that the effect of microbiota on the adverse effects of CTLA-4 blockers can be separated from their effect on therapeutic efficacy, thus potentially enhancing the effect of CTLA-4 blockers by reducing the collateral toxicity of the targeted microbiota [27]. Mao *et al.* in a study correlating gut microbes with clinical response to anti-PD-1 immunotherapy in hepatobiliary cancer found that immunotherapy-associated adverse events (irAE) immunotherapy-associated colitis, influenced by the gut microbiome, with higher diversity and relative abundance of thick-walled phylum taxa may be a protective factor against irAE.

5. Conclusion

The special anatomical relationship between the intestine and the liver exposes

the liver directly to bacteria and endotoxins, thus promoting liver damage and hepatocarcinogenesis. Recent theoretical basis and research advances have confirmed that intestinal flora plays an important role in the development of hepatocellular carcinoma. The diversity of intestinal flora and the relative abundance of specific strains can promote the body's anti-tumor immune response and image the efficacy of immune checkpoint inhibitors. Flora transplantation can improve the efficacy of tumor immunotherapy and reduce the side effects of immunotherapy. In the future, it is worth our expectation to improve the efficacy of immune checkpoint inhibitors in patients with intermediate and advanced hepatocellular carcinoma by fecal bacteria transplantation.

Conflicts of Interest

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

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