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The Gut Microbiota, Tumorigenesis, and Liver Diseases

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ABSTRACT

In recent decades, diseases concerning the gut microbiota have presented some of the most serious public health problems worldwide. The human host's physiological status is influenced by the intestinal microbiome, thus integrating external factors, such as diet, with genetic and immune signals. The notion that chronic inflammation drives carcinogenesis has been widely established for various tissues. It is surprising that the role of the microbiota in tumorigenesis has only recently been recognized, given that the presence of bacteria at tumor sites was first described more than a century ago. Extensive epidemiological studies have revealed that there is a strong link between the gut microbiota and some common cancers. However, the exact molecular mechanisms linking the gut microbiota and cancer are not yet fully understood. Changes to the gut microbiota are instrumental in determining the occurrence and progression of hepatocarcinoma, chronic liver diseases related to alcohol, nonalcoholic fatty liver disease (NAFLD), and cirrhosis. To be specific, the gut milieu may play an important role in systemic inflammation, endotoxemia, and vasodilation, which leads to complications such as spontaneous bacterial peritonitis and hepatic encephalopathy. Relevant animal studies involving gut microbiota manipulations, combined with observational studies on patients with NAFLD, have provided ample evidence pointing to the contribution of dysbiosis to the pathogenesis of NAFLD. Given the poor prognosis of these clinical events, their prevention and early management are essential. Studies of the composition and function of the gut microbiota could shed some light on understanding the prognosis because the microbiota serves as an essential component of the gut milieu that can impact the aforementioned clinical events. As far as disease management is concerned, probiotics may provide a novel direction for therapeutics for hepatocellular carcinoma (HCC) and NAFLD, given that probiotics function as a type of medicine that can improve human health by regulating the immune system. Here, we provide an overview of the relationships among the gut microbiota, tumors, and liver diseases. In addition, considering the significance of bacterial homeostasis, we discuss probiotics in this article in order to guide treatments for related diseases.

1. Introduction

The microecosystem, which exists in all organisms with complicated structures and forms, comprises mainly bacteria, along with archaea, eukarya, and viruses. In humans, the microbiota attaches to the mucosal surfaces of almost all organs. Among these organs, the gut, which contains trillions of bacteria, is the primary organ in charge of microbe-host communications. Microbial density increases from the proximal to the distal end of the intestine and comprises a biomass of 1.5–2.0 kg, dominated by strictly anaerobic bacteria [1]. There are more microbial cells in the gut than human cells in the entire body. Approximately 1200 different bacterial species have been recognized in the human gut microbiota, and each human individual has a distinguished set of almost 160 species in the gut [1–5]. Although the vast majority of the gut microbial community is composed of only five phyla (Bacteroidetes, Firmicutes, Actinobacteria,

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Proteobacteria, and Verrucomicrobia), considerable diversity has been identified at the species level and in their relative abundances, although their relevance for human health has been less studied.

The composition of the gut microbiota is mediated by genetic and environmental factors, starting early in life [6]. Moreover, the host's physiological status is influenced by the intestinal microbiome, thus integrating external factors, such as diet, with genetic and immune signals [7]. Microbial signals can regulate crucial functions of the healthy human body, ranging from host metabolism to brain function [8]. Accumulating data also suggests that many human diseases have their origin in distorted gut microbiota composition or, potentially, in microbial metabolites that signal to distant organs, including the adipose tissue, liver, pancreas, cardiovascular system, brain, lungs, and many others. The intestinal microbiota communicates with peripheral organs in the body and influences many physiological processes. More importantly, tumors in related organs are being reported with much higher frequency than ever before.

In the following sections, we discuss recent findings on how gut microbiotic signals influence organs that are distant from the gut, and how this communication affects tumorigenesis and liver diseases.

2. Tumorigenesis

Statistically speaking, altered constructions or disorders of the gut microbiota are often related to tumorigenesis. For example, factors influencing gut bacteria, including dietary formula, living habits, and immunity, can obviously exacerbate colorectal malignancies. When compared with healthy people, patients with colorectal cancer have higher amounts of *Enterococcus, Escherichia, Klebsiella,* and *Streptococcus,* and lower amounts of *Rothia* and butyrate-producing bacteria [8]. People who are susceptible to colorectal cancer have more species that metabolize to generate secondary bile acids, but fewer that produce butyrate [9]. Studies on mice with bowel cancer induced by a carcinogen combined with gene deletion revealed that germ-free mice showed no tumorigenesis, thus indicating that the gut microbiota is indispensible for colorectal malignancies. Colorectal carcinogenesis is triggered by a combination of microbiota- and host-dependent mechanisms. Stepwise data has confirmed the carcinogenesis of gut microorganisms, mostly as a result of the activation of Toll-like receptor (TLR)/MyD88 signaling [10].

Certain bacteria promote carcinogenesis directly (Fig. 1), by secreting substances that lead to DNA damage [11]. Accumulating examples include *Helicobacter hepaticus*, *Enterococcus faecalis*, and *Bacteroides fragilis*. *Helicobacter hepaticus* can trigger immune cells to release nitric oxide excessively, and *Enterococcus faecalis* can produce reactive oxygen species (ROS). *Bacteroides fragilis* secretes an enterotoxin that activates a classical oncogene, *c-myc*. Other bacteria promote carcinogenesis indirectly (Fig. 1) by maintaining a persistent pro-inflammatory microenvironment; for example, the virulence factor FadA produced by *Fusobacterium nucleatum* increases the permeability of colonic epithelial cells. The mechanism by which chronic inflammation drives carcinogenesis has been widely established for various tissues. Inflammation may also exacerbate community-level alterations in the microbiota and facilitate bacterial translocations from the gut into the neoplastic tissue, which further promotes the expression of inflammatory cytokines and leads to the increased progress of tumors [12]. For example, microbiota dysbiosis that arises in the absence of NLRP6 promotes the development of cancer through IL-6-induced epithelial proliferation [13]. However, many questions regarding the roles of the microbiota in the occurrence of tumorigenesis remain to be answered.

3. Hepatocellular carcinoma

The gut microbiota plays a vital role in the progress of hepatocellular carcinoma (HCC). In human anatomy, the liver is the first downstream organ affected by the gut microbiota; thus, the gut microbiota has a significant influence on the liver, depending on the portal system and bacterial metabolites. For epithelial and immune cells, microbe-associated molecular patterns (MAMPs) such as peptidoglycan, flagellin, lipopolysaccharide (LPS), or other structural components are recognized by pattern-recognition receptors (PRRs), including TLRs, NOD-like receptors (NLRs), or RIG-I-like receptors (RLRs) [14], and generally inhibiting translocations across the epithelial barrier. Research shows increased LPS in the serums of patients with liver cancer and liver cirrhosis [15], suggesting

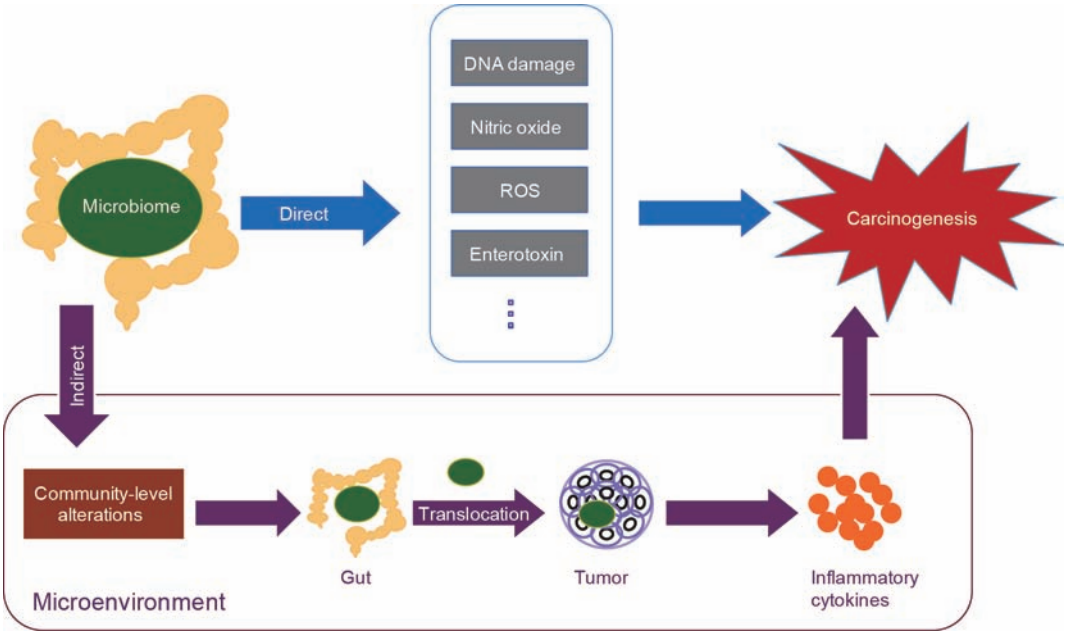

Fig. 1. The gut microbiota promotes tumorigenesis both directly and indirectly.

that the gut microbiota has strong correlations with liver diseases. Remarkably, further studies have verified the phenomenon of mice exhibiting a persistent imbalance of the gut microbiota during the construction of an HCC model induced by chemical carcinogens; the mice also exhibited the pathologic features of altered gut microflora, destruction of the intestinal mucosa, and increased gut permeability [16]. Numerous reports have suggested that gut microflora dysbiosis generated by the administration of low-dose antibiotics or intestine mucosa injuries can further accelerate the progression of HCC, mainly due to the over-expression of IL-6, the activation of NF-κB, and the phosphorylation of STAT3, and the activation of the TLR pathway by increasing levels of LPS [15,16]. It was also proved that the mechanism of HCC acceleration by gut microbiota dysbiosis depends on the progression of chronic inflammation, which subsequently initiates flagellin, peptidoglycan, and LPS, and activates the TLR4 signaling pathway, thus sheltering cancer cells from apoptosis [17]. As expected, the above-mentioned symptoms are mitigated by the administration of probiotics [15,18,19]. Obesity is also a vital factor in the progression of HCC. Data obtained from mice implies that the gut microbiota may contribute to obesity [20–23], and vice versa. Thus, researchers have determined that liver cancer induced by obesity or a high-fat diet (HFD) is likewise mediated by gut microbiota dysbiosis, which contributes to assimilating and reserving energy for organisms [17,24]. The gut microbiota also generates deoxycholic acid and activates the TLR4 signaling pathway, in addition to widely expressing aging-related genes (i.e., *IL-6*, *GRO-α*, *CXCL9*, *DES*, *53BP1*, *p21*, *p16*, and *γH2AX*) [25,26]. Therefore, the gut microbiota is extremely significant in the progression of HCC, despite the diversified pathogenesis of HCC. Recent research findings describe therapies that adjust the gut microbiota in order to alleviate the symptoms of liver cancer. Unsurprisingly, professionals around the world have reached a widely accepted consensus regarding the importance of bacterial disorders [19,27].

Recent data on mice exposed to chemical carcinogens suggests that the oral administration of probiotics (i.e., VSL#3, *Lactobacillus*, or combinations of *Streptococcus thermophilus* and *Bifidobacterium*) may play a role in guarding against HCC by lowering serum LPS levels, protecting the mucosa and microbiota homeostasis of the gut, and alleviating chronic inflammation [15]. Further studies confirmed that probiotics increase the levels of *Prevotella* and *Vitreoscilla*—bacteria that help to prevent inflammatory responses and facilitate the differentiation of immune cells—thus changing the tumor microenvironment and inhibiting the growth of cancer cells [27,28]. Aflatoxin is a metabolite created by fungi that produces strong liver carcinogenic effects. To date, some studies have found that taking probiotics (a combination of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii*) orally can defend humans against absorbing aflatoxins, thus giving rise to promising therapeutics for HCC [28]. However, multi-center clinical trials are urgently required in order to demonstrate the role that intervention of the gut microbiota, or administration of probiotics, plays in preventing liver tumorigenesis. Indeed, the development of probiotic preparations targeting hepatocarcinogenesis, with a focus on tackling the high heterogeneity of HCC, is a subject that remains to be thoroughly studied.

4. Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) includes variable degrees of simple steatosis (fatty liver), nonalcoholic steatohepatitis (NASH), and cirrhosis. During its progression, steatosis may be benign; however, NASH is characterized by hepatocyte injury, inflammation, and fibrosis, and can result in cirrhosis, liver failure, and HCC [29]. About 7% of subjects with a combination of NAFLD and compensated cirrhosis will develop an HCC within 10 years [30]. HCC frequency in NAFLD-related cirrhosis is comparable with that in cirrhosis associated with hepatitis C or alcoholic factors [31].

It has been shown that people with obesity are more likely to have chronic liver diseases such as NAFLD. Furthermore, 20% of NAFLD patients proceed to NASH [32]. Articles have reported that the gut microbiota induces the transportation of inflammatory molecules to the liver and accelerates the transition from NAFLD to NASH [33].

Evidence also suggests that certain food components and lifestyle factors that are known to influence the progress of NAFLD do so partly by changing the gut microbiota. When germ-free mice were given a diet lacking in methionine-choline, they showed high TLR4 and TLR9 agonists of bacteria in the portal blood, and were susceptible to colitogenic gut microflora. This observation can mainly be attributed to the missed bacterial identification via inflammasome. TNF-α signaling was subsequently activated to exacerbate hepatotoxicity and promote the transition, as well as increasing TLR levels in the liver. However, there is no direct proof of bacterial translocation; no increase in TLR2 agonists was observed in the circulation after a methionine-choline diet [33,34]. Thus, it remains to be clarified whether or not the higher TLR agonists (i.e., TLR4 and TLR9) are derived from a certain bacterium. It is notable that researchers have found Porphyromonadaceae in mouse models, and one such bacterium in the human gut microbiota, whose member *Porphyromonas* contributes to metabolic diseases in humans, as well as in mice [35,36]. Further studies are necessary to clarify the reason why Porphyromonadaceae can grow in the colitogenic gut, and how the intestinal microbiota composition alters in order to facilitate the carriage of specific bacterial molecules into circulation. Based on studies on mice, in which the gut microbiota can be manipulated to alter gut bile acids and farnesoid X receptor (FXR) signaling, researchers have shown that mice lacking FXR in the intestine are resistant to HFD-induced obesity, insulin, and NAFLD, thus verifying that intestinal FXRs participate in the potentiation of metabolic diseases [37]. Targeting a receptor in the intestine is a promising strategy for the treatment of metabolic diseases because it can avoid systemic exposure to the resultant compound. However, it remains to be determined whether the activation [38] or the inhibition [39] of FXR is a viable approach for devising therapies. The following topics are yet to be unveiled: the relationships between the microbiota and FXRs; improved methods of analysis of the gut microbiome; the interactions between diet, dysbiosis, and environmental factors; and the effects of these factors on the gut-liver axis. These areas of interest, once demystified, will hopefully provide some insight into methods of improving treatments of this common liver disease and its associated disorders.

5. Discussion

In 2007, the US government launched the Human Microbiome Program around the world. Since then, the gut microbiota has been a widely discussed topic. To understand how microbes affect human health and cause various diseases, researchers involved in the National Institutes of Health's Human Microbiome Project have collected metagenome samples from individuals with a variety of health conditions and from different parts of the human body, and have constructed metagenome datasets. The results garnered from this project have sparked a series of studies to be carried out. In particular, stepwise accumulating studies indicate that microbial disorders have vital relationships with chronic diseases. These findings have directed public attention to gut homeostasis. Microbiota dysbiosis, in particular, is of concern to researchers all over the world. By definition, microbiota dysbiosis means the alteration of the quantity or structure of microorganisms; the term mainly refers to a reduction of beneficial bacteria, whose proportions simultaneously change. Given that factors such as diet, obesity, and chronic inflammation

can all contribute to the promotion of cancer, it is reasonable to assume—and accumulating evidence indicates—that the above-mentioned hazards accelerate tumorigenesis. When put under scrutiny by a considerable amount of research, the gut microbiota is seen to play a vital role in initiating neoplasm. Furthermore, preliminary data has shown that it may be necessary to maintain gut bacterial homeostasis in order to increase the efficiency of tumor therapeutics [40,41].

Probiotics function as a type of medicine that can improve human health by regulating the immune system, decreasing serum cholesterols, reconditioning energy metabolism, guarding the intestines against tumorigenesis, and so forth [42,43]. It is notable that the oral ingestion of probiotics (*Bifidobacterium*) combined with anti-PD-L1 agents almost completely restrains tumor growth [44]. Mechanisms include enhancing T lymph cells' infiltration into the tumor microenvironment, adjusting the initiation of cytokine receptors, and germinating monocytes and increasing the number of cells that secrete INF-γ [45]. However, multi-center clinical trials are urgently required in order to demonstrate the effects of probiotics on the prevention of liver tumorigenesis. Meanwhile, the development of probiotics-based treatments targeting hepatocarcinogenesis, with a focus on solving the high heterogeneity of HCC, is a subject that remains to be thoroughly researched.

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Compliance with ethics guidelines

Guishuai Lv, Ningtao Cheng, and Hongyang Wang declare that they have no conflict of interest or financial conflicts to disclose.

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