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The Human Microbiota in Health and Disease

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ABSTRACT

Trillions of microbes have evolved with and continue to live on and within human beings. A variety of environmental factors can affect intestinal microbial imbalance, which has a close relationship with human health and disease. Here, we focus on the interactions between the human microbiota and the host in order to provide an overview of the microbial role in basic biological processes and in the development and progression of major human diseases such as infectious diseases, liver diseases, gastrointestinal cancers, metabolic diseases, respiratory diseases, mental or psychological diseases, and autoimmune diseases. We also review important advances in techniques associated with microbial research, such as DNA sequencing, metabonomics, and proteomics combined with computation-based bioinformatics. Current research on the human microbiota has become much more sophisticated and more comprehensive. Therefore, we propose that research should focus on the host-microbe interaction and on cause-effect mechanisms, which could pave the way to an understanding of the role of gut microbiota in health and disease, and provide new therapeutic targets and treatment approaches in clinical practice.

1. Introduction

More than 100 trillion symbiotic microorganisms live on and within human beings and play an important role in human health and disease. The human microbiota, especially the gut microbiota, has even been considered to be an "essential organ" [1], carrying approximately 150 times more genes than are found in the entire human genome [2]. Important advances have shown that the gut microbiota is involved in basic human biological processes, including modulating the metabolic phenotype, regulating epithelial development, and influencing innate immunity [3–6]. Chronic diseases such as obesity, inflammatory bowel disease (IBD), diabetes mellitus, metabolic syndrome, atherosclerosis, alcoholic liver disease (ALD), nonalcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma have been associated with the human microbiota [7,8] (Fig.1).

In recent decades, a tremendous amount of evidence has strongly suggested a crucial role of the human microbiota in human health and disease [7,9–23] via several mechanisms. First, the microbiota has the potential to increase energy extraction from food [24], increase nutrient harvest [9,10], and alter appetite signaling [25,26]. The microbiota contains far more versatile metabolic genes than are found in the human genome, and provides humans with unique and specific enzymes and biochemical pathways [9]. In addition, a large proportion of the metabolic microbiotic processes that are beneficial to the host are involved in either nutrient acquisition or xenobiotic processing, including the metabolism of undigested carbohydrates and the biosynthesis of vitamins [10]. Second, the human microbiota also provides a physical barrier, protecting its host against foreign pathogens through competitive exclusion and the production of antimicrobial substances [11–13]. Finally, the microbiota is essential in the development of the intestinal mucosa and immune system of the host [14,16]. For example, germ-free (GF) animals have abnormal numbers of several immune cell types, deficits in local and systemic lymphoid structures, poorly formed spleens and lymph

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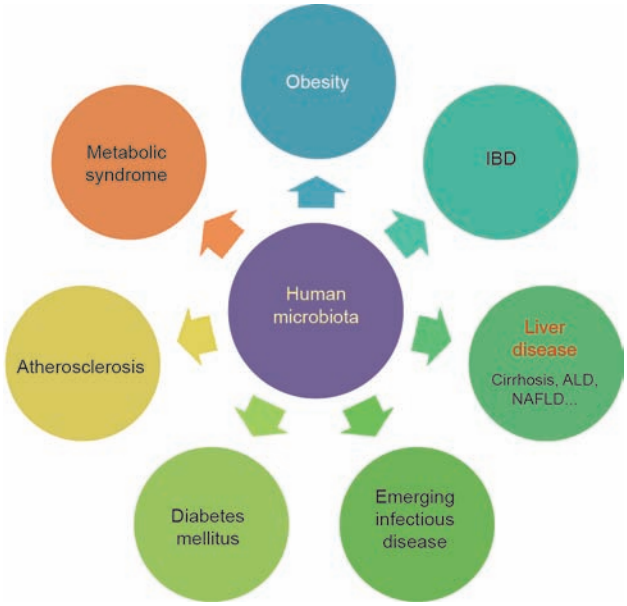

Fig. 1. Human microbial symbiosis has a close relationship with diseases of different systems.

nodes, and perturbed cytokine levels [16]. Studies on GF animals have suggested that the immune modulation functions of the microbiota are primarily involved in promoting the maturation of immune cells and the normal development of immune functions [14]. In addition, studies have revealed the central role of microbial symbiosis in the development of many diseases [17], such as infection [18], liver diseases [19], gastrointestinal (GI) malignancy [20], metabolic disorders [7], respiratory diseases [21], mental or psychological diseases [22], and autoimmune diseases [23].

In this article, we provide an overview of the role of the human microbiota in health and disease, the advent of microbiome-wide association studies, and potential and important advances in the development of clinical applications to prevent and treat human disease.

2. The human microbiota in health

The human microbiota affects host physiology to a great extent. Trillions of microbes colonize the human body, including bacteria, archaea, viruses, and eukaryotic microbes. The body contains at least 1000 different species of known bacteria and carries 150 times more microbial genes than are found in the entire human genome [2]. Microbiotic composition and function differ according to different locations, ages, sexes, races, and diets of the host [27].

Commensal bacteria colonize the host shortly after birth. This simple community gradually develops into a highly diverse ecosystem during host growth [28]. Over time, host-bacterial associations have developed into beneficial relationships. Symbiotic bacteria metabolize indigestible compounds, supply essential nutrients, defend against colonization by opportunistic pathogens, and contribute to the formation of intestinal architecture [29]. For example, the intestinal microbiota is involved in the digestion of certain foods that cannot be digested by the stomach and small intestine, and plays a key role in maintaining energy homeostasis. These foods are primarily dietary fibers such as xyloglucans, which are commonly found in vegetables and can be digested by a specific species of *Bacteroides* [30]. Other non-digestible fibers, such as fructooligosaccharides and oligosaccharides, can be utilized by beneficial microbes, such as *Lactobacillus* and *Bifidobacterium* [31]. Studies have clarified the role of the gut microbiota in lipid and protein homeostasis as well as in the microbial synthesis of essential nutrient vitamins [32]. The normal gut microbiome produces 50–100 mmol·L^{-1} per day of short-chain fatty acids (SCFAs), such as acetic, propionic, and butyric acids, and serves as an energy source to the host intestinal epithelium [33]. These SCFAs can be quickly absorbed in the colon and serve many diverse roles in regulating gut motility, inflammation, glucose homeostasis, and energy harvesting [34,35]. Furthermore, the gut microbiota has been shown to deliver vitamins to the host, such as folates, vitamin K, biotin, riboflavin (B_2), cobalamin (B_{12}), and possibly other B vitamins. A previous study demonstrated that B_{12} can be produced from delta-aminolevulinate (ALA) as a precursor [36].

In addition, gut-colonizing bacteria stimulate the normal development of the humoral and cellular mucosal immune systems [37]. The signals and metabolites of microorganisms can be sensed by the hematopoietic and non-hematopoietic cells of the innate immune system and translated into physiological responses [38]. Studies comparing normal mice with GF mice have found that GF mice show extensive defects in the development of gut-associated lymphoid tissue and antibody production [29,39]. A report has also demonstrated that the gut microbiota generates a tolerogenic response that acts on gut dendritic cells and inhibits the type 17 T-helper cell (Th17) anti-inflammatory pathway [40]. However, not all microbiota lead to health benefits. Some induce inflammation under certain conditions.

3. The human microbiota in disease

3.1. The human microbiota and infectious diseases

Infection is one of the most common diseases caused by dysbiosis of the microbiota. Importantly, infectious disease and its treatment have a profound impact on the human microbiota, which in turn determines the outcome of the infectious disease in the human host (Fig. 2). Offending pathogens colonize the intestinal mucosa, thus resulting in the induction of a strong inflammatory response, followed by the translocation of the intestinal bacteria [41,42]. Numerous studies have demonstrated the intimate relationship between infection and dysbiosis of the microbiota, and have shown that infection is associated not only with the microbiome, but also with viruses [43,44]. For example, the intestinal microbiota of patients with *Clostridium difficile* (*C. difficile*) infection (CDI) is significantly altered [45,46]. Disturbance of the microbiota is also associated with the progression of human immunodeficiency virus (HIV) [44,47], hepatitis B virus (HBV) [48], and other diseases [49,50].

3.1.1. *Infection with* Clostridium difficile

The pathological overgrowth of *C. difficile* is usually related to antibiotic-associated diarrhea, which is one of the most frequent complications following antibiotic administration and which is now a growing public health threat [45]. *C. difficile* is an anaerobic, gram-positive, spore-forming bacillus that is a component of the human gut microbiota. Antibiotics disturb intestinal mucosa homeostasis, thus decreasing resistance against toxin-producing *C. difficile* and promoting the progression of CDI [45]. Gu et al. [45] found that fecal bacterial diversity is reduced and the microbial composition dramatically shifts in patients following antibiotic administration, whether or not CDI is present. A decrease in putative butyrate-producing anaerobic bacteria and an increase in endotoxin-producing opportunistic pathogens and lactate-producing phylotypes have been detected in patients following antibiotic administration, whether or not CDI is present [45]. Putative butyrate-producing anaerobic bacteria are significantly depleted

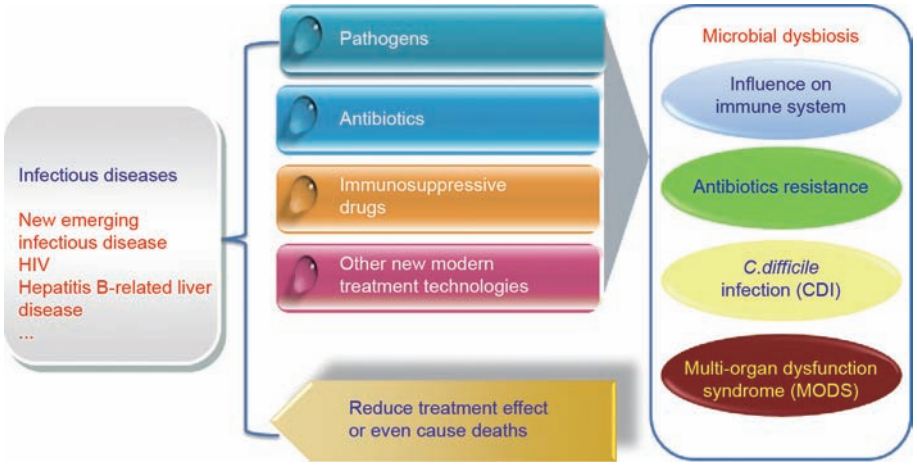

Fig. 2. Infectious diseases have a profound impact on the human microbiota. The wide use of antibiotics, immunosuppressive drugs, and other new treatment technologies for infectious diseases such as frequently emerging infectious diseases, HIV infection, and CDI has a profound impact on the human microbiota, which in turn determines the outcome of the infectious disease in the human host.

in patients with antibiotic treatment when compared with healthy controls. The above changes in microbial communities may increase susceptibility to *C. difficile* colonization. Ling et al. [46] found that different toxigenic *C. difficile* strains have different effects on fecal microbiota in children. *C. difficile* strains that are both toxin A-positive and toxin B-positive reduce fecal bacteria diversity to a greater degree than strains that are only toxin B-positive.

3.1.2. Infection with Helicobacter pylori

Helicobacter pylori (*H. pylori*) is a pathogen that induces peptic disease. It was recently found to be related to the progress of periodontitis [49]. Hu et al. [49] investigated the correlation of *H. pylori* infection with periodontal parameters, periodontal pathogens, and inflammation. Their study showed that the frequencies of *Porphyromonas gingivalis*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Treponema denticola* are significantly higher in patients infected with *H. pylori* than in those without infection, whereas the frequency of *Aggregatibacter actinomycetemcomitans* is lower. The results indicate that patients with *H. pylori* show significantly higher probing depth and attachment loss, and that *H. pylori* might promote the growth of some periodontal pathogens and aggravate the progress of chronic periodontitis [49].

3.1.3. Bacterial vaginosis

Another important infection called bacterial vaginosis (BV) is associated with numerous adverse health outcomes including pre-term birth and the acquisition of sexually transmitted infections. BV is regarded as an ecological disorder of the vaginal microbiota. Using culture-independent polymerase chain reaction (PCR) denaturing gradient gel electrophoresis (DGGE) and barcoded 454 pyrosequencing methods, Ling et al. [50] observed a profound shift in the absolute and relative abundances of bacterial species present in the vagina. In a comparison of populations associated with healthy and diseased conditions, three phyla and eight genera were clearly and strongly associated with BV. These genera may be used as targets for clinical BV diagnosis by means of molecular approaches [50].

3.1.4. Infection with HIV

At present, HIV continues to be a major global public health issue. The gut microbiomes in patients with HIV are significantly disturbed, and there are significant increases in the Firmicutes/Bacteroidetes ratio of patients infected with HIV-1 [47]. Although the viral loads of HIV-1 are reduced after a short-term course of effective highly active anti-retroviral therapy (HAART), the diversity and composition of the fecal microbiota are not completely restored, and the dysbiosis remains [47]. South African teenage girls and young women have extremely high rates of HIV infection, a phenomenon that has been considered to be associated with biological factors. Recently, one study found that the vaginal microbiome might affect the risk of HIV infection. A bacterium in the vagina named *Prevotella bivia* has been identified as causing inflammation. A microbicidal gel is available that decomposes the anti-HIV drug tenofovir, thus leading to tenofovir treatment failure [44]. Through close examination of the vaginal microbiome, Cohen [44] recently found an unusual bacterium named *Gardnerella* in the vagina; this finding potentially explains the high infection rates in South African women and strongly suggests that the vaginal microbiome affects HIV risk. Cohen [44] found that *Gardnerella* "gobbles up" tenofovir, thus rapidly decreasing the levels of the drug and leading to tenofovir treatment failure.

3.2. The human microbiota and liver diseases

Growing evidence demonstrates the close interaction of the GI tract (GIT) and the liver, as well as the chronic exposure of the liver to gut-derived factors including bacteria and bacterial components, thus fostering the use of the term "gut-liver axis" [51]. The intestinal microbiota produces ethanol, ammonia, and acetaldehyde; these products may influence liver function through endotoxin release or liver metabolism [52].

Alterations in the intestinal microbiota play an important role in inducing and promoting liver damage progression as well as in direct injury resulting from different causal agents (e.g., viral, toxic, and metabolic agents) [53] through mechanisms such as the activation of Kupffer cells by bacterial endotoxins. The gut microbiota participates in the pathogenesis of liver cirrhosis complications, such as infections, spontaneous bacterial peritonitis, hepatic encephalopathy, and renal failure. Patients with liver cirrhosis have an altered bacterial composition in their gut; patients in Child-Pugh classes B/C have a higher prevalence of bacterial overgrowth than those in class A [54,55]. Fecal microbial communities are distinct in patients with cirrhosis compared with healthy individuals. By bacterial 16S rRNA gene sequencing, microbial diversity, and especially Bacteroidetes species, is shown to be reduced in cirrhotic patients, while the number of species of Proteobacteria and Fusobacteria are increased [56]. In line with the previous study, *Bacteroides* has also been found to decrease at

the genus level, according to a metagenomics technique. In this study, a gene catalog of the gut microbiomes of Chinese patients with liver cirrhosis was constructed for the first time. Furthermore, *Veillonella*, *Streptococcus*, and *Clostridium* were found to be enriched in patients with liver cirrhosis [57]. On the basis of our previous findings, we further detected dysbiosis of the duodenal mucosal microbiota in liver cirrhosis patients. In this research, we found that cirrhotic patients were colonized by a remarkably different duodenal mucosal microbiota in comparison with the controls. Twelve operational taxonomic units (OTUs) were identified as the key microbes contributing to the differentiation between the cirrhosis and control duodenal microbiota. Regarding the etiology of cirrhosis, two OTUs were found to discriminate between types of liver cirrhosis, with different etiology results for HBV-related cirrhosis and primary biliary cirrhosis (PBC). These findings indicated that duodenum dysbiosis might be related to alterations in the oral microbiota and to changes in the duodenal microenvironment [58]. The oral microbiota is one of the most important microbial communities in the human body. This study was also the first to show that the diversity and composition of the oral microbiota in patients with liver cirrhosis are significantly different from those of healthy controls and from those of patients with HBV-related chronic diseases. Harmful bacteria may be derived from the oral cavity. In addition, patients with chronic liver disease show oral diseases [43].

Acute-on-chronic liver failure (ACLF) syndrome is characterized by the acute decompensation of cirrhosis, with high 28-d mortality. Based on the final clinical outcome at 90 d, we were the first to identify gut dysbiosis in ACLF patients, and to demonstrate its predictive value for mortality. We found a marked difference between the gut microbiota of the ACLF group and that of the control group. Our study indicated that there are correlations between specific bacterial families and inflammatory cytokines in ACLF patients. We have demonstrated that the relative abundance of Pasteurellaceae and the model of end-stage liver disease (MELD) score are independent factors that predict the mortality rate, thus indicating that gut dysbiosis is associated with the mortality of patients with ACLF [59].

Although the exact reason for these changes in liver cirrhosis remains unclear, these changes are certainly associated with reduced intestinal motility and pancreatobiliary secretions, an impaired intestinal barrier, and decreased gastric acidity. In addition, 80% of hepatocellular carcinoma (HCC) develops in a microenvironment of chronic injury, inflammation, or fibrosis [60]. Changes in the composition of the gut microbiota promote HCC by contributing to hepatic inflammation through increased intestinal permeability and the activation of Toll-like receptors [60].

The incidence and prevalence of primary sclerosing cholangitis (PSC), PBC, and autoimmune hepatitis increases every year [61]. Autoimmune liver diseases are presumed to involve environmental factors in individuals with genetic susceptibility; however, the gut flora is relevant to pathogenesis. Recently, a study showed that patients with PSC-IBD have distinct gut microbiota and a significant increase in the abundance of *Escherichia*, Lachnospiraceae, and *Megasphaera*, along with a near-absence of *Bacteroides*, as compared with IBD patients and control patients [62]. Another study found patients with PBC-altered gut bacterial taxa that exhibited potential interactions through their associations with altered metabolism, immunity, and liver-function indicators [63]. There is evidence that bacterial antigens translocate across a leaky and inflamed gut wall into the portal and biliary system; thus, they may induce an abnormal immune response and initiate autoimmune liver disease [64].

NAFLD is a multifactorial disorder comprising a group of diseases. Genetic, epigenetic, and environmental factors interact with one another during the development of these diseases. Nonalcoholic steatohepatitis (NASH) is a hepatic feature of metabolic syndrome. Obesity and insulin resistance are often factors promoting NASH. The accumulation of triglycerides in hepatocytes is the most commonly observed phenotype in NAFLD [65]. Alterations in the gut microbiota are considered to be a key factor contributing to NAFLD, and the interplay of metabolic syndrome, diabetes, and liver disease in NAFLD patients influences the microbiota in complementary ways [66]. Because the body mass index (BMI) may be a major determinant of compositional changes in microbial communities [7], Wang et al. [8] directly assessed the fecal microbial composition and its correlation with liver biochemistry in non-obese adult patients with NAFLD. In a human study of gut dysbiosis across the spectrum of NAFLD lesions, comprising 57 patients with biopsy-proven NAFLD, significant fibrosis was found to be associated with large amounts of *Bacteroides* and *Ruminococcus* and decreased levels of *Prevotella*. Along with metabolite information from patients, a microbiota analysis is useful for predicting NAFLD classes and severity. For example, *Bacteroides* abundance is independently associated with NASH severity, and *Ruminococcus* abundance is associated with significant fibrosis [67].

Thus, liver disease is usually accompanied by an increase in Enterobacteriaceae and a decrease in *Bifidobacterium*. Gut dysbiosis can lead to endotoxemia in patients through bacterial translocation (BT). Endotoxemia may induce immune dysfunction, thus leading to further liver cell necrosis and liver failure. Therefore, we propose the development of new probiotics specifically for the prevention and treatment of the progression of liver diseases (Fig. 3).

3.3. The human microbiota associated with gastrointestinal malignancy

GI malignancy is a leading cause of human morbidity and mortality worldwide. Aside from widely accepted genetic factors, non-genetic factors for cancer risk, especially the residential microbes in the GIT, exert a broad impact on the development of cancers that arise within the GIT. Recent advances in microbial research on GI malignancies, such as gastric cancer, colorectal cancer, and esophageal cancer, provide new insight into the role of the human microbiota in tumorigenesis.

3.3.1. Gastric cancer

H. pylori-associated chronic inflammation is considered to be the strongest risk factor for gastric cancer. Each year, approximately 660 000 new cases of gastric cancer are caused by *H. pylori* infection, which results in the loss of acid-producing parietal cells, thus leading to the development of gastric atrophy, metaplasia, dysplasia, and, finally, carcinoma formation [68]. It is interesting that the elimination of *H. pylori* before the onset of chronic atrophic gastritis may protect against gastric cancer [69]. As a distinct causative factor for gastric cancer, *H. pylori* has been classified as a class I carcinogen by the World Health Organization (WHO). However, only 1%–2% of people with an *H. pylori* infection develop stomach cancer [70]. The carcinogenic risk may be related to the genetic diversity of the *H. pylori* strain, variations in host responses, and specific host-microbe interactions [71]. Importantly, the phylogenetic origin of *H. pylori* is a good predictor of the risk for gastric cancer [72].

The two best-studied *H. pylori* determinants, cytotoxin-associated antigen A (CagA) and vacuolating cytotoxin (VacA), have been shown to be associated with a higher risk of cancer [73]. It has been reported that VacA promotes the apoptosis of gastric epithelial cells, in a specific host response to *H. pylori* that may be

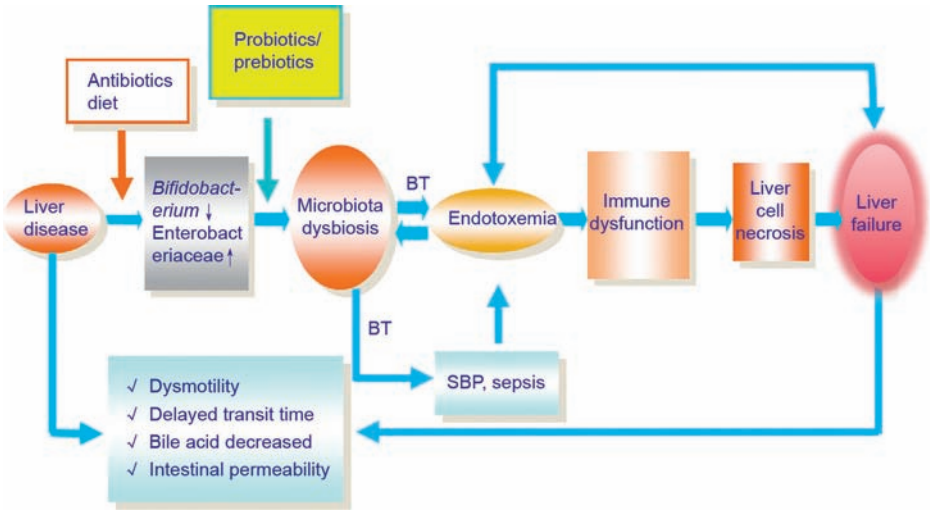

Fig. 3. Our hypothetical pathway for the role of gut microbiota dysbiosis in liver diseases. Evidence shows that chronic liver disease is usually accompanied by intestinal dysbiosis, which is characterized by the increase of Enterobacteriaceae and the decrease of *Bifidobacterium*; this can lead to BT, then to endotoxemia and even spontaneous bacterial peritonitis (SBP), and finally to progression of the liver disease. Importantly, the maintenance of the normal microbial community by means of probiotics/prebiotics could greatly improve the prevention and treatment effect of liver disease.

responsible for gastric carcinogenesis by interfering with mitochondrial function [74]. In addition, VacA suppresses the host immune response by inducing dendritic cells to express and release the anti-inflammatory cytokines interleukin (IL)-10 and IL-18. This compromised immune response promotes *H. pylori* evasion and enhances tumor survival [75]. Unlike the *VacA* gene, the *cag* pathogenicity island (PAI), which is present in some strains of *H. pylori*, is associated with a significantly increased risk of developing adenocarcinoma of the stomach [76]. Genes within the *cag* PAI encode proteins that form a bacterial type IV secretion system (T4SS). T4SS exports CagA and peptidoglycan from adherent *H. pylori* into host cells, thus activating the PI3K pathway, stimulating cell migration, and contributing to carcinogenesis [77]. After tyrosine phosphorylation, CagA interacts with and activates several host cell proteins, thereby leading to morphological alterations, including cell scattering and elongation [78]. Beyond *H. pylori*, Lertpiriyapong et al. [79] noted that the synergetic colonization of altered Schaedler's flora (ASF) causes more pronounced gastric pathology in insulin-gastrin (INS-GAS) mice, including gastric corpus inflammation, epithelial hyperplasia, and dysplasia.

3.3.2. Colorectal cancer

The interaction of the gut microbiome with the development of colon cancer has recently become a major focus of research. Microbial dysbiosis has been implicated in the etiology of colorectal adenomas and colorectal cancer (CRC). A pathological imbalance in the microbial community has been observed in subjects with adenomas compared with normal controls [80,81]. Despite the varied results among different studies, the microbiota in cases of adenomas or CRC is characterized by a high proportion of potential pathogens, such as *Pseudomonas*, *Helicobacter*, and *Acinetobacter*, and by a lower richness of beneficial bacteria, such as butyrate-producing bacteria [80]. Zackular et al. [82] observed that the gut flora from tumor-bearing mice promotes inflammation and tumorigenesis in recipient animals, thus directly contributing to CRC. This study has provided mechanistic insight into the relationship between the gut microbiome and CRC development. However, it is still unclear from human studies whether the alteration in the microbial community is a cause or consequence of adenomas and CRC.

In addition, the contribution of specific bacterial species to cancer risk remains to be fully established. *Fusobacterium nucleatum*, a periodontal pathogen, has been suggested to be overabundant during disease progression from adenomas to cancer [83]. A significant increase in *Bacteroides massiliensis*, *Bacteroides ovatus*, *Bacteroides vulgatus*, and *Escherichia coli* (*E. coli*) has also been observed from advanced adenoma to carcinoma [84]. The potential mechanism underlying this development includes the promotion of inflammation and the induction of tumorigenesis [85,86]. Kostic et al. [86] observed that *Fusobacterium nucleatum* modulates the tumor-immune microenvironment by promoting the myeloid infiltration of intestinal tumors in an adenomatous polyposis coli (APC) multiple intestinal neoplasia (Min) mouse model of CRC and increasing the expression of pro-inflammatory genes such as *PTGS2* (*COX2*), *SCYB1*(*IL8*), *IL6*, *TNF* (*TNFα*), and *MMP3*. In addition, many studies have elucidated a link between bacterial antigens, virulence factors, and colon malignancy. Enterotoxigenic *Bacteroides fragilis* (ETBF) produces a toxin known as fragilysin (*B. fragilis* toxin, BFT), which activates the Wnt/β-catenin signaling pathway and NF-κB; consequently, it increases cell proliferation and induces the production of inflammatory mediators [87–89]. The role of ETBF in colorectal carcinogenesis was further illustrated by Wu et al. [90], who showed that mice colonized with ETBF exhibit a marked increase in colon adenomas and tumors as compared with normal controls. *Enterococcus faecalis* and *E. coli* may induce DNA damage by promoting the release of extracellular superoxide in host cells and encoding the enzymatic machinery that generates colibactin via the polyketide synthase (PKS) genotoxic island [91,92]. Although these observations show an etiological contribution by intestinal microbiota in colorectal neoplasia, additional investigations are needed to determine their potential as CRC biomarkers, or their utility as diagnostic and therapeutic targets.

Moreover, many bacteria-derived metabolites have been implicated in the suppression of colon cancer development; these include SCFAs, which are produced through the microbial fermentation of complex polysaccharides, including acetate, propionate, and butyrate, which serve as energy sources for colonic epithelial cells. Butyrate, which is primarily produced by species within the Lachnospiraceae and Ruminococcaceae, has been shown to be protective against colonic neoplasia. A high fiber intake reportedly leads to a reduction in the risk of developing colon malignancy because of the production of butyrate [93,94]. In an *in vitro* study of cancer lines, butyrate was found to exert a tumor-suppressing

effect by inducing apoptosis, inhibiting proliferation, causing epigenetic changes in gene expression, and modulating inflammatory responses and cytokine levels [95]. Therefore, modulation of the gut microbiome through dietary control or antibiotic treatment may offer great therapeutic potential. The manipulation of gut microbiota to favor and enhance the production of SCFAs through the use of prebiotic or non-digestible food ingredients may be a promising approach to program host metabolism, and may consequently influence cancer risk.

3.3.3. Esophageal cancer

Recent studies confirm that chronic inflammation at the end of the esophagus that is caused by gastroesophageal reflux is closely related to esophageal adenocarcinoma (EA). The overall pathophysiology of this development process can be described as "gastroesophageal reflux disease–Barrett's esophagus–esophageal adenocarcinoma" (GERD–BE–EA) [96–98]. Regional differences in its incidence appear to be correlated with economic development. Therefore, researchers have suggested that the morbidity from EA may be related to the use of antibiotics worldwide. Long-term changes in esophageal microecology after frequent antibiotic exposure may lead to a higher incidence of GERD, thus resulting in an increasing morbidity from EA [99]. A large number of descriptive studies have reported observing esophageal microecological changes in patients with GERD [100]. However, the local microbiome does not distinguish between squamous cell carcinoma and adenocarcinoma [101]. In addition, the role of *H. pylori* in the pathogenesis of GERD and EA remains unclear and controversial. *H. pylori* was first identified by the WHO as a carcinogen associated with gastric cancer in the 1990s, and eradication treatments against this bacterium are widely performed. In addition, researchers have found that, with the decline in *H. pylori* infection, GERD incidence has increased [102]. A series of case-control studies also suggested that *H. pylori* may play a protective role in the development of GERD and associated EA. However, the eradication of *H. pylori* treatment does not worsen GERD or increase new GERD [103].

3.4. The human microbiota and metabolic disorders

The composition of the gut microbiota is influenced by the use of antibiotics and by the lifestyle of the human host, including exercise, diet, and hygiene preferences. In turn, the dysbiosis of intestinal flora affects the production of immune mediators and induces both chronic inflammation and metabolic dysfunction [104]. Obesity and its associated metabolic complications, such as type 2 diabetes (T2D) and cardiovascular disease, have become a global epidemic health problem and are considered to be the consequences of a complex multidirectional interaction among host genetics, diet, environment, and the gut microbiota [105].

3.4.1. Obesity

An increasing number of *in vivo* and human studies have indicated that interactions between the gut microbiota and host genotype or dietary changes may be crucial factors that contribute to obesity and related metabolic disorders [106,107]. Ridaura et al. [108] demonstrated that the microbiota from lean or obese co-twins induces similar adiposity and metabolic phenotypes in mice. Moreover, the lean co-twin's microbiota can prevent adiposity gain in obese-recipient mice, if the mice are fed with an appropriate diet [108]. Several studies on the gut microbiota indicated that diet modulates the composition and function of microbes in humans [109] and rodents [110]. For example, a mouse study revealed that mice that were fed with lard for 11 weeks exhibited increased Toll-like receptor activation and white adipose tissue inflammation, along with reduced insulin sensitivity, compared with mice that were fed with fish oil [110]. However, phenotypic differences between the dietary groups can be partly attributed to differences in microbiota composition. Increasing evidence shows that the gut microbiota is an important modulator of the interaction between diet and the development of metabolic diseases [111]. Furthermore, recent studies have shown that the gut microbiota influences the circadian clock and undergoes circadian oscillations [112]. Disruption of the host circadian clock induces dysbiosis, which is associated with host metabolic disorders [113]. Obesity, which is associated with gut microbiota dysbiosis and altered metabolic pathways, induces impaired gut epithelial barrier function and has significant influences on physiological processes [114], such as gut and immune homeostasis [115], energy metabolism [116], acetate [25] and bile acid metabolism [117], and intestinal hormone release [118].

3.4.2. Type 2 diabetes

T2D is a prevalent metabolic disease worldwide; the link between the gut microbiome composition and the development of T2D is gradually being uncovered [119–121]. Growing numbers of studies indicate that an altered gut microbiome characterized by lower diversity and resilience is associated with diabetes. The mechanisms that cause the disease may be related to the translocation of microbiota from the gut to the tissues, thus inducing inflammation [122]. Pedersen et al. [123] recently demonstrated that the human gut microbiome may affect the serum metabolome and induce insulin resistance through species such as *Prevotella copri* and *Bacteroides vulgates*. Metformin is one of the most widely used antidiabetic drugs and is thought to confound the results of metagenomics data analysis [121]. The gut microbiota may directly affect T2D through its effect on the metabolism of amino acids; thus, future antidiabetic treatment strategies may target bacterial strains that cause imbalances in amino acid metabolism [121,124]. Therefore, obesity and its associated metabolic complications may be a result of complex gene-environment interactions. Microbiome interventions aimed at restoring the homeostasis of the gut microbiome have recently emerged, such as the ingestion of specific fibers or therapeutic microbes. These are promising strategies to reduce insulin resistance and related metabolic diseases.

3.5. The human microbiota and other diseases

Growing evidence indicates that alterations in the microbiota are implicated in the pathogenesis of a number of other diseases, such as severe asthma, food allergies, autism, and major depressive disorder (MDD) [125–130], all of which have recently received considerable scientific interest. Interestingly, these diseases may not involve direct interactions with the microbiota. However, the regulating function of the microbiota, such as the microbiota-gut-brain axis, may participate in the specific pathways of the diseases. The complex microbiota-host interactions are dynamic, involving a variety of mechanisms that include immune, hormonal, and neural pathways. Therefore, changes in the microbiota may result in the dysregulation of host homeostasis and in an increased susceptibility to these diseases. On the basis of these well-established connections between disease and the disruption of homeostatic interactions in the host, microbiota-targeted therapies may alter the community composition, and microbiota restoration might be used for treating these diseases.

3.5.1. The microbiota and allergic diseases

An early-life, antibiotic-driven low diversity in gut microbiota enhances susceptibility to allergic asthma [131], and thus may

also affect asthma development in childhood after long-term follow-up. Of course, the mode, place of delivery, and infant feeding also affect the GI microbiota composition and subsequently influence the risk of atopic manifestations [132]. Bunyavanich et al. [128] found that infants with a gut microbiota enriched in Clostridia and Firmicutes at a host age of 3–6 months are associated with the resolution of cow's milk allergy (CMA) by the age of 8 years. Because the intestinal microbiota of an infant evolves rapidly in the first year, the early-life gut microbiota composition may be one of the determinants for CMA outcomes in childhood. The gut microbiota interacts with the immune system intimately, providing signals to promote the maturation of regulatory antigen-presenting cells and regulatory T cells (Tregs), which play a crucial role in the development of immunological tolerance. The specific members of the microbiota, such as *Clostridium* species, interact with Treg and regulate immunoglobulin E (IgE) levels [133]. Saarinen et al. [127] showed that the clinical course and prognosis of CMA are highly dependent on the milk-specific IgE status. A previous study also found that specific microbiotic signatures, such as that of *Clostridium sensu stricto*, can distinguish infants with IgE-mediated food allergies from those with non-IgE-mediated ones, and that *Clostridium sensu stricto* is positively correlated with specific IgE level in serum [126].

3.5.2. The microbiota and psychiatric diseases

Psychiatric diseases have posed a severe threat to human health throughout history [134]. They are caused by a combination of biological, psychological, and environmental factors [135–137]. The existence of a gut-brain axis has been acknowledged for decades. The gut-brain axis plays a key role in maintaining normal brain and GI function. More recently, the gut microbiota has emerged as a critical regulator of this axis. The concept of this axis has been extended to the "microbiota-gut-brain axis," and is now seen to involve a number of systems, including the endocrine system, neural system, metabolic system, and immune system, all of which are engaged in constant interaction [138]. Gut microbiota dysbiosis may increase the translocation of gut bacteria across the intestinal wall and into the mesenteric lymphoid tissue, thereby provoking an immune response that can lead to the release of inflammatory cytokines and the activation of the vagus nerve and spinal afferent neurons [139,140]. Autism spectrum disorder (ASD) has been reported as correlated with an altered gut microbiota, and low relative abundances of the mucolytic bacteria *Akkermansia muciniphila* and *Bifidobacterium* spp. have been found in the feces of children with autism [125]. Our previous study found an altered fecal microbiotic composition in patients with MDD. Most notably, the MDD groups had increased levels of Enterobacteriaceae and *Alistipes*, but reduced levels of *Faecalibacterium* [130]. These studies suggest the role of the gut microbiota in autism and MDD as a part of the gut-brain axis; this suggested role should form a basis for further investigation of the combined effects of microbial, genetic, and hormonal changes in the development and clinical manifestation of autism and MDD.

4. Advancements in microbiota technology

Over the past few decades, human microbiome research has been revolutionized by high-throughput sequencing technology. High-throughput sequencing provides an opportunity for studies to focus on complex microbial systems without the need to clone individual genes. Initially, microbiota studies focused on compositional studies (i.e., answering the question: what is there?) and functional studies (i.e., what are they doing?). With the development of sequencing technology and bioinformatics analysis, it has become increasingly interesting to study the activity of microbes within microbial communities. It is widely accepted that the microbes with the highest abundance are not always the most active ones. RNA sequencing (RNAseq) permits the analysis of gene expression, adding valuable expression data to compositional data sets. Gosalbes et al. [141] performed the first metatranscriptomic analysis of the healthy human gut microbiota in 2011. The analysis of 16S transcripts showed the phylogenetic structure of the active microbial community. Lachnospiraceae, Ruminococcaceae, Bacteroidaceae, Prevotellaceae, and Rickenellaceae were the predominant families detected in the active microbiota. The primary functional roles of the gut microbiota were found to be carbohydrate metabolism, energy production, and the synthesis of cellular components. A systematic comparison of the gut metagenome and metatranscriptome revealed that a substantial fraction (41%) of microbial transcripts was not differentially regulated relative to their genomic abundances. The metatranscriptional profiles were significantly more individualized than the DNA-level functional profiles but were less variable in their microbial composition [142]. A transcriptome analysis of bacteriophage communities in the periodontal microbiota was recently performed using RNAseq. Oral phages were found to be more highly expressed in individuals with relative periodontal health [143].

To achieve precise microbiome-based medicine in the future, it is necessary to understand which individual microorganisms mediate vital microbiome-host interaction(s) under health or disease conditions. Most gut microbes are currently uncultivable. Even with the use of recent technologies, such as gnotobiotic mice and anaerobic culturing techniques, it is possible to culture only approximately half of the bacterial species identified by 16S rDNA high-throughput sequencing [144]. In addition, species-level identification may not reflect the real situation because most of the functional diversity can be reflected only at the strain level. Therefore, it will be crucial to develop technologies to identify and isolate these microorganisms and/or microbial consortia.

Compared with traditional microbiology approaches, the use of anaerobic conditions and gnotobiotic animals largely facilitates the cultivation of difficult-to-grow microbes. Numerous previously uncultivable microbes can now be cultured in a laboratory setting [145]. A chip-based isolation device (the iChip) was recently developed and was specifically designed to identify uncultivable microbes within complex microbial ecosystems [146]. The iChip is composed of hundreds of miniature diffusion chambers, each of which is inoculated with a single environmental cell. The capacity for microbial recovery using the iChip is many times higher than that of standard cultivation, and the resulting species are of significant phylogenetic novelty [146]. A new device for *in situ* cultivation (the I-tip) was subsequently developed. The principal of the I-tip is similar to that of the iChip; however, the I-tip traps individual microbes within a gel, thus allowing for the passage of metabolites and nutrients. The *in situ* isolation of microbes from invertebrate organisms using the I-tip has recovered isolates from 34 novel microbial species [147].

Simulating GIT conditions can greatly facilitate *in vitro* cultivation. The Simulator of the Human Intestinal Microbial Ecosystem (SHIME) has succeeded in establishing stable, reactor-grown GIT microbial communities. More importantly, this system is able to precisely simulate different regions of the human GIT, thus allowing the diversity of the community to be studied *in vitro* in a different niche. The power of SHIME has been estimated by numerous studies [148–150]. For example, it has been found that different regions of SHIME are colonized by different unique microbial communities when cultured with microbes. The distribution is highly similar to that of the living host, such as the prevalence of *Bacterioides/Prevotella* spp. and *Lactobacillus* spp. in the colon [148].

Identifying single cells that produce metabolites of interest within complex microbial ecosystems is very important for understanding microbiome-host interactions. With this purpose, a flexible high-throughput approach using a combination of microfluidics and fluorescence-activated cell sorting has recently been developed [151]. This system has successfully identified xylose-overconsuming *Saccharomyces cerevisiae* and *L*-lactate-producing *E. coli* cells from a population. This system also allows for the screening of mutants in known pathways.

The use of traditional animal models in microbiome studies continues to provide insight into host-microbiota interactions. However, animal models often do not predict the results obtained in humans, thus posing a particular problem when considering challenges relating to the oral absorption of drugs and nutrients. Here, we introduce two methods of *in vitro* simulation based on stem cells: the gut-on-a-chip system and colonic stem cell construction. The gut-on-a-chip system takes advantage of biomaterial engineering and provides an optional approach to study the complex interactions occurring within the gut microbiome. This system is an *in vitro* living cell-based model of the intestine that mimics the properties of the human gut along with crucial microbial symbionts. Biomimetic human gut-on-a-chip micro-devices are usually composed of microfluidic channels and a porous flexible membrane that are coated with an extracellular matrix and lined with human intestinal epithelial cells [152]; such devices mimic the complex structure and physiology of a living intestine. Microfluidic devices can also be used to study microbe-microbe interactions, such as chemotaxis/chemical attraction and quorum sensing [153]; such interactions have been shown to be more effectively studied using microfluidic devices than using traditional capillary-based assays [154]. In addition, given the recapitulation of many complex functions of the normal human intestine, it may also become an essential platform for drug screening and toxicology testing.

Colonic stem cell construction is a recently developed *in vitro* system that is used to grow 3D organoid colonic epithelium structures that are guided by microstructures without the utilization of microfluidics technology [155]. Within a Matrigel overlay, spherical 3D structures grown from colonic stem cells or intestinal stem cells are collected from an array containing individually grown structures. These membrane-free 3D stem-cell-derived organoids, which contain various differentiated cell types, form a barrier similar to that of intestinal or colonic epithelia [156,157]. These organoids have recently been used to demonstrate that the *Salmonella enterica* serovar Typhimurium can successfully invade the epithelial cell layer [157], and that *C. difficile* can disrupt the epithelial barrier function [158]. This technology also provides more novel and valuable methods for higher throughput microbiome studies than existing models, although this technique is still in its infancy.

5. Application of the human microbiota

The human microbiome can be considered as an important origin of resources for genetic diversity, a modifier of disease, an essential component of immunity, and a functional entity that influences metabolism and modulates drug interactions. On one hand, there are many potential probiotics or beneficial bacteria that may prevent or treat certain diseases, although most of them cannot be cultivated at present [159]. For example, some of these gut microbes belong to genera that contain many probiotics such as *Lactobacillus* and *Bifidobacterium*. Some are novel potentially beneficial bacteria, such as *Faecalibacterium prausnitzii* for treating IBD and irritable bowel syndrome (IBS), and *Akkermansia muciniphila* for improving metabolic health [160]. On the other hand, as our second genome, the human microbiome must produce a large number of metabolites. Some isolated metabolites have important potential applications, although it still remains a great challenge to isolate and identify all the metabolites of the human microbiome. For example, Chu et al. [161] discovered methicillin-resistant *Staphylococcus aureus*-active antibiotics by using primary sequencing from the human microbiome.

With the increased understanding of the relationship between the human microbiome and a variety of diseases, the use of these findings to predict or diagnose diseases has attracted a great deal of attention [162]. Enrichments of some microbes are noted as potential biomarkers in some research; however, these alterations are often observed in other research as well, and cannot be distinguished among different diseases. In contrast, clinical models based on tens of genes within a metagenome analysis perform better in diagnostics and predicting diseases. In addition, we found that the *Bifidobacterium*/Enterobacteriaceae (B/E) ratio indicates the microbial colonization resistance of the bowel, and that this ratio is considered to be an indicator of human microbiome heath. The B/E ratio is higher than 1 in people with healthy microbiomes, whereas it is far below 1 in patients with cirrhosis and patients with the avian influenza H7N9 infections [163,164].

The prevention and treatment of diseases by targeting the microbiome have been widely investigated, and some therapies have been successfully applied in the clinic. The administration of probiotics is reported to help restore the health of H7N9 patients more quickly [164]. Fecal microbiome transplantation has exhibited better clinical efficacy than antibiotics in the treatment of *C. difficile* infections [165]. Substantial progress has also been made in the treatment of liver diseases by modulating the gut microbiome. A clinical trial showed that probiotic VSL#3 reduces liver disease severity and hospitalization in patients with cirrhosis [166]; the administration of *Lactobacillus salivarius* LI01 or *Pediococcus pentosaceus* LI05 improves the acute liver injury induced by *D*-galactosamine in rats [167]. Furthermore, the regulation of the human microbiome plays important roles in the treatment of GI diseases, such as infectious diarrhea, antibiotic-associated diarrhea, inflammatory bowel disease, and necrotizing enterocolitis. For example, the oral administration of a mixture of 17 Clostridia strains from the human microbiota to adult mice was found to attenuate disease in models of colitis and allergic diarrhea [168]. Modulation of the gut microbiome may also contribute to the treatment of cancer. Iida et al. [169] reported that optimal responses to cancer therapy require an intact commensal microbiota that mediates the therapy effects by modulating myeloid-derived cell functions in the tumor microenvironment. Viaud et al. [170] reported that the gut microbiota helps to shape the anticancer immune response of cyclophosphamide. In addition, many clinical studies have shown that probiotics and their products have outstanding effects on the treatment of allergic diseases, especially those in infants [171].

6. Future perspectives

The human microbiota plays an important role in the well-being of the human host, and participates actively in the development of a wide variety of diseases. Given the extensive influence of microorganisms throughout the human body, we propose that research on host-microbiota interactions should go beyond a characterization of the community composition and an investigation of the community members' associations. From the structure to the function of the microbiota, future research should move microbiome investigations toward providing explanations of causality. With new techniques for microbiota function prediction, new microbiota interaction models, and novel analytical and simulation approaches, future advances will help to clarify the interactions

between the microbiota and human development, and the potential roles of those microbiota involved in the mechanisms of various diseases, such as liver diseases, bacterial infection, cancer, psychiatric diseases, and metabolic diseases. The crucial roles of the human microbiota should be investigated at a much deeper level, and microbiome-based diagnosis and treatment strategies will be used for future personalized medicine work.

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Compliance with ethics guidelines

Baohong Wang, Mingfei Yao, Longxian Lv, Zongxin Ling, and Lanjuan Li declare that they have no conflict of interest or financial conflicts to disclose.

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