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APPLICATION OF INTESTINAL MICROECOLOGICAL RESEARCH TECHNOLOGY IN METABOLIC SYNDROME

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Abstract: The mammalian intestine is home to a large number of complex microbiota, which together constitute the intestinal microbiome. In recent years, people have gradually realized that intestinal microorganisms are closely related to the occurrence and development of some diseases, such as metabolic diseases, inflammatory bowel diseases, tumors, immune system and neurological diseases, etc., making intestinal microorganisms a hot spot in research. The rapidly developing microbial research technology has provided us with an efficient and powerful technical platform, promoted the systematic understanding of intestinal microecology, and opened up new ideas for the diagnosis and treatment of diseases. This article aims to summarize and analyze the latest progress and limitations of commonly used microecological research technologies, provide a reference for further intestinal microbiome research, and briefly introduce the research results related to intestinal microecology and metabolic syndrome.
Keywords: Intestinal microbiome; Single cell analysis; Culture method; Metabolic syndrome

1INTESTINAL MICROECOLOGY

The intestine is the main place where humans digest and absorb nutrients. It is home to a large number of bacteria, fungi, archaea, viruses and protozoa. They interact, influence and co-evolve with the host to form a relatively stable community structure. That is, gut microbiota. In recent decades, microbial research technology has developed rapidly, resulting in epoch-making progress in this field. A large number of studies have shown that the intestinal microbiota plays an important role in the occurrence and development of metabolic syndrome, tumors, inflammatory bowel disease, and allergic diseases. Among them, intestinal microorganisms play an important role in the development of metabolic syndrome (metabolic syndrome). The role it plays has received the most widespread recognition and attention, and it is also an area of relatively clear research at present. Based on reading a large amount of literature, this article summarizes the current status of intestinal microbiome research technology in order to provide information for researchers in related fields; at the same time, it also analyzes the role of intestinal microbiome in metabolic syndrome such as obesity, diabetes, and non-alcoholic fatty liver disease. An overview of the characteristics, functions and related mechanisms in symptomatic states.

The intestinal microbiota of an adult individual under normal physiological conditions contains about 1014 microorganisms, which is equivalent to more than 10 times the total number of host cells, and the total mass can reach 2kg; there are more than 2000 different strains, and the number of genes carried is equivalent to the entire human genome 150 times[1]. Such a large number of intestinal microorganisms play an important role in intestinal homeostasis and host health. Most of them have a symbiotic relationship with the host and co-evolve with the host, participating in nutrient metabolism, drug metabolism, pathogen resistance, and immune regulation. Intestinal microorganisms can perform a large number of metabolic reactions that the host itself cannot complete but are necessary for host health, such as producing a large number of enzymes and fermenting carbohydrates that have not been fully digested and decomposed by the intestine, including macromolecular plant polysaccharides, some oligosaccharides, and Endogenous mucus produced by epithelial cells produces short-chain fatty acids (SCFAs) - acetic acid, propionic acid and butyric acid, as well as gases such as CO_2, H_2 and CH_4[2]. In addition, intestinal microorganisms can also produce a variety of vitamins, synthesize essential and non-essential amino acids, etc. Although gut microbes prefer carbohydrates as a growth substrate, they can also utilize protein, especially in the distal colon, where carbohydrates that reach this site are almost exhausted. After protein reaches the colon, it is first cleaved into peptides and amino acids, and then further degraded by intestinal microorganisms. In the colon, bacterial amino acid degradation involves oxidation reactions and deamination reduction reactions, followed by decarboxylation to produce SCFA. However, it also produces a series of harmful substances such as ammonia, amines, p-cresol, and hydrogen sulfide. These substances are released in the intestine. It plays an important role in tract leakage, inflammation, DNA damage, and tumor progression.

The presence of intestinal microorganisms is crucial to the development and regulation of host innate immunity and adaptive immunity. About 70% of the human body's immune system is located in the gastrointestinal tract, including its glands, mucosa and mucosa-related lymphatic system[3]. Comparative studies between germ-free animals and conventionally raised animals[4] revealed that the immune components of germ-free animals are immature and imperfectly developed, and the development and functional integrity of the mucosal immune system are adversely affected, especially the gastrointestinal system, which is manifested as follows Lymph follicles decreased.

Peyer's lymph nodes and mesenteric lymph nodes became smaller, phagocytes were scattered, and the expression of antimicrobial peptides decreased. The impact of intestinal microorganisms on host immunity is not limited to the gastrointestinal tract, but also affects immune responses in the respiratory system, nervous system, and skin. A significant increase in the number of iNKT cells and a significant increase in IgE levels are commonly found in germ-free animals. These components are related to the spread of inflammatory responses and high respiratory tract responses[5].

2 INTESTINAL MICROECOLOGICAL RESEARCH TECHNOLOGY

2.1 Artificial Culture Method

Artificial microbial culture methods have been used for more than 130 years. Traditional microbial identification mainly relies on biochemical reactions, staining, and morphological characteristics. Culture and identification are time-consuming, heavy workload, and cumbersome operations. Normally, this method is mainly used for the isolation and identification of pathogenic microorganisms harmful to humans; however, this is also the first generation method used in microecological research. The initial research on intestinal flora components mainly used Gram staining microscopy. Pure culture. Since most of the intestinal flora are caustic anaerobes, which are more than 100 times more numerous than aerobes and facultative anaerobes, a brief exposure to the air will cause death [6]. We have used culture methods and quantitative polymerase chain reaction (PCR) to study the relationship between intestinal flora and sepsis, but the valuable data obtained are limited [7]. This traditional microbial culture method can only obtain about 1% of the microorganisms in the environment [8]. Therefore, artificial culture alone is far from meeting the needs of intestinal microecological research.

2.2 Next-Generation Sequencing Technology (NGS)

Currently, NGS is considered a mainstream method for studying microbiota, especially in the study of human intestinal microbiota. Compared with culture methods, this method has obvious advantages and can provide direct and in-depth understanding of the structure of intestinal flora. There are two main options for using NGS technology to study biological communities: (1) 16S rRNA gene sequencing. The bacterial gene encoding ribosome 16S is only 1.5 kb and is highly conserved. There are 9 hypervariable regions that are sufficient to distinguish microbial species. It is commonly used in bacteria. The species identified were variable regions such as V3, V4, V6 and V8 or full-length sequencing of the 16S gene. This technology is relatively cheap, but the selection of sequencing variable regions has a great impact on the relative abundance detection at the phylum level. For example, in the study of intestinal flora, the V4-V5 hypervariable region is selected to reveal the intestinal flora using 454 or Illumina sequencing platforms. It is dominated by Bacteroidetes, and the selection of V3-V4 region indicates that Firmicutes is dominant [9]. Hiergeist et al. [10] conducted a performance evaluation on 9 independent centers qualified for 16S rDNA sequencing and found that DNA extraction, amplification primer selection, 16S rDNA sequencing hypervariable region selection and subsequent bioinformatics processing have a greater impact on the microbiome analysis results. Large, while the sequencing platform has less impact on the sequencing results. According to the evaluation by Turnbaugh et al. [11], the detection threshold of 16S rRNA gene sequencing is 106 microorganisms per gram of feces. This means that bacteria that are below this threshold but may be harmful to humans cannot be detected. Moreover, 16S rRNA sequencing can only detect known microorganisms, but many undiscovered intestinal microorganisms have no corresponding reference sequences. (2) Metagenomic sequencing is the sequencing and analysis of all nucleic acids obtained from specific environmental samples. Metagene sequencing can be used to analyze all genes in a specific environment, including the identification and changes of genes encoding carbohydrates, energy metabolism, secondary metabolite synthesis, signal transduction, fermentation pathways, etc., to understand the developmental composition and composition of microbial systems. Functional differences lay the foundation for further research on the impact of the microbiome on human health and disease. For example, metabolic network analysis of fecal microbiome metagenomic sequencing data from 124 unrelated individuals and 6 pairs of identical twins and their mothers found that topological differences at the gene level and network level are related to obesity and IBD [12]. In addition, metagenomic analysis was performed on 252 stool samples from 207 subjects, and it was found that there are huge differences in different individuals at the genetic level, and 107, 991 short insertions/deletions and 10.3 million single nucleotide diversity (SNP) were identified.) and 1051 structural variations; in addition, it was also found that even if the composition of the intestinal flora changes dramatically over time, the body's unique SNPs are still transiently stable, which also suggests that everyone has a unique metagenome, which can be used for further exploration. Provide reference for personalized treatment and dietary nutrition [13]. A detailed metagenomic sequencing analysis of intestinal flora can help us better understand the pathogenesis and identify new therapeutic targets.

2.3 Microbial Single Cell Genome (SCC) Technology

SCC technology is newly developed after metagenomics and can effectively obtain the genetic information of a large number of uncultivated microorganisms. This technology involves steps such as single cell isolation, whole genome amplification, whole genome sequencing and data analysis. The current application of this technology in microbial research mainly focuses on exploring new functional genes that have not been detected by metagenomic technology or other conventional technologies. In recent years, single-cell genome sequencing technology has developed rapidly and was named Technology of the Year by Nature Methods in 2013. It is believed that this technology will change many fields in biology and medicine. The difficulty in applying this technology is single cell separation. Current single cell separation methods include limiting dilution, microfluidics, flow cytometry, micromanipulation, optical tweezers grabbing, flow cytometry analysis, and microfluidic chips [14]. Through single-cell isolation, microorganisms with low content in the sample can be targeted and selected, complete genome sequencing, and supplement the reference database for newly discovered microorganisms [15]. For example, the whole genome sequencing of the uncultivable microorganism TM7 and two Flavobacteria has been completed using this technology [16, 17]. However, this technology has certain operational difficulties, such as single-cell separation effect, whole-genome amplification accuracy, and susceptibility to the influence of free nucleic acids in specimens. As the technology improves, wider use will become possible.

2.4 Microbial Cultureomics

Culture methods use minimal amounts of host-specific microorganisms and facilitate detailed and standardized studies of intestinal microbiota and microbe-host interactions. Molecular methods can detect species differences and potential functions of microorganisms. Metagenomic data can predict the growth conditions of bacteria that are difficult or impossible to culture, making isolation and identification possible. Lagier et al. [18] established a high-throughput microbial culture omics based on this principle, that is, using 212 culture conditions for microbial culture, and then performing mass spectrometry and 16S rDNA sequencing on the obtained colonies. And used this method to analyze 340 microorganisms from two lean Africans and one fat European, 174 of which have not been described in the human intestine, and 5 fungi and a virus, Senegalvirus, were also discovered for the first time, with only 51 Species 16S rRNA V6 sequencing obtained results that were consistent with the culture species. Using this approach, hundreds of new species have been identified [19].

In short, microecological research has received more methodological support. Culture methods are very necessary to describe new species, improve taxonomic levels and enrich databases. They can also be used to isolate and study low-abundance bacteria, which is helpful for intestinal Studies of bacteria and interactions with their hosts. Although there is no consensus in this field, it is currently estimated that nearly half of the intestinal prokaryotes can be obtained through culture methods [20]. High-throughput sequencing technology can directly obtain huge amounts of data from different biological specimens, which is a major factor in promoting microecological research, while culture-omics technology is an important supplement to molecular technology. Therefore, high-throughput molecular technology and culture-omics technology will coexist, complement each other and further develop, allowing microecological research to develop to a higher level.

3 INTESTINAL MICROECOLOGY AND METABOLIC SYNDROME

The International Diabetes Federation (IDF) defines metabolic syndrome as a cluster of physiological, biochemical, metabolic and clinical factors that increase the risk of cardiovascular disease, type 2 diabetes, etc., mainly obesity, insulin resistance, hyperglycemia, and hyperglycemia. blood pressure etc. Also includes non-alcoholic fatty liver disease. The prevalence rate in different regions ranges from 10% to 84%. It is a high-risk factor for cardiovascular disease and has become a serious public health problem worldwide, seriously threatening human health [21]. Although the occurrence of metabolic syndrome is traditionally believed to be mainly related to lifestyle, a large number of literatures have reported that the intestinal microbiota is closely related to metabolic syndrome. Here, the research results in this area are also briefly introduced.

3.1 Intestinal Microecology and Obesity and Insulin Resistance

Bäckhed et al. [22] obtained first-hand information on the relationship between intestinal microecology and the occurrence of obesity and insulin resistance from a comparative study of germ-free mice and conventionally raised mice. Under the same conditions, the body fat content of conventionally raised mice was 42% higher than that of germ-free mice; and two weeks after the feces of conventionally raised mice were transplanted into germ-free mice, their body fat content increased by 60% even though their food intake decreased. Insulin resistance also occurs. Germ-free mice were transplanted with intestinal microorganisms from obese mice and lean mice, and it was found that the weight and adipose tissue of the former increased significantly compared with the latter [23]. After SD rats with metabolic syndrome model fed high-fructose were transplanted with feces from normally raised rats or treated with antibiotics, it was found that blood lipids, blood sugar, etc. were significantly reduced, and glucose tolerance was improved [24]. This

not only shows that the intestinal microecology is involved in the regulation of obesity and insulin sensitivity, but also shows that some phenotypes of the intestinal microecology can be transferred.

Studies in humans and animals have found that obesity and insulin resistance are related to the structure of the gut microbiome. The content of Bacteroidetes in the intestinal microorganisms of obese individuals decreases, while Firmicutes increases significantly. After dietary restriction, the abundance of Bacteroidetes in the intestine increases, accompanied by weight loss [25]. Jumpertz et al. [26] found that the energy in feces was positively correlated with the Bacteroidetes content in the feces and negatively correlated with the Firmicutes content, suggesting that the intestinal microbiome is related to the host's energy intake. Whole-genome analysis revealed that the intestinal flora of ob/ob mice were rich in related genes that are beneficial to obtaining energy from food, proving that the intestinal microbiome of obese mice can promote the body to obtain energy from food more efficiently. However, research by Schwiertz et al. [27] showed that obese or overweight individuals had a significant increase in Bacteroidetes. These differences may be related to the clinical judgment criteria of the disease or the choice of microbiome research techniques. In addition, it was found that the intestinal contents of Staphylococcus aureus and Escherichia coli in overweight people were higher than those in normal-weight people, while the intestinal contents of Bifidobacterium in normal-weight people were higher [28] . At the same time, studies have found that increased levels of Prevotella copri are related to the imbalance of sugar homeostasis in the body [29]. As for Akkermansia municiphila[30], Lactobacillus gasseri BNR17[31], lactobacillaceae and alcaligenaceae[32], increased levels of these bacteria can improve insulin sensitivity. and blood sugar levels.

3.2 Intestinal Microecology and Non-Alcoholic Fatty Liver Disease

Non-alcoholic fatty liver disease (NAFLD) is a manifestation of metabolic syndrome in the liver. It refers to the liver's ability to function without excessive alcohol consumption (men >20 g/d, women >10 g/d). Simple fatty liver (SS), non-alcoholic steatohepatitis (NASH), and even complications such as cirrhosis and liver cancer may occur; obesity, insulin resistance, and dyslipidemia are high risk factors [33]. From an anatomical point of view, the liver is connected to the intestines through the portal venous system, and 70% of the blood supplied by the liver comes from the intestines, which means that the liver is more susceptible to bacteria and their products derived from the intestines [34]. Changes in the intestinal flora structure are related to the occurrence and development of NAFLD. A study was conducted on C57BL/6J mice transplanted with the feces of NAFLD and non-NAFLD mice respectively. It was found that the former developed NAFLD phenotype, and the 16S sequencing results showed that the former feces contained Barnesiella intestinihominis and Lachnospiraceae. Excessive growth may promote the occurrence of NAFLD; the latter has a higher content of Bacteroides vulgatus [35]. Zhu et al. [36] analyzed the intestinal flora of children with NASH, obese children and normal children through 16S sequencing and found that each group has its own unique intestinal flora structure. Proteobacteria and Enterobacteriaceae in healthy and obese individuals The contents of Escherichia and Escherichia are similar, and they are indeed significantly increased in children with NASH. Moreover, the blood alcohol concentration in children with NASH is significantly increased compared with healthy and obese controls. However, this difference is found in SS patients and healthy controls. There does not exist between them [36]. At the same time, it was found that Escherichia coli in the intestines of patients with liver cancer was significantly higher than that of patients with liver cirrhosis [37]. It can be seen that intestinal microorganisms play an important role in the occurrence and progression of non-alcoholic liver disease.

In summary, advances in microbiological research technology have gradually revealed the role of the gut microbiome in human disease and health, but there are inconsistencies among research results. Therefore, standardized experimental methods, operating procedures and data analysis, a complete microbial database, and complementary advantages between different experimental technologies are needed to better provide strong support for microecological research.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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