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Advanced Application of Bioinformatics in Gut Microbiota

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Abstract: The changes of the intestinal microbiota are closely related to animal health. Intestinal microbiota can regulate energy metabolism and heterologous material metabolism, repair cells, and improve immune function. Metagenomics can be used to detect the dynamic changes of intestinal microflora in animals. Human gastrointestinal tract is inhabited by large and complex microorganisms, which is closely related to human health and disease. Analyzing intestinal microorganisms by using metagenomics technology can reveal the relationship between intestinal microorganisms and their hosts at a higher level. This paper summarizes the research progress of metagenomics in the field of intestinal microorganisms. Exhibition and Its Function in the Treatment of Diseases.

1. Introduction

Intestinal microorganisms refer to the living bacteria in the human intestinal tract. The number of intestinal microorganisms is 10 times that of human cells. There are more than 1000 kinds of intestinal microorganisms which are beneficial to the development and maturation of human natural immunity and adaptive immunity. Intestinal microorganisms can participate in the metabolism of carbohydrates and proteins and promote the absorption of mineral elements such as iron, magnesium and other mineral elements, to synthesize various vitamins and non-essential amino acids. The human intestine is symbiotic with trillions of microorganisms, which play an essential role in maintaining the host's normal physiological functions. Changes in its composition and function can lead to severe intestinal and systemic diseases. Metagenomics research based on new generation sequencing technology and bioinformatics analysis has not only significantly promoted the overall understanding of human intestinal microbes, but also deepened the understanding of the mechanism by which intestinal microbial metabolites promote human health, and provided new ideas for the diagnosis and treatment of human diseases such as intestinal inflammation, metabolic diseases, and cancer.

Previous studies on intestinal microbial community diversity mainly used denaturing gradient gel electrophoresis (DGGE) and other molecular biological methods and biochips to study intestinal microflora. Because DGGE can detect only a dozen dominant bacteria in environmental samples, it has no reaction to trace microorganisms. Electrophoresis strips contain many 16SrDNA sequences, so as to understand specific strains. Information needs cloning, sequencing and so on. The experimental operation is tedious and can not reflect the abundance of microorganisms. Microbial diversity information can be obtained by a biochip probe, and the accuracy of judging microbial abundance by signal strength is not high. Therefore, the study of intestinal microorganisms is limited.

In recent years, metagenomes has gradually become the main research method of intestinal microorganisms. Metagenome is a new concept proposed by Handelsman et al. It is defined as the sum of all microorganism genetic materials in the environment, including the genomes of all culturable and uncultured microorganisms. Tagenomies is a new subject developed in recent years. It takes microbial population genome in environmental samples as the research object, functional gene screening, and sequencing as the research means, microbial diversity, population structure, evolutionary relationship, functional activity, interaction, and relationship with environmental factors as the research object. In general, all individuals of any microbial community are regarded

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as a single gene pool in metagenomes. Metagenomics technology can be divided into four steps: extraction of total DNA from microbial communities in the environment, construction of metagenomes libraries, sequencing, and sequencing. In recent years, 454 sequencing, Illumina sequencing, and ABI-SOLID have developed into the mainstream high-throughput sequencing technology, which has promoted the development of metagenomes. The metagenomes method has obvious advantages. The systematic, physical, and functional characteristics of complex microbial communities are comprehensively analyzed. Fully understand the dynamic changes of microorganisms metagenomics studies clone the total DNA of all microorganisms in a specific environment, and obtain new physiological active substances by constructing a metagenome library and screening; or design primers according to rDNA database, and obtain the inheritance of microorganisms in that environment through systematic analysis. Diversity and molecular ecology information can explain the diversity of microbial communities, evolutionary relationships, population structure, functional activities and environmental relationships, greatly expanding the scope of Microbiology research.

2. Influencing Factors of Intestinal Microorganisms Based on Metagenomies

2.1 Physiological Status and Dietary Habits

The fetus is in a sterile environment in the maternal body, and bacterial colonization begins after birth. The types of bacteria are affected by the host genotype and external environment, the physiological stages of age and dietary habits, and the intestinal microorganisms will be affected. Claeson MJ et al. collected 161 elderly people over 65 years old and 9 adults with intestinal microflora. The V4 region of 16SrRNA gene was sequenced by 454 pyrophosphate sequencing technique in biological samples. It was found that the proportion of Bacteroides in the main intestinal microorganisms of the elderly was larger than that of the young. The microbial diversity of the elderly receiving long-term care was significantly lower than that of the community elderly, which may be related to diet. The results showed that the number of pathogenic bacteria in the intestine of pregnant women increased sharply during the first three months and the last three months of pregnancy, the number of beneficial bacteria decreased significantly, and the number of inflammatory markers increased. Therefore, the supplementation of probiotics to pregnant women during the perinatal period can not only change the disappearance of their mothers. Chemicals and immune functions are beneficial to the growth of beneficial intestinal bacteria and the maturation of intestinal immunity in offspring.

2.2 Food and Drugs

Diet has a great impact on the metagenome. One of the reasons is that different microorganisms have different food types. They often eat meat, and their individual cellulose intake is low, which leads to an inadequate intake of cellulose-like bacteria and a large number of deaths. Filippo CD and other 454 high-throughput sequencing techniques were used to study the difference of intestinal microbial communities between European and African rural children. Prevotella and Xylanibacter strains containing a large number of cellulose and xylan hydrolysis genes were found only in African children and accounted for a high proportion. Western dietary groups also rich in fructose and artificial sweeteners, and the diversity of intestinal microorganisms decreased. This is a metabolic reaction beneficial to the host. Once diet changes, intestinal microorganisms will rebuild themselves. A balanced and diverse diet can promote intestinal homeostasis, prevent, and prevent obesity. Eating an alkaline diet containing dietary fibre and supplementing probiotics can effectively improve intestinal microbial disorders. After 8 weeks of antibiotic treatment, infants were found to be in spite of each fine diet. The number of bacteria can be restored to normal level, but the diversity of gastrointestinal microorganisms is significantly reduced. In infants treated with antibiotics, pathogenic bacteria in the intestine are in the dominant position. In addition, studies have shown that continuous oral administration of antibiotics can cause intestinal microbial imbalance and then affect rat behavior, which may lead to depression and anxiety. The increase of brain-derived neurotrophic factor (BDNF) is related to mental symptoms. If antibiotics are stopped, rats' behavior and BDNF level will return to normal after intestinal microorganisms return to normal.

2.3 Psychological Factors

Bailey et al. studied the effects of stress on the diversity of intestinal microbial communities in mice. By measuring the V4 region of 16S rRNA gene of intestinal microorganisms in mice, it was found that long-term and stress environment resulted in the increase of intestinal anaerobic microorganisms and the decrease of microbial richness and diversity in mice. This may be related to the damage of gastrointestinal mucosa caused by long-term stress. Stress often leads to the decrease of beneficial bacteria such as Lactobacillus in intestine and the increase of conditional pathogens, which have adverse effects on human health.

3. The Relationship between Intestinal Microorganisms and Host Health

When intestinal microorganisms are in a dynamic equilibrium state, the pathogenic bacteria are few or difficult to exist, which will not harm the host. They can play autoimmunity roles, regulate blood pressure, treat allergies, and mediate the application of chemotherapeutic drugs. Once the equilibrium state is broken, the health of the host will be impaired, leading to diseases of the digestive system and even the whole body. Recent studies have shown that microbial disorders are closely related to some diseases. Depression, as an epidemic in current society, indirectly affects various diseases. The intestinal microecology of depressed patients changes and is closely related to the occurrence of cancer. It will be a new way to study by improving the intestinal flora of patients and replacing the traditional surgical chemoradiotherapy to achieve the goal of treatment. At present, probiotics (probiotics, lactic acid bacteria) can help alleviate mental illness effectively, but whether they can be cured remains to be explored. In the future, larger clinical studies are needed to validate this association. In 2006, the American Genome Research Institute first studied the gene function of intestinal microorganisms, and found that the intestinal microbial genome is rich in genes involved in the metabolism of nutrients such as carbohydrates, amino acids, vitamins, most of which are not available to the human body itself. In 2007, Japanese scholars reported that 25% of the 237 gene families enriched in adult intestinal microorganisms were related to carbohydrate metabolism. Glycosidases in intestinal microorganisms are necessary to degrade non-digestible polysaccharides in food and ferment to produce short-chain fatty acids. The resulting SCFAs can not only transmit signals to immune cells through G-protein-coupled receptor 43 (GPR43) to suppress inflammation. It also acts on L-enteroendocrine cells in the small intestine terminal and colon, regulates the production of the hormone glucagon-like peptide 1 (GLP-1), increases insulin secretion, and SCFAs can promote the expression of some specific genes in the intestine, such as tumor suppressor genes, by inhibiting the activity of histone deacetylase (HDAC).Butyrate in SCFAs is a vital energy source for intestinal epithelial cells, and has an essential impact on the development of intestinal epithelial cells, which can reduce the occurrence of inflammation, reduce oxidative stress, and improve intestinal barrier function; propionate plays a positive role in regulating blood pressure. In addition, intestinal microorganisms alter the hepato-intestinal circulation of bile acids in the human body by breaking down the conjugates of bile acids and glycine, thereby regulating fat and glucose metabolism. While intestinal microbial metabolites have beneficial effects on human health, they also have some adverse effects on health, such as lipopolysaccharides (LPS), an endotoxin produced by Gram-negative bacteria in the intestine, which can cause inflammatory response, leading to obesity and insulin resistance.

3.1 Intestinal Immunity

Professor M. Nepelska and his team applied metagenomes to explore the relationship between intestinal microorganisms and intestinal immunity. Founding that a clone of sclerenchyma intestinalis could stimulate the expression and secretion of interleukin-8 (IL-8) by stimulating nuclear factor-KB and cohesion factor-associated protein complex 1 (AP1). In vitro experiments showed that the clone could be used. To effectively resist the harmful effects of Salmonella on intestinal tract may play a protective role in dextran sodium sulfate-induced colitis animal model. Immunosuppressive receptor PD-1 has the function of maintaining the quality of immunoglobulin A and controlling the composition of intestinal microorganisms. Researchers took the experimental mice with the deletion of PD-1 receptor as the target and found that PD-1 receptor has a protective role. The binding ability of immunoglobulin A to intestinal bacteria was weakened in the absence of experimental mice, but the decrease of probiotic bifidobacteria to Enterobacter harmful was greatly increased. Therefore, the results suggest that microbial changes may lead to overactive immune system and autoimmune diseases. Crohn's disease refers to the immune tissue of the organism. Chronic persistent inflammatory response to external stimuli.

3.2 Obesity and Diabetes

Bacterial infections may cause obesity, and certain bacteria (Enterobacter cloacae) may cause obesity. When mice fed a high-fat diet, and obese mice were injected with a human bacterium and fed a rich diet, their weight increased rapidly. This bacterium, called Enterobacter cloacae, is abundant in the intestines of morbidly obese volunteers. After 9 weeks of probiotic fiber diet, the bodyweight lost more than 30 kg, and the intestinal bacteria also decreased significantly. Fiaf-gene is a gene related to obesity, while the activity of Fiaf-gene in the intestinal tract of obese mice induced by bacteria is very low, suggesting that Enterobacter cloacae may promote by closing Fiaf-gene and inhibiting fat consumption. Fat storage, excessive fat accumulation

Diabetes mellitus, especially type 2 diabetes mellitus, is not only related to abnormal glucose metabolism. Microbial imbalance may also be an influencing factor. In the intestinal tract of diabetic model animals, changes in the number of specific bacteria can be detected. The number of two kinds of lactic acid bacteria decreased significantly. Supplementation of lactic acid bacteria preparation can alleviate the symptoms of diabetes mellitus in model animals. Certain types of lactic acid bacteria may be involved in the occurrence and development of diabetes mellitus. Therefore, the change of intestinal microorganisms is not only the result of diabetes mellitus, but also an important inducement of diabetes mellitus.

3.3 Cancer

Studies have shown that intestinal microorganisms can enter the circulatory system through damaged intestinal mucosa in patients undergoing chemotherapy and then stimulate the production of immune cells, which can help kill tumor cells. The use of a large number of antibiotics can also weaken the effect of chemotherapeutic drugs, which may also be related to intestinal microbial damage. However, some types of intestinal microorganisms do not. Marchesi JR et al. used 454 high-throughput sequencing technology to study the difference of microbial composition between the tumorous colon and normal colon mucosa. The results showed that there were significant differences in microbial colonization, number and distribution between the two groups. The study also found that patients with colon cancer were foraging for tumor cell-related bacteria in the intestine and might be able to obtain them. The generation of pathogenic bacteria is involved in the etiology of colon cancer. However, the causal relationship between cancer and intestinal microorganisms remains unclear in current studies. It is becoming increasingly clear that the symbiotic microbiota promotes overall immunity. In addition, there is now evidence that these microorganisms may confer susceptibility to certain cancers through direct effects localized in the tumor microenvironment or through systemic effects of distant microbiota, such as the intestine and skin. The latter is particularly related to the ability of the intestinal microbiota to modulate toxic responses to traditional chemotherapies and immunotherapies, and may affect patient outcomes. A comprehensive understanding of the bacteria within a specific tumor type and an in-depth understanding of the specific enzymes and cellular activities of each bacteria are just beginning, but we look forward to the possibility of developing new anticancer agents in the future. For the microbiota in immunotherapy, antibiotics combined with immunotherapy should be considered in the future to regulate the composition and function of microbiota to improve immunotherapy and develop microbiota as a prognostic marker. Efforts are currently being made to develop next-generation biotherapies involving single or multiple bacterial consortia with strong scientific basis. Many biotechnology companies, like branches of large pharmaceutical companies, are working in this area.

4. Advances in the Study of Intestinal Microbial Metagenome in Major Developed Countries

Metagenomics is a challenge for microbiologists to study the relationship between intestinal diseases and/or intestinal health associated with the metabolic transformation of specific intestinal microbial communities and their products. Intestinal microbial disorders are closely related to the intestinal, liver, pancreas or even brain-specific diseases. Direct inducement, but the human intestine contains a large number of microorganisms, it is impossible to isolate each microorganism to study their role. By studying the genetic structure of human and its microorganisms - metagenomes, we can provide a large amount of incalculable genetic information Relman Laboratory and the American Institute for Genom (The Institute for Genom). IC Research (TIGR) members carried out the first comprehensive study of human intestinal metagenomes in 2005 to improve our understanding of the diversity of gastrointestinal microbial communities. 13335 16S rRNA sequences were generated from the analysis of three healthy adult colon mucosa biopsy samples and fecal samples from proximal to distal end, and 395 species of the habitat were identified. The largest 16S rRNA sequence database (11831 bacterial sequence, 1524 archaeological sequence) was formed by the identification of bacteria and one archaeological fungus. Cloning and sequencing of 16S rDNA products amplified by PCR revealed that the majority of 395 known bacteria were Bacteroidetes (48%) and Firmicutes (51%) and the rest were Proteobacteria, Verrucomicrobia, and Fusba. In 2006, Gill et al.constructed two metagenome libraries from stool samples of a 28-year-old healthy woman and a 37-year-old healthy man. Through large-scale shotgun sequencing, 33,753,08 BP sequence information was obtained and 5,164 open-reading samples were included. Frame functional analysis revealed that the intestinal microbial genome was rich in genes involved in carbohydrate, amino acid, methane, vitamin and isoprene metabolism, which was a major breakthrough in the study of intestinal microbial function.

In 2010, the EU-funded Human Intestinal Metagenome Project conducted the largest genetic study of intestinal bacteria to date. Qin et al.collected 124 samples of intestinal microorganisms from Europeans (including healthy, overweight or obese, people with inflammatory bowel disease) and imported total fecal DNA based on short reading frame Illumina GA technology. A total of 3.3 million non-redundant human intestinal metagenome reference genes were obtained by sequencing, which is 150 times the total number of human genes. This gene information not only contains most of the known genes of human intestinal microorganisms, but also contains more genes of unknown microorganisms. Each human individual contains about 536 000 common genes. At the same time, almost all the genes (99.1%) originated from bacteria, which indicated that there were 1000 to 1150 common bacteria in this population and there were at least 160 common bacteria in each person, followed by archaea, which accounted for 0.8%, and other fungi and diseases. Toxicity, accounting for 0.1%. These results provide a new basis for studying the relationship between microbial genes and human phenotypes or living environment factors and diseases such as diet from birth to old age. However, these studies do not involve the actual activities of microbial communities or the interaction between microbial communities and their hosts under physiological conditions, so it is impossible to use these bases at present. The relationship between microorganisms and their metabolic processes and human physiological conditions and disease occurrence is directly deduced from the data.

5. Conclusion and Prospect

Intestinal microecology is a hot spot in the research of digestive tract diseases at present. The maturity of intestinal metagenomics makes the research of intestinal microorganisms clearer and deeper. Maintaining the stability of intestinal microorganisms and rebuilding imbalanced intestinal microorganisms are of great significance to human health. Intestinal microorganisms play an important role in human body, and their composition and metabolites are closely related to human health. It not only affects food digestion and nutrient absorption, but also has physiological functions such as regulating host metabolism and immune system. It has been clearly recognized that the human body's own metabolism is not only controlled by its own genes, but also regulated by intestinal microorganisms. People are increasingly demanding a healthy diet. In the relationship between diet and health, the role of intestinal microorganisms as a bridge has also been paid more and more attention. With the development of the Human Genome Project, a large amount of data and information on intestinal microorganisms have been obtained in recent years, and biologists have paid more and more attention to the study of intestinal microorganisms, a “microbial organ”. However, there are still some shortcomings in the processing methods of massive data generated by omics technology. There is still room for further development on how to mine meaningful data from these huge data to explain and solve problems. In the future, the most effective means for the interpretation and information mining of this complex system of intestinal microorganisms should be to establish a complete knowledge system by using biological big data, combining clinical experience and relevant literature, and build corresponding knowledge bases (such as intestinal microbial metabolites database, gene regulation database, etc.) on this basis. Intestinal microorganisms are closely related to human health, and the imbalance of intestinal microorganisms can lead to the occurrence of a variety of diseases. There are many factors that affect the structure of intestinal microorganisms, but the most important factor is diet. Diets not digested by the gastrointestinal tract enter the hindgut (cecum) for microbial fermentation, which will produce substances beneficial to human health (such as short-chain fatty acids) or harmful (such as lipopolysaccharide, phenol, etc.); at the same time, it will also change the structure of intestinal microorganisms, further affecting human health. However, there are few studies on the effects of diet on intestinal flora and its metabolites, and few studies have been conducted on the relationship between these effects and human health. It is believed that in the near future, there will be new breakthroughs in the mechanism of how diet regulates the structure of intestinal microflora and its metabolites; in health regulation or disease treatment, changing the structure of intestinal microflora and its metabolites through diet may be a new idea for researchers. In order to further elucidate the mechanism and principle of the relationship between host and microorganism in disease state, metagenomies can be widely used to monitor the composition of intestinal microorganisms and their metabolites in different diseases, to find out the specific changes of intestinal microorganisms in specific diseases, so as to provide biomarkers for disease diagnosis and new evidence for the pathogenesis of diseases. Diagnostic methods, such as blood tests and endoscopy, can cause certain damage to the human body. Dynamic monitoring of microbial composition in fecal samples may become a non-invasive method for clinical assessment of human health. In addition, in human nutrition research, intestinal microbial metagenomes is also expected to coincide. To design and develop new health food, food and medicine to promote human health by interfering with the interaction between host and microorganism.

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