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OPINION ARTICLE

Human Microbiomics

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Abstract The sequencing of the human genome has driven the study of human biology in a significant way and enabled the genome-wide study to elucidate the molecular basis of complex human diseases. Recently, the role of microbiota on human physiology and health has received much attention. The influence of gut microbiome (the collective genomes of the gut microbiota) in obesity has been demonstrated, which may pave the way for new prophylactic and therapeutic strategies such as bacteriotherapy. The significance and recent understandings in the area of "human microbiomics" are discussed here.

Keywords Human microbiome · Metagenomics · Microbial diversity · 16S rRNA · Obesity · Bacteriotherapy

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Introduction

Human microbiomics is an emerging discipline, which deals with the microorganisms that live in and on humans and their roles in human physiology and health. A human infant acquires the microbiota from the environment. Colonization, succession and diversification occur in various microbial habitats in the body, ranging over the first weeks, months or years of life [1]. The genomes of the microbiota, collectively defined as the "human microbiome", provide traits that humans did not evolve on their own. Therefore, human beings are considered as ''superorganisms'' with the trillions of associated microorganisms [2].

The human microbiota is estimated to outnumber human cells by at least one order of magnitude and composed of more than 1000 different species level phylotypes. The aggregate size of microbiome may be equivalent to the human genome, and the number of genes in the microbiome may exceed the total number of human genes by two orders of magnitude [3]. A long coevolutionary process has led to mutualistic interactions between the microbiota and the host. For instance, the plant polysaccharides commonly consumed in the diet are rich in xylan-, pectin-, and arabinose-containing carbohydrate structures. The human genome lacks most of the enzymes required for degrading these glycans. On the other hand, plant polysaccharides that are not digestible by humans are the main substrates for microbial growth in the colon. Our microbiome has significantly enriched with genes for the metabolism of glycans, amino acids, xenobiotics, methanogenesis and biosynthesis of vitamins, etc [4]. Thus, the superorganismal view of our genetic landscape should include the genes in the human genome and the affiliated microbiome.

In this direction, the International Human Microbiome Project (HMP) has been recently launched [5]. The HMP is another rational extension of the human genome project. It is an interdisciplinary effort consisting of multiple projects launched concurrently worldwide, including in the United States, Europe and Asia. Ultimately, the goal is to associate differences in microbiome with differences in metabolic function and/or disease.

The microbial diversity routinely are reported based on the sequence analysis of small subunit ribosomal RNA (16S rRNA) genes. The 16S rRNA genes are directly amplified from the human metagenomic DNA using broad-range PCR primers, and used to make libraries of clones. Each clone in a library represents a 16S rRNA gene from a prokaryotic organism. The clones are then differentiated through fingerprinting methods such as denaturant gradient gel electrophoresis (DGGE) or amplified ribosomal DNA restriction analysis (ARDRA) and the non-redundant clones are sequenced. In the recent years, either randomly selected clones or all the clones in a library are being sequenced [6–10]. After sequencing, 16S rRNA genes are clustered into groups and a threshold of sequence similarity (>97%) is established to distinguish species level phylotypes. The 16S rRNA gene sequence based surveys of bacterial communities that reside on or in the human body, including on the skin and in the mouth, oesophagus, stomach, colon and vagina have been recently reported [6–10]. Interestingly, among the 70 divisions of Bacteria and 13 divisions of Archaea, the human gut microbiota is dominated by just two bacterial divisions, the Bacteroidetes and the Firmicutes, which made up to 90% of the identified phylotypes [4].

The 16S rRNA based bacterial diversity analysis reveals the composition of the microbial community. However, establishing linkages between microbial diversity and the human physiology depends on understanding their genome content. For instance, many common human pathogens are closely related to non-pathogenic strains; examples are found in the genera *Staphylococcus*, *Streptococcus*, *Neisseria*, *Enterococcus*, and in the family Enterobacteriaceae including *Escherichia coli*. Genomes of the pathogenic *E. coli* strain O157:H7 and the commensal strain *E. coli* K12 are more different than that of any two mammals [11]. Therefore, it is essential to consider the role of genes coded in the microbiome rather than just as individual species.

One of the major goals of the HMP is to determine whether there is an identifiable 'core microbiome' of shared organisms, genes or functional capabilities found in a given body habitat of all or the vast majority of humans. Comparison of individuals from the same family revealed that the human gut microbiome is shared among family members, but that each person's gut microbial community varies in the specific bacterial lineages present. It has been shown that even identical twins had significant differences at the species level phylotypes [12]. Similarly, the Chinese family shared a similar division-level phylogenetic landscape with the American individuals. However, the Chinese and American microbiomes were clearly different at the species level of composition [2]. However, a core gut microbiome exists at the level of shared genes rather than at the organismal level. Thus, a molecular link between host metabolism and gut microbiota genes, rather than just species identity should be established.

Comparative analysis of existing genome sequences of human gut bacteria revealed that each genome contains a large repertoire of genes involved in acquisition and metabolism of polysaccharides. These genes are organized as polysaccharide utilization loci (PUL) that encode functions necessary to detect, bind, degrade and import carbohydrate species encountered in the gut habitat either from the diet or from host glycans associated with mucus and the surfaces of epithelial cells [13]. Sequencing of more reference genomes would provide more insight into the evolution of human microbiota and their diverse metabolic functions. The HMP is aimed to sequence 1000 reference microbial genomes, which will serve as the resource for investigators interested in exploring the human microbiome [5]. Recently, single cell genomics approach has been developed to obtain reference-genome sequences of uncultured organisms [14]. In this approach, single microbial cells are isolated by flow cytometry; the whole-genome of the single cell is amplified by multiple displacement amplification (MDA) and sequenced by shotgun approach. This approach has been used to obtain a partial genome assembly of a member of the candidate phylum TM7 from human mouth, which is a group of microorganisms with no culturable representatives [15].

A better understanding of the microbiota's contribution to human health requires characterization of microbial molecular signals, which drive the interactions among the members and with the host. For example, identification of commensal organisms, which can inhibit the pathogens, known as pathogen interference, may be useful for therapeutic applications. Bacteriotherapy, use of harmless bacteria to displace pathogenic organisms, has been proposed as an alternative and promising way of combating against infections caused by multidrug resistant organisms [16]. Corr et al. [17] have demonstrated that a probiotic bacterium, *Lactobacillus salivarius* UCC118, protects against infection by *Listeria monocytogenes*, a food borne pathogen that can be fatal in pregnant women and immunocompromised individuals. Roos et al. [18] have showed commensal α-haemolytic streptococci can be used to replace the normal nasopharyngeal flora in children with recurrent otitis media.

Similarly, nonpathogenic *E. coli* have successfully been used to treat ulcerative colitis [19].

Identification of mechanisms of microbe-host signaling is also essential to understand the human biology. For example, the regeneration and proliferative activity of the human intestinal epithelium are modulated by the gut microbiota. Recently, a functional screening of metagenomic libraries revealed the candidate loci involved in modulation of eukaryotic cell growth [20]. The modulating clones were represented from the four phyla namely, Bacteroidetes, Firmicutes, Proteobacteria and Actinobacteria. More than 75% of these clones could not be affiliated to a cultured species. Most of the inserts from Bacteroidetes were stimulatory and inserts from Proteobacteria including the genus *Escherichia* were found to be inhibitory. The candidate loci identified include ABC transporter systems, a RecD gene homologue, a glutamate synthase subunit, a V-type ATPase subunit, etc [20].

The influence of human microbiota in obesity has been postulated [21]. Obesity in humans is a major risk factor for a number of chronic diseases, including diabetes, cardiovascular diseases and cancer. The excess caloric intake compared with expenditure results in obesity. Recent findings have suggested that individuals predisposed to obesity are associated with gut microbiota that promote more efficient extraction and/or storage of energy from a given diet, compared with these communities in lean individuals [21, 22]. The involvement of microbiome in the development of obesity has been experimentally proved in mouse model. As in humans, the Bacteroidetes and the Firmicutes make more than 90% of the mouse gut microbiota. The relative abundance of the Bacteroidetes in obese (ob/ob) mice was lower by 50%, whereas the Firmicutes were higher by a corresponding degree. Moreover, the increase in Bacteroidetes was significantly correlated to weight loss but not to total caloric intake. Therefore, it has been proposed that the microbiota of obese individuals may be more efficient at extracting energy from a given diet than the microbiota of lean individuals [21].

Turnbaugh et al. [22] have examined the association of microbial community gene content with obesity by random shotgun sequencing of the distal gut microbiomes of ob/ob, ob/+, and +/+ littermates. The results revealed that the ob/ob microbiome is enriched with several glycoside hydrolase families capable of degrading dietary polysaccharides. Similarly, proteins that import the products of these glycoside hydrolases (ABC transporters), metabolize them, and generate the major end products of fermentation, butyrate and acetate were found to be significantly enriched in the ob/ob microbiome. The predicted increased capacity for dietary energy harvest by the ob/ob microbiome was subsequently validated by microbiota transplantation [22]. The microbiota transplantation experiments were performed to test whether the ob/ob microbiota has an increased capacity to harvest energy from the diet and to determine whether increased adiposity is a transmissible trait. Adult germ-free C57BL/6J mice were colonized with a microbiota harvested from the caecum of obese (ob/ob) or lean (+/+) donors. Colonization of germ-free mice with an obese (ob/ob) microbiota resulted in a significantly greater increase in total body fat than colonization with a lean (+/+) microbiota. Since the gut microbiome of obese humans is comparable to that of obese mice, it could be considered as a biomarker and a new therapeutic target for people suffering from the obesity.

To conclude, the significance of the human gut microbiome is now well established. The influence of the gut microbiome in the risk of common age-related diseases such as cancer and cardiovascular diseases through the classical risk factors such as obesity has been suggested. However, it is not yet known which of the many hundreds of microbial species and their genes are of key importance in the human health, and little is known on the molecular host-microbiome interactions. The major objective of the HMP is to explore the role of microbiome in human diseases. Before the effect of the microbiota on disease predisposition and pathogenesis is evaluated, the 'normal' states are expected to be defined in the forthcoming years. Overall, the HMP is expected to provide a broader view of human biology and the outcome of this project may lead to the development of new prophylactic and therapeutic strategies against infectious as well as non-infectious diseases.

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