

Кишечный микробиом — основной фактор поддержания здоровья и развития болезней человека

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Intestinal microbiome — a leading factor in human health and diseases

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Цель обзора. Отразить современные представления о роли микробиома в поддержании здоровья человека и развитии различных заболеваний.

Краткое содержание. Благодаря современным достижениям молекулярной и клеточной биологии существенно расширились наши представления о патогенезе многих заболеваний человека и разработаны новые подходы к их диагностике, лечению и профилактике. На основе результатов современных молекулярных, генетических, эпигенетических, микробиологических и биохимических исследований стало возможным, с одной стороны, изучать точечные мутации и варианты полиморфизма отдельных нуклеотидов в рамках полногеномного анализа, с другой — с помощью высокоточного анализа и других методик проводить одновременное исследование тысяч генов (анализ ДНК) или их производных (РНК и белки) с созданием индивидуального профиля самих генов или их экспрессии («генетическая подпись»), а также осуществлять анализ индивидуального микробиома пациента с оценкой его патогенного потенциала. Подобные исследования позволяют все чаще проводить оценку индивидуальной предрасположенности к заболеваниям, прогноза болезни и эффективности выбранной стратегии лечения («персонализированная медицина»).

Заключение. Исследование микробиома человека, а также полногеномные исследования способствуют

Aim: to present the modern concept of physiological and pathophysiological impact of microbiome.

Summary: Major advances in molecular and cell biology significantly improved our understanding of disease pathogenesis as well as in novel strategies for the diagnosis, therapy and prevention of human diseases. Based on modern molecular, genetic, epigenetic microbiologic and biochemical studies it is, on the one hand, possible to identify disease-related point mutations and single nucleotide polymorphisms within genome-wide association analyses (GWAS). On the other hand, high throughput array and other technologies made it possible to simultaneously analyze thousands of genes (DNA) or gene products (RNA and proteins), resulting in an individual gene or gene expression profile ('signature') or to characterize the individual microbiome and its pathogenetic potential. Such data increasingly allow to define the individual disease predisposition and to predict disease prognosis as well as the efficacy of therapeutic strategies in the individual patient ('personalized medicine').

Conclusion: Studying of human microbiome along with GWAS contributed greatly to the recent advances in the diagnosis, treatment and prevention of human diseases.

Key words: microbiota, fecal transplantation, inflammatory bowel disease, obesity, atherosclerosis, neurodegenerative diseases

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Поступила: 25.04.2017 / Received: 25.04.2017

достижению новых успехов в диагностике, лечении и профилактике заболеваний человека.

Ключевые слова: микробиота, пересадка кала, воспалительные заболевания кишечника, ожирение, атеросклероз, нейродегенеративные заболевания.

Для цитирования: Блюм Г.Э. Кишечный микробиом — основной фактор поддержания здоровья и развития болезней человека. Рос журн гастроэнтерол гепатол колопроктол 2017; 27(5):4-10
DOI: 10.22416/1382-4376-2017-27-5-4-10

For citation: Hubert E. Blum. Intestinal microbiome — a leading factor in human health and diseases. Ross z gastroenterol gepatol koloproktol 2017; 27(5):4-10
DOI: 10.22416/1382-4376-2017-27-5-4-10

The basic aspects of molecular and cell biology are not only integral part of biomedical research but are also translated into patient care. Several global consortia have been launched and in part completed during the last decades. All of them continuously transform basic biomedical research and translate into medical applications and, after evaluation in randomized clinical trials, enter clinical practice with a tremendous potential to advance the diagnosis, treatment and prevention of human diseases.

More than 15 years ago, the international human genome organization (HUGO) project established the complete sequence of the ca. 3 billion base pairs that make up the human genome [1, 2]. In order to utilize these data from the HUGO project for research as well as for clinical applications and to define the functions of newly identified genes, collectively termed 'functional genomics', strategies were developed to globally analyze genomic DNA sequences as well as their cell-, tissue- or organ-specific expression profile. Using chips, so-called 'microarrays', thousands or ten thousands of single-stranded DNA species, reverse transcribed RNA (cDNA) or oligonucleotides of known sequence can provide a global gene (genomics), gene expression (transcriptomics, proteomics) or metabolite (metabolomics) profile ('signature') that is characteristic for the disease of individual patients, including its natural course, prognosis and response to therapy.

In 2005 the international haplotype map (HapMap) project was initiated to identify via genome-wide association studies (GWAS) in ethnically different populations, single nucleotide polymorphisms (SNPs) and their association with specific human diseases and individual phenotypic characteristics [3, 4]. Through GWAS an increasing number of gene loci have been identified that are associated with individual (future) phenotypic traits, such as hair or eye color, height, body mass index and others as well as with the predisposition

to specific disease [3, 4]. Further, genetic variants are associated with the individuals' response to drug treatment, e.g., to lithium [5]. Overall, GWAS allow an increasingly better understanding of disease pathogenesis and a more accurate assessment of the individual risk to develop a specific disease. Clinically, this may eventually translate into clinical advances in disease prevention, early diagnosis and therapy. It should be cautioned, however, that the contribution of a defined SNP to the risk assessment for a given disease must be weighed by established clinical parameters and needs to be carefully evaluated before clinical utilization.

The Human Microbiome Project

The human microbiome project (HMP) was established as another global consortium [6–10]. The HMP and the 'Metagenomics of the Human Intestinal Tract (Meta-HiT) Consortium Europe' aim at the sequencing of all microbes (eukaryotes, archaea, bacteria, viruses) that inhabit specific body sites, such as the mouth, throat and airways, stomach and intestine, the urogenital system and the skin, respectively. Recent data demonstrate that specific compositions of the microbial community are associated with health and disease and suggest that the detailed characterization, function and variation of the microbial communities will reveal important commensal host-microbe as well as microbe-microbe interactions with diagnostic, therapeutic and preventive implications [11, 12].

While the HMP has meanwhile developed into a major field of biomedical research, the intestinal microbial community in particular has turned out to play a major role in human health as well as in and disease pathogenesis as will be discussed in more detail below [13].

The intestinal microbial community

In recent years the intestinal microbial community has been studied in great detail. It represents a microbial ecosystem consisting of

trillion microbial cells with an aggregate 9.9 million microbial genes across the fecal microbiome [14]. While until recently, the environment *in utero* has been considered sterile, DNA-based analyses identified bacterial species in maternal placenta, amniotic fluid and meconium. The colonization of the human gut begins at birth with a rapid expansion of bacterial diversity and is characterized by a successively changing composition that eventually becomes relatively stable in adulthood [15]. While the specific microbial species and subspecies and their proportions vary greatly from person to person the individual microbiome is unique and becomes more diverse in the elderly.

Important factors for the composition of the intestinal microbial community are endogenous and exogenous factors [16, 17]. Examples are the mode of delivery of the neonates, diet (dietary supplements, breast-feeding, formula-feeding), xenobiotics, including antibiotics and other drugs [18–21]. Further, infections and exposure to environmental microbial agents are established risk factors for childhood diseases, such as obesity and allergy [22, 23]. Recent evidence further suggests that human genetic variation also influences the abundance of specific members of the intestinal microbial community [24].

Taken together, the emerging data suggest that the detailed characterization of the human intestinal microbiome composition, function and variation across different body sites will reveal important commensal host-microbe as well as microbe-microbe interactions that may play a role in human health and disease.

In view of the numerous and diverse physiological functions of the intestinal microbiota in human health (Table 1) it is not surprising that it is also involved in gastrointestinal as well as non-gastrointestinal diseases, such as obesity/metabolic syndrome, and atherosclerosis/cardiovascular as well as neurologic/psychiatric and neurodegenerative diseases, making it one of the most dynamic current topics in biomedical research (Table 2). In the following, a few examples will be discussed in more detail.

Inflammatory bowel diseases and colon cancer

The inflammatory bowel diseases (IBD) in humans include ulcerative colitis (UC) and Crohn disease (CD). These are characterized by inflammation limited to the mucosal layer of the colon in UC and the transmural involvement of the gastrointestinal tract, including extraintestinal sites in CD. While the pathogenesis of IBD is not fully understood [25], it is clear that its pathology depends among others on the intestinal microbial community [26, 27]. Further, a case-control study identified 'IBD-specific' alterations of the intestinal microbiota that

may serve as biomarkers for the prediction of disease predisposition, activity/severity and responsiveness to therapy [28, 29].

Host genes with effects on the composition of the intestinal microbiota are the IgA locus and the HLA genes as well as the defensin genes, the NOD2 gene, the resistin-like molecule beta gene, the apolipoprotein I gene, the MEFV gene and the myeloid differentiation primary response protein 88 gene. The three components – environment, host genetics and the microbial community – interact to maintain homeostasis in the intestine [7]. The disruption of the stability of this interaction may be a trigger for disease development. Two recent publications shed a new light on the pathogenesis of IBD through the change of the intestinal microbial composition involving two different pathways: helminth invasion [30] and lipocalin-2 expression [31], respectively.

Helminth invasion, microbial community and IBD. Epidemiologic studies demonstrated a major increase of the incidence of IBD in the developed world, suggesting a change in the environment, including an alteration of the intestinal microbiome [32] and a decreased exposure to intestinal parasites, such as helminths [33]. In mice deficient for the CD susceptibility gene Nod2 (Nod2^{-/-} knockout) [34] it could be demonstrated that small intestinal abnormalities develop at sustained colonization by the inflammatory bacterium *Bacteroides vulgatus*, an ubiquitous member of the intestinal microbial community [35]. Chronic infection of Nod2^{-/-} mice with the parasitic worm *Trichuris muris*, however, inhibited colonization with inflammatory *Bacteroides* species and promoted the establishment of a protective microbial environment enriched in *Clostridiales* [30]. Further, the authors demonstrated that individuals from helminth-endemic regions harbour a similar protective microbial community and deworming treatment reduced *Clostridiales* and increased *Bacteroidales*, resulting in an increased IBD incidence. These data support the 'hygiene hypothesis' whereby certain individuals are genetically susceptible to the consequences of a changing intestinal microbial community that favors IBD development.

Lipocalin-2 protection from IBD and colon cancer. Lipocalin-2 (Lcn2) is an antimicrobial peptide with high mucosal and fecal concentrations in patients with IBD. It is produced by various cell types, including epithelial cells, and acts as an antimicrobial defense mediator by binding to a subset of bacterial siderophores, thereby preventing bacterial iron acquisition and growth of siderophore-dependent strains. While it has been implicated in several biologic processes, such as acute phase response, erythropoiesis and iron metabolism, its functional role in contributing to IBD development remained unclear.

To decipher the role of Lcn2 in colon inflammation, mice double deficient in Lcn2 and IL-10 (Lcn2^{-/-}/IL10^{-/-} double knockout) were generated and compared to single knockout and wild-type animals. The experimental data indicate that Lcn2 expression protects from early onset colitis and from the spontaneous emergence of right-sided colonic tumors that result from IL-10 deficiency. Inflammation is driven by IL-6 which controls tumorigenesis as well. The Lcn2^{-/-}/IL10^{-/-} double knockout mice showed major alterations of their intestinal microbial community, especially with respect to the facultative pathogenic *Alistipes* spp. These contribute to inflammation and tumorigenesis as shown by the transmissibility of the phenotype and by protective effect of antibiotic therapy. Taken together, the authors demonstrate that Lcn2 protects against intestinal inflammation and tumorigenesis in the face of an altered intestinal microbial composition [31].

Recently, it was discovered that the probiotic *Lactobacillus casei* strain ATCC334 produces ferrichrome that inhibits colon cancer progression by apoptosis mediated through the c-Jun N-terminal kinase pathway [36], possibly representing a novel tumor-suppressing strategy.

Obesity

Obesity [37], insulin resistance [38] and kwashiorkor [39, 40] are examples for which a correlation between intestinal microbial dysbiosis and the clinical state has been demonstrated. Further, in genetically susceptible hosts, the transplantation of fecal microbiota from healthy donors to patients resulted in clinical improvement [41]. The underlying concept is a 'common ground hypothesis' that involves a leaky mucosa caused by various endogenous or exogenous factors, the expansion of opportunistic microbes (dysbiotic pathobionts) with the generation of pathogenic microbial gene products which can be transferred to genetically susceptible individuals [13].

Atherosclerosis and thrombosis risk

Recent studies suggest that intestinal microbes are involved in atherosclerosis development. In this context, foods rich in choline, phosphatidylcholine and carnitine such as meat, egg yolk and high-fat dairy products, serve as precursors of trimethylamine (TMA) and TMA N-oxide (TMAO) that accelerates atherosclerosis [42]. Elevated TMAO blood levels are associated with an increased risk for atherosclerotic heart disease and major adverse cardiac event incidence. Further, TMAO enhances platelet hyperreactivity and thrombotic events in animal models, employing dietary choline or TMAO, germ-free mice and microbial transplantation, collectively confirming the key role of intestinal microbiota [43]. Taken together, these

results reveal a previously unknown link between specific dietary nutrients, intestinal microbes and thrombosis risk.

The intestinal TMAO formation is a two-step process involving the generation of TMA by intestinal microbes after food ingestion and the hepatic conversion of TMA to TMAO by host flavin monooxygenases. Wang et al. [44] demonstrated that 3,3-dimethyl-1-butanol (DMB), choline structural analog, blocks the intestinal TMA formation inhibiting microbial TMA lyase that results in reduced TMAO levels. Thus, 'drugging the microbiome' with DMB may be a novel approach for the prevention/treatment of atherosclerosis.

Neurodevelopmental, psychiatric and neurodegenerative diseases

Studies investigating the intestinal microbial-brain communication (gut-brain axis) demonstrate a critical role of the intestinal microbial community in modulating the maturation and function of tissue-resident immune cells in the central nervous system (CNS) as well as the activation of peripheral immune cells involved in neuroinflammation, brain injury, autoimmunity and neurogenesis [45]. Germ-free mice raised under sterile conditions or mice depleted of their intestinal microbiota by antibiotics show major alterations in behaviours or neuropathologies that characterize neurodevelopmental, psychiatric and neurodegenerative disorders [46] like autism spectrum disorders, depression and Alzheimer's or Parkinson's disease (Table 2).

An impressive example for a pathogenic role of the intestinal microbial community is Parkinson's disease (PD). In patients with PD, plaques in brain cells as well as in the intestine containing neurotoxic protein alpha-synuclein (AS) are a hallmark of the disease. For example, in PD patients gastric motility is frequently impaired [47] and intestinal AS levels are elevated [48].

In a mouse model overexpressing AS the animals indeed develop neurologic deficits resembling those of PD patients. Recently, three lines of evidence demonstrated a central role of the intestinal microbial community in the pathogenesis of PD: (1) in the PD mouse model germ-free animals developed fewer plaques and almost no neurological deficits as compared to conventionally colonized controls, (2) treatment of PD mice with antibiotics resulted in an improvement of the neurological deficits and (3) fecal transplantation from patients with PD to germ-free mice resulted in neurological deficits resembling PD [49].

The underlying concept is the central contribution of the microbial community to a defect of the microglia *via* short-chain fatty acids (SCFAs) that represent bacterial fermentation products [50]. While germ-free mice showed reduced microglia, SCFAs modulated microglia and enhanced PD

Table 1. Examples of the intestinal microbial community functions in human health.

Табл. 1. Основные функции кишечного микробного сообщества у здорового человека

	References
Host Physiology	
Adaptive immunity	[51]
Autoimmunity	[52]
Innate immunity	[53]
Cell proliferation	[54]
Bone density	[20]
Vascularization	[55]
Neurological signalling	[56]
Biosynthesis	
Neurotransmitters	
Steroid hormones	
Vitamins	
Metabolism	
Dietary components	
Bile salts	
Drugs	
Xenobiotics	

pathophysiology [49]. The identification of the disease-causing bacteria and the mechanism leading to the deposition of the neurotoxic AS plaques await further clarification.

Basic biomedical research has made major advances in recent years and provides increasing amounts of individual diagnostic, preventive as well as therapeutic options for patients with inherited or acquired, malignant or non-malignant diseases. Apart from an increasing number of host genetic susceptibility loci and environmental factors, the individual microbial community is central for the barrier between microbes and hosts. In particular, the intestinal microbial community is involved in a large number of normal biological functions in health (**Table 1**) as well as in numerous common, gastrointestinal and non-gastrointestinal diseases, such as obesity/metabolic syndrome,

Table 2. Examples of diseases associated to intestinal microbial community.

Табл. 2. Основные заболевания, связанные с состоянием кишечного микробного сообщества (примеры)

	References
Allergies/ Allergy protection	[23, 52, 57–59]
Atherosclerosis/ Thrombosis/ Cardiovascular Disease	[42–44, 60–63]
Cancer	[64, 65]
Diabetes mellitus	[38, 66]
Immune-Mediated Inflammatory Diseases	
Inflammatory bowel diseases	[26, 27, 30, 31, 67, 68]
Multiple sclerosis	[69, 70]
Rheumatoid arthritis	[71]
Psoriasis	[72]
Kwashiorkor	[39, 40]
Liver Diseases	[73, 74]
Metabolic Syndrome/ Obesity	[37, 75–78]
Neurodevelopmental, psychiatric and neurodegenerative diseases	
Autism	[45, 79]
Depression	[45, 80]
Alzheimer's Disease, Parkinson's Disease	[45, 49, 50, 81, 82]

atherosclerosis/thrombosis or neurodevelopmental, psychiatric and neurodegenerative diseases (**Table 2**). Dietary interventions targeting intestinal microbiota, such as caloric restricted diets rich in fiber and vegetables as well as fecal microbial transplantation are examples of health benefits in humans. In recent years, the intestinal microbiome thus has become one of the most dynamic areas of biomedical research that holds an enormous potential for interventions regarding human health and diseases.

Conflict of interests. The author declares no conflict of interest.

Financial disclosure. The author has no financing to disclose.

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