Trends in
Molecular Medicine

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VSI: Bottlenecks and Breakthroughs in Molecular Medicine

Forum

Breakthroughs and Bottlenecks in Microbiome Research

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Over the past 15 years, the research community has witnessed unprecedented progress in microbiome research. We review this increasing knowledge and first attempts of its clinical application, and also limitations and challenges faced by the research community, in mechanistically understanding host–microbiome interactions and integrating these insights into clinical practice.

Humans, akin to all mammals, carry diverse communities of commensal microorganisms (hence termed the microbiome). Over the past 15 years, the research community has witnessed unprecedented breakthroughs in microbiome research, which, however, are associated with notable limitations, bottlenecks, and challenges, which need to be tackled by this young research community in its next chapter of exciting basic and biomedical research.

Metagenomic Sequencing and Analysis

High-throughput sequencing endowed an unmatched ample view on complex commensal communities. Initiatives such as the Human Microbiome Project (HMP), MetaHIT, American Gut, and Flemish Gut have uncovered links between microbial taxa and human health outcomes [1]. Notwithstanding the opportunities next-generation sequencing provides (NGS), it also exposes the limits of sequencing-based community profiling. A compelling question is whether quantitative and transcriptional microbiome profiling is more revealing than the usual study of microbial composition alone? Are more microbiome-based readouts needed? Indeed, pioneering studies in human inflammatory bowel disease (IBD) provide evidence that in addition to proportional compositional alterations, the microbial load may be a key driver of microbiome alterations [2], and that certain microbes despite being metagenomically abundant may be in a dormant state with little to no gene expression (Figure 1) [3]. Among human diseases linked to microbes, phenotypes are often associated with only a subset of strains within microbial clades. Emerging methods allow for strain-level analysis based on high-throughput metagenomic sequencing [4]. (See Box 1.)

Metagenomic sequencing is fundamentally limited by the fact that it is unable to account for the functional activity of the community directly. To accurately model health-related outcomes associated with microbial configuration, it has become incumbent to divert the attention from the isolated study of different data types to an integrative, systemic

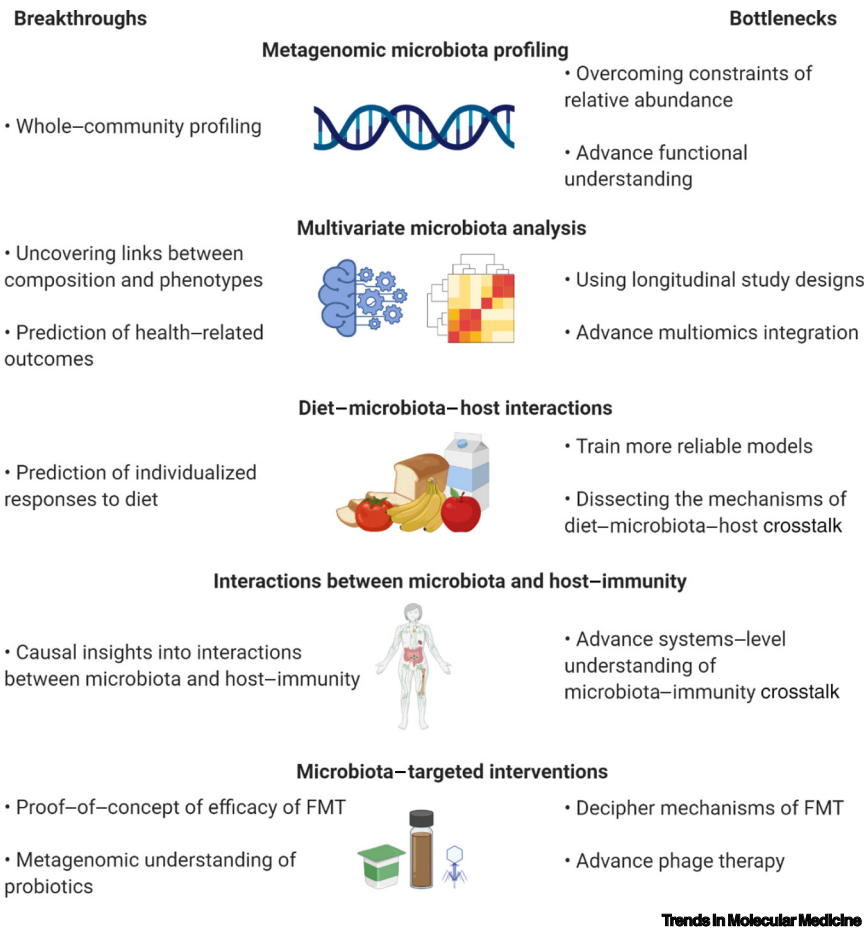

Figure 1. Breakthroughs and Bottlenecks in Microbiome Research. The figure portrays major breakthroughs (left) and bottlenecks (right) in different areas of microbiome research. Created with BioRender.com. Abbreviations: FMT, fecal microbiota transplantation.

Box 1. Major Challenges in Microbiome Research

High interindividual variability: a typical intestinal metagenomic sample includes between several hundred to several thousand different taxa with different individuals displaying a high degree of variability in these taxa abundances. Ethnic, geographic, and lifestyle differences are just some of the major sources of variation that limit the generalization of microbiome-related findings across populations. Ideal statistical analysis requires a relatively large sample size in order to overcome such variability and generate meaningful conclusions.

Reliance on relative abundances: a profound limitation of culture-independent methods such as NGS is that they do not reliably provide information regarding absolute microbial abundances or functionality. Culture-based methods provide information on absolute abundances but are limited in their capacity to characterize complex microbiomes such as that of the mammalian intestine owing to technical limitations (i.e., number of required cultures and nonculturable microorganisms).

Reliance on stool samples: collecting stools is relatively convenient and noninvasive; however, the stool microbiome is not identical in composition or function to the more physiologically relevant microbiome of the gastrointestinal tract.

Focus on bacteria: often overlooked in microbiome studies, commensal microorganisms of other kingdoms are increasingly acknowledged as relevant in health and disease.

Focus on the gut: microbiome analysis of specimens with a low microbial load such as skin, urinary/reproductive tract, saliva, or tumors are technically challenging since they are prone to artifacts as a result of contamination during specimen collection and processing. Such samples are less frequently included in microbiome studies.

approach that incorporates a wealth of covariables from different data domains. In consequence, additional multiomics data are required to fully describe a microbiome, such as community RNA abundance (metatranscriptomics), proteins (metaproteomics), and metabolites (metabolomics), preferably in an integrated manner [5]. Integrated analysis of multiple disparate data types (i.e., NGS and mass spectrometry), of which each is comprised of relative abundances with a different underlying distribution, constitutes a formidable statistical challenge. Caution is warranted regarding the expectation of a direct translation of such complex and delicate scientific results into clinical practice.

Diet–Microbiota–Host Interactions

Diet is of paramount importance among the factors shaping the gut microbiome configuration. Dietary habits represent a crucial source of interpersonal gut microbiome variation. In turn, the gut microbiome plays the role of a 'signaling hub' generating a wealth of diet-derived signals to the host. Over the last years, it has become increasingly salient that there may be no 'one-size-fits-all' dietary recommendations to achieve a health benefit. Microbiome-based machine learning predictions of individual-specific responses to certain foods were suggested to be feasible by several studies [6,7]. Machine learning and other artificial intelligence approaches require the training of models on data sets of microbial and clinical features to learn the effect of specific foods on a given health-related outcome. In principle, any kind of person-specific quantifiable data can be utilized to this end without the necessity of understanding the complicated mechanistic links underlying this prediction. The critical challenge to bring the concept of personalized diet to real-world applications is to gather adequately large cohorts with sufficiently in-depth and comprehensive profiling of the microbiome and other data domains to train models of high reliability. In an equally important parallel step, mechanistic dissection of the features predicting a given physiological response could be of high value for the development of preventive or therapeutic interventions, as was demonstrated in a multiomic investigation of undernourished children suffering from enteropathy [8].

Towards Metagenomic Clinical Diagnostics

An individual's commensal microbial repertoire also holds great promise as a clinical diagnostic modality. Metagenomics sequencing of stool or blood has a powerful, noninvasive, diagnostic capacity in infections or malignancy. In the case of infections, for example, metagenomic sequencing of infected body fluids was demonstrated to both expedite pathogen detection and to have potentially greater diagnostic sensitivity compared to gold-standard culture-based methods [9]. A prompt pathogen detection can optimize antimicrobial coverage, and reduce the use of wide empiric antibiotic coverage to minimize the emergence of antibiotic resistance, a global public health threat. NGS platforms are currently not widely available in some medical centers and their implementation into clinical care will require some protocol adaptions in both sample collection and data analysis, to render them clinically interpretable and affordable.

Resurgence of Phage Therapy

Much of the microbiome research has focused on bacterial community members, mainly due to the technical challenges by which the study of commensal representatives of other kingdoms is burdened. Nevertheless, emerging evidence points towards a crucial role of commensal protozoa, fungi, archaea, and viruses in homeostasis and disease. Over 100 years have passed since the discovery of bacteriophages (phages), prokaryotic viruses that attack bacteria in a host-specific manner. However, reproducible protocols to interrogate phages on a metagenomic level are emerging only since recently. The imminent threat of multidrug-resistant bacterial infections has revived the interest in phages as an alternative to conventional antibiotics. The mechanisms of population dynamics between phages and bacteria in the intestine are mostly elusive and a field of ongoing research [10]. An essential challenge to advance phage therapy for infectious and

possibly lifestyle-associated diseases is the identification and isolation of crucial phage consortia featuring efficacy and genomic safety to the host.

Fecal Microbiota Transplantation and Beyond

The metagenomic leap has revitalized interest in the 'new old tool' of fecal microbiome transplantation (FMT). The interest in FMT is exemplified by more than 350 completed or planned clinical trials (NIH, December 2020). The most credible data to date on FMT is available for recurrent *Clostridioides difficile* infection, and potentially for ulcerative colitis. Currently, its applicability is limited only to selected patients with a previous failure of conventional treatment [11]. All randomized controlled trials available so far feature significant limitations. Therefore, the efficacy and safety of FMT cannot be sufficiently appraised yet. A source of uncertainty is the possibility of iatrogenic transmission of infectious agents from donors to recipients. Another source of uncertainty is the heterogeneity between studies in patient selection and technical procedures. It is crucial to decipher the mechanisms underlying the purported effects of FMT. A series of studies have pointed out that factors other than the engraftment of live bacteria could be decisive (such as bile acids or phages) [12,13]. The identification of these factors will enable us to move beyond the relatively simplistic procedure of FMT to more controllable and potent precision interventions.

Probiotics in the Era of Metagenomic Sequencing

The metagenomic breakthrough has shed new light on the old notion of probiotics, live microorganisms that upon consumption may confer a health benefit [14]. A vast body of studies dissects the clinical outcomes associated with the consumption of various types of commercially available probiotics strains. However, the mixed results and the consequent lack of regulatory approval as medical interventions is in part explained by insufficient characterization, lack of a mechanistic understanding of potential modes of action, and lack of identification of host factors predicting response. It is imperative to move beyond the empirical application of candidate probiotic bacteria to the identification of rationally defined beneficial bacterial consortia, dietary factors fostering their growth and pinpointing microbial-derived metabolites conferring beneficial effects. Emerging technologies will allow for large-scale mining of such metabolites significantly interacting with the host's physiology [15].

Concluding Remarks

Research on the human microbiome has so far yielded valuable insights but has also produced an abundance of insufficiently characterized observations and correlations. Although microbiome research has matured remarkably over the last couple of years and can no longer be considered in its infancy, it has yet to fulfill the high hopes of revolutionizing clinical practice and prevention of dysbiosis-associated diseases. We strongly believe that the advent of more mechanistically focused microbiome research, striving at demonstrating and explaining causality rather than producing associations, correlations, and predictions, will lead to the incorporation of microbiome-derived data into precision medicine.

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Declaration of Interests

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