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Mini review

Challenges of metabolomics in human gut microbiota research

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ABSTRACT

The review highlights the role of *metabolomics* in studying human gut microbial metabolism. Microbial communities in our gut exert a multitude of functions with huge impact on human health and disease. Within the meta-omics discipline, gut microbiome is studied by (meta)genomics, (meta)transcriptomics, (meta)proteomics and metabolomics. The goal of metabolomics research applied to fecal samples is to perform their metabolic profiling, to quantify compounds and classes of interest, to characterize small molecules produced by gut microbes. Nuclear magnetic resonance spectroscopy and mass spectrometry are main technologies that are applied in fecal metabolomics. Metabolomics studies have been increasingly used in gut microbiota related research regarding health and disease with main focus on understanding inflammatory bowel diseases. The elucidated metabolites in this field are summarized in this review. We also addressed the main challenges of metabolomics in current and future gut microbiota research. The first challenge reflects the need of adequate analytical tools and pipelines, including sample handling, selection of appropriate equipment, and statistical evaluation to enable meaningful biological interpretation. The second challenge is related to the choice of the right animal model for studies on gut microbiota. We exemplified this using NMR spectroscopy for the investigation of cross-species comparison of fecal metabolite profiles. Finally, we present the problem of variability of human gut microbiota and metabolome that has important consequences on the concepts of personalized nutrition and medicine.

1. Introduction

Gut microbiota plays a crucial role in human health and disease. Translational research with rodents demonstrated that gut microbiota can be involved in different physiological functions such as energy harvesting (Bäckhed et al., 2004), shaping and maintaining of the intestine function (Hooper and Gordon, 2001), or regulation of the host immune system (Kau et al., 2011). Furthermore, the microbial composition can influence the host response to pathogens and the predisposition to diseases (Vrieze et al., 2010). Since microbes in our gut possess their own genome, they undergo the same machinery of transcription, translation, and metabolism, as depicted in Fig. 1A (Qin et al., 2010). In order to conduct studies on human gut microbiota, samples obtained by mucosal biopsy should be ideally utilized (Fraher et al., 2012). However, due to challenges related to the corresponding sampling procedure, stool samples are mainly collected. Although fecal microbiota only partly can represent gut microbiota, fecal genome, transcriptome, proteome, and metabolome can be potentially used to define specific members within the gut microbial ecosystem and investigate their functions by interpreting gene expression patterns and behavior of proteins and metabolites. Large-scale 'omics' studies are performed separately or in an integrated way in order to get a holistic overview of the processes taking place in a dynamic system (Fig. 1B). Omics studies regarding complex microbial communities and their interactions in a habitat, *e.g.* within a human intestine, are often accompanied by the prefix "meta" such as *metagenomics*, *metatranscriptomics*, *metaproteomics*, or *(meta)metabolomics* with the latter only rarely used in literature (Turnbaugh and Gordon, 2008; van Baarlen et al., 2013). Several studies attempted to combine these techniques or to integrate them on different levels of data processing and evaluation, thereby going beyond only taxonomic profiling (Daniel et al., 2013; Tong et al., 2014; Zhang et al., 2015b).

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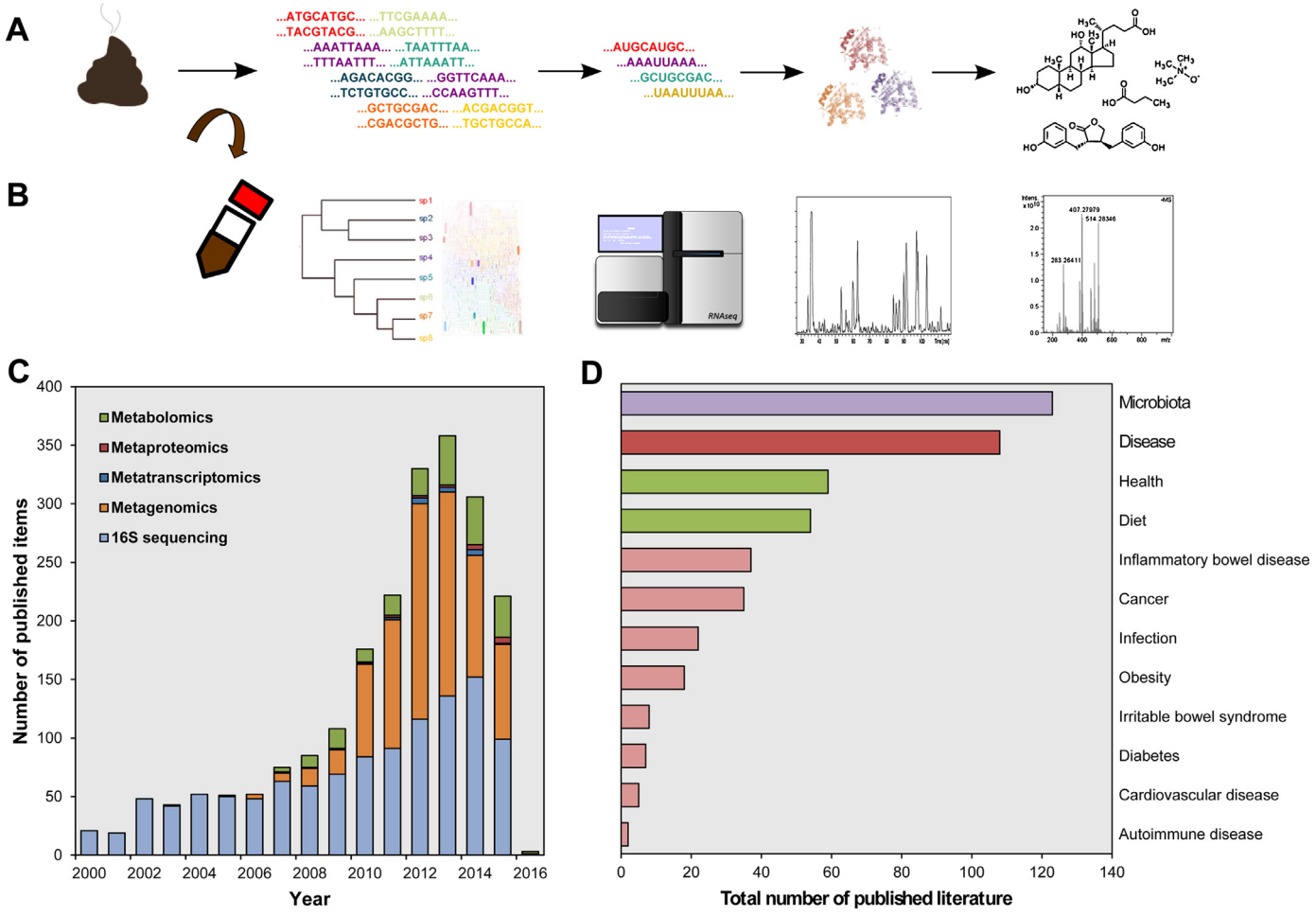

Fig. 1. A meta-omics overview on studying functional properties of human gut microbiota. (A) Human gut microbiota owns its own genome, transcriptome, proteome, and metabolome that can be investigated independently or in combination with other omics disciplines. (B) Existing fields of metagenomics, metatranscriptomics, metaproteomics, and metabolomics with some typical representatives. (C) Emerging of meta-omics field is expressed through the number of published literature for each year starting from the year 2000. (D) Fecal metabolomics has been dealing with topics related to microbiota, disease, health, and diet, outlined by the number of publications found for each topic. The disease related issues are further represented by eight different disease categories.

Metabolomics is defined as a comprehensive analysis of all metabolites in a biological system with their identification and quantification (Fiehn, 2002). To conduct a metabolomics study, spectroscopic or spectrometric techniques have been applied. Various biological matrices have been analyzed comprising urine, plasma, feces, or biopsies in order to monitor metabolites from host, microbes, and their co-metabolism (Storr et al., 2013). Metabolomics on fecal samples for studying gut microbial metabolism is just a rising but promising field, since stool is an easily accessible and non-invasive matrix with metabolites originating from host, its gut microbiota, and food components (Marchesi et al., 2007). To obtain a quick overview of the published literature on studying microbial ecosystem using fecal samples via metagenomics, metaproteomics, metatranscriptomics, or metabolomics, an ISI Web of Science search was conducted using the following queries: **TOPIC: (fec* hum* metabolom(or nom)*)** for human fecal metabolomics; **TOPIC: (fec* hum* metatranscriptom*)** for human fecal metatranscriptomics; **TOPIC: (fec* hum* metagenom*)** for human fecal metagenomics; **TOPIC: (fec* hum* metaproteom*)** for human fecal metaproteomics; **TOPIC: (fec* hum* 16S sequencing)** for 16S sequencing of genome present in human fecal samples. Next to the 16S sequencing, the omics area of metagenomics has the greatest number of publications followed by metabolomics whereas a relatively small number of publications is observed for metatranscriptomics and metaproteomics (Fig. 1C). Despite of their prevalence, a decreasing trend either for metabolomics, metagenomics, or 16S sequencing can be seen after a certain time point (Fig. 1C). The majority of publications regarding metabolomics using fecal samples are focused on microbiota, health, disease related issues, and diet (Fig. 1D). In turn, disease related issues have their main focus on inflammatory bowel disease, cancer, infection, and obesity (Fig. 1D).

2. Fecal metabolomics in inflammatory bowel disease

Inflammatory bowel disease (IBD) was found as one of the major diseases described in relation with metabolomics studies and gut microbiota (Fig. 1). IBD is an idiopathic disease that mainly affects gastrointestinal tract. Two main forms, ulcerative colitis (UC) and Crohn's disease (CD), have a complex etiology (Erickson et al., 2012). A disbalance of commensal microbiota is discussed in connection to IBD with a decrease of diversity and altered metagenome and metaproteome (Erickson et al., 2012; Kostic et al., 2014). For example, *Faecalibacterium prausnitzii*, which is a prominent short chain fatty acid (SCFA) producer in human gut, is decreased in IBD patients (Duncan et al., 2002; Miquel et al., 2015), which shows the connection between bacteria and metabolites. In order to get an overview of altered metabolites in IBD patients revealed by non-targeted metabolomics, the corresponding data is listed in Table 1 . In total, there are 9 studies that dealt with human and searched