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Review

Unraveling the environmental and genetic interactions in atherosclerosis: Central role of the gut microbiota

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ABSTRACT

Recent studies have convincingly linked gut microbiota to traits relevant to atherosclerosis, such as insulin resistance, dyslipidemia and inflammation, and have revealed novel disease pathways involving microbe-derived metabolites. These results have important implications for understanding how environmental and genetic factors act together to influence cardiovascular disease (CVD) risk. Thus, dietary constituents are not only absorbed and metabolized by the host but they also perturb the gut microbiota, which in turn influence host metabolism and inflammation. It also appears that host genetics helps to shape the gut microbiota community. Here, we discuss challenges in understanding these interactions and the role they play in CVD.

1. Introduction

Studies of the role of microbes in common diseases have exploded in recent years, due in part to technical advances such as the use of next-generation sequencing to profile samples. Such studies have now linked gut microbiota to ulcers, colitis, obesity, diabetes, cancer, fatty liver, autism, kidney stones, cardiovascular disease (CVD) and other disorders (reviewed in [1–3]). Among the most convincing of these associations are those for atherosclerosis and its risk factors, with experimental evidence of causal interactions in insulin resistance, bile acid metabolism, inflammation, and obesity. In addition, studies of certain microbiota derived metabolites have recently revealed novel pathways affecting atherosclerosis and cholesterol metabolism. A particularly exciting aspect of this work is the possibility for the development of new therapeutic interventions as well as diagnosis.

Our group, among others, has been interested in dissecting genetic and environmental interactions in atherosclerosis, and these recent findings introduce an additional key component, namely, the microbiota, that must be considered. Thus, in analyzing how a particular diet or genetic background influences the disease process, one must not only consider direct effects but also how the diet or genetic background perturbs the microbiome (Fig. 1). The great complexity of the gut microbiome and its sensitivity to environmental factors make such analyses challenging.

Our review is divided into three parts. First, we briefly summarize some of the tools for studying microbiota, with an emphasis on gut microbiota. Second, we review the literature implicating microbes in atherosclerosis and its risk factors. And, third, we discuss approaches to unraveling the complex interactions between gut microbiota composition, diet, and genetics.

2. Studying microbiota–host interactions

Human skin and mucosal surfaces and the gut are colonized by an untold number of microorganisms, comprised of bacteria, archaea, viruses, fungi, and other protozoans, collectively known as the microbiota (their genes are known as the microbiome). The human gut, for example, is colonized by about 100 trillion bacteria including at least 1000 distinct species [4]. The bacteria tend to associate with one another and the host in a mutualistic/commensal or opportunistic/parasitic manner. In the case of gut microbiota, it is clear that a symbiotic relationship has evolved. The host provides the microbiota with nutrients while the microbiota make possible the digestion of complex carbohydrates, balance immune and metabolic functions, and provide protection against

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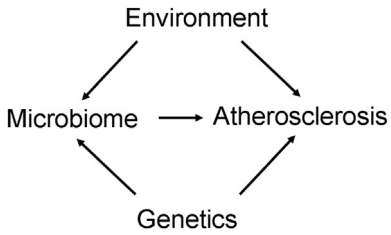

Fig. 1. Genetic and environmental interactions in atherosclerosis: Role of the gut microbiota. Environmental factors, particularly the diet, and host genetics can contribute to atherosclerosis and other CVD either directly or by perturbing microbiota.

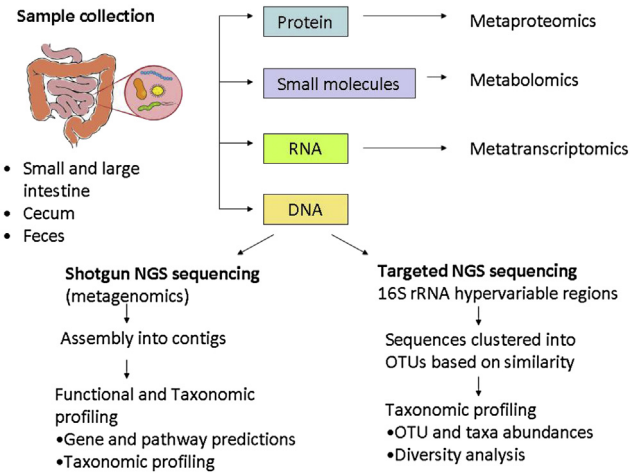

Fig. 2. Overview of culture-independent methods for microbial analysis. High-throughput analysis of gastrointestinal tract microbiota via sequencing of DNA using targeted or shotgun sequencing or using other "omics" approaches. NGS, next generation sequencing; OTU, operational taxonomic units.

opportunistic pathogens [4,5]. The compositions of these microbial communities vary widely in the population due to differences in environmental factors and host genetic factors and, as discussed below, these differences appear to contribute to CVD.

The microbiota of an individual are "seeded" at birth through maternal physical contact, becoming more diverse within weeks and eventually stabilizing in adolescents or young adults. The gut microbiota play an important role in intestinal development and in shaping the immune system. Birthing, breastfeeding and weaning methods significantly impact the gut microbiota profiles of infants. Infants delivered by Caesarean section have bacterial composition more similar to maternal skin, unlike vaginally delivered infants, who have more *Lactobacillus* [3,6]. Through development the predominantly aerobic bacteria are replaced by anaerobes, until approximately 2–3 years of age, where the profile more closely resembles that of an adult [7].

A very small fraction of gut microbiota have been cultured or studied in any detail. Most are obligate anaerobes rapidly killed by exposure to air and they live as communities highly interdependent on other species. The distribution of microbes throughout the gut is highly structured and specialized in biological functions, varying considerably between different parts of gastrointestinal tract. In studies of mice the most commonly used sites are the cecum and different parts of small intestine (duodenum, jejunum and ileum), whereas in human studies stool samples are usually used.

Because it is challenging to culture gut microbiota, culture-independent genomic methods have greatly stimulated this area of research (Fig. 2). Particularly important has been the use of next-generation sequencing for taxonomic profiling of samples, together with the development of bioinformatics techniques. Taxonomic profiling has revealed enormous interindividual variability due to factors such as diet [8] and other environmental exposures and factors such as age [7], sex [9], and host genotype [10–13]. Shotgun metagenomic sequencing, in which total DNA is extracted and sequenced, has the advantage of providing data on functional potential present in a given sample. Despite the considerable variability of gut microbiota composition, metagenomic shotgun sequencing has shown that these diverse communities share a core set of gene functions [14]. In concert with sequencing methods, new approaches for assessing the functionality of bacteria have been developed, such as screening for metabolites derived from microbiota [15].

The most common approach to taxonomic profiling of gut microbiota is to amplify and then sequence hypervariable regions of bacterial 16S rRNA genes followed by clustering of the sequences into Operational Taxonomic Units (OTUs) (Fig. 3). Several analytic tools have been developed for analyzing next generation of DNA sequence data from targeted 16S and metagenomic analysis, such as QIIME [16], mothur [17] and VAMPS [18]. These enable an accurate downstream analysis of organism distribution, which can then be correlated with disease status or other phenotypes of interest.

Mice harbor microbes that roughly resemble those found in humans and are the most widely used organism for experimental studies (reviewed in [19]). Mouse models allow perturbations in gut microbiota to be studied in highly controlled environments and thus are useful in assessing causality of the complex microbiota–host interactions and in developing mechanistic hypothesis. Fig. 4 summarizes different experimental procedures with mice that are commonly used for studying gut microbiota. Germ-free mice or mice previously treated with antibiotics are often used for transplantation with human microbiota (humanized mice) or other microbes (conventionalized or gnotobiotic mice). Germ free mice are raised in the absence of any resident microorganisms, representing a defined assay system for studying the impact on the host of colonization of the gut by microbiota members. In addition, mice can be cross-fostered following Caesarian delivery, and since they are coprophagic, simply co-housing two strains allows transfer of gut microbiota.

An exciting aspect of studies of the gut microbiome in disease is the possibility of manipulating the microbiota composition through alterations in diet and the use of probiotics and prebiotics. "Probiotics" refers to the administration of live dietary supplements such as *Lactobacilli* or *Bifido* bacteria in yogurt. These can, for example, help alleviate symptoms of lactose intolerance [20]. Prebiotics are nondigestible food ingredients such as oligomers of fructose (fructans) and fibers such as Psyllium husk that can be utilized by selective microbiota. Such prebiotics have been shown to affect the absorption of minerals and the levels of plasma lipids [21]. Administration of fructans in mice on a high fat diet has been shown to decrease fat storage in white adipose and liver, improve glycemic control, and decrease systemic inflammation [22]. A high fat diet has been shown to downregulate the intestinal expression of certain antimicrobial peptides, such as Reg3g, and prebiotic treatment was shown to reverse this, providing one possible explanation for the effect on the microbiome composition [23].

The complexities of investigating the role of gut microbiota in disease are illustrated by two studies of atherosclerosis in germ-free mice. In an early study, Wright and colleagues [24] compared atherosclerosis development in germ-free versus conventional apolipoprotein E null (*Apoe*$^{-/-}$) mice maintained on a Western-type high cholesterol, high fat diet. No differences in atherosclerosis