Published in final edited form as:
Circ Res. 2012 September 28; 111(8): 948–950. doi:10.1161/CIRCRESAHA.112.277277.

An anti-atherosclerotic signaling cascade involving intestinal microbiota, microRNA-10b, and ABCA1/ABCG1 mediated reverse cholesterol transport

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A sobering fact is that human DNA represents less than 10% of the total DNA within each of us. Every mucosal surface harbors a diverse and distinct microbial composition, with the human intestines containing by far the largest and most complex microbial ecosystem (intestinal microbiota). Serving as the filter for our largest environmental exposure-what we eat-the human intestinal microbiota is enormous-at least 3.3 million non-redundant microbial genes, making it approximately 150 times larger than the human gene complement[1]. Analyses of fecal metagenomes (genomic analysis of a community of microorganisms) from individuals, both with distinct long-term dietary patterns[2] and from around the world[3], demonstrate the existence of a more limited number of stable clusters of microbial compositions, termed enterotypes, which represent relatively durable host-microbial symbiotic states. Metabolomics analyses reveal intestinal microflora exert a significant effect on mammalian blood metabolites[4], raising the possibility that distinct enterotypes may respond differently to environmental (dietary or drug) exposures.

Consistent with this notion, significant mounting evidence indicates that numerous cardiometabolic phenotypes within the “human superorganism” are influenced by a complex interplay among environmental (typically dietary nutrient) exposures, intestinal microbiota composition, and host factors. One of the earlier examples demonstrating the importance of intestinal microflora in a complex cardiometabolic phenotype was first reported by Turnbaugh et al[5], who showed that differences in the efficiency of energy harvest from food from distinct microflora compositions within lean versus obese inbred strains of mice could contribute to the development of obesity. Importantly, the obese phenotype was shown to be a transmissible trait, with ‘obese’ versus ‘lean’ cecal microbiota transplant into germ free mice resulting in significantly greater body fat accumulation despite equivalent caloric intake[5]. Metabolomics studies by Nicholson and colleagues suggested that intestinal microbiota may similarly play an active role in the development of complex metabolic abnormalities such as susceptibility to insulin resistance and fatty liver disease[6]. Subsequent, examination of germ free versus conventional mice on high fat diet revealed both insulin sensitivity and cholesterol metabolism are metabolic targets influenced by the intestinal microbiota[7]. And more recently, studies with mice defective in NLRP3 and NLRP6 inflammasome sensing demonstrate that inflammasome-mediated intestinal dysbiosis (imbalance in microbial composition) enhances susceptibility to hepatic steatosis, inflammation and obesity[8]. Remarkably, co-housing of inflammasome-deficient mice with wild-type mice conferred hepatic steatosis and obesity phenotypes to the wild type mice,

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Disclosures:
None

suggesting some aspects of metabolic syndrome may be communicable[8]. A study comparing different toll like receptor (TLR) deficient mice found markedly different gut microbiotas, but that these differences were due to divergence during extended husbandry in isolation, with maternal transmission of the gut microbiota yielding the observed differences rather than the specific gene deficiencies[9]. Thus, mice from different colonies may have different phenotypes based upon different gut flora rather than on genetic differences, and comparisons of separately bred knockout and wild type colonies should be avoided and replaced by studies of littermates bred from hemizygotes, who share maternally transmitted gut microflora. A mechanistic link between nutrient consumption, intestinal microflora and atherosclerosis pathogenesis was recently reported by Wang and colleagues where phosphatidylcholine consumption, the major dietary source of choline, through intestinal microflora metabolism, was shown to produce a metabolite that accelerates atherosclerosis in rodent models[10]. Complementary clinical studies further demonstrated that elevated circulating levels of the gut flora metabolite within subjects predicted increased cardiovascular risk independent of traditional cardiovascular risk factors[10]. There is thus a growing body of evidence suggesting that intestinal microflora, through a variety of processes, can influence physiological processes important to development cardiovascular disease.

The dietary anthocyanin cyanidin-3-*O*-B-glucoside (Cy-3-G) is a pigmented polyphenol commonly found in fruits and berries, vegetables, red wine, pigmented cereals and tea[11]. Numerous epidemiological studies suggest enhanced consumption of foods rich in anthocyanins (e.g. strawberries, blueberries and red wine) is associated with reduced risk of developing cardiovascular disease (reviewed in[11, 12]). Further, animal model studies report beneficial health effects, including reduction in atherosclerosis, from diets supplemented with either Cy-3-G or anthocyanin enriched extracts[13–15]. In their intact form, anthocyanins like Cy-3-G are poorly absorbed, and consequently, following ingestion, achieve only low circulating systemic levels[16]. The limited bioavailability of ingested anthocyanins has raised questions of a plausible mechanistic rationale for how these polyphenols might promote such biological effects. More recently, it has become appreciated that a significant portion of ingested polyphenols reach the cecum and large bowel, where microbiota mediated biotransformation can potentially produce metabolites that achieve significant plasma levels following dietary ingestion[17]. One of the major metabolites of Cy-3-G, protocatechuic acid (PCA), was recently shown to inhibit monocyte adhesion and atheroscleorsis in animal models[18]. While suspected to be a metabolite formed by intestinal microbiota action on Cy-3-G, direct demonstration of an obligatory role for intestinal microbiota in PCA formation from dietary Cy-3-G has been lacking. Moreover, a convincing mechanistic rationale for how Cy-3-G, PCA, or some other down stream metabolite might mediate their anti-atherosclerotic effects has yet to be revealed.

In this issue of *Circulation Research*, Wang and colleagues[19] conclusively demonstrate that PCA is an intestinal microbiota metabolite of ingested Cy-3-G. Further, they demonstrate that dietary Cy-3-G, through an intestinal microflora and PCA dependent pathway, promotes an anti-atherosclerotic effect via a newly defined signaling cascade in reverse cholesterol transport (RCT) involving miRNA-10b (miR-10b) dependent enhancement in ABCA1 and ABCG1 mediated cholesterol efflux. Specifically, Wang et al. demonstrate that PCA at physiological concentrations represses macrophage miR-10b and induces ABCA1 and ABCG1 mRNAs along with cholesterol efflux activity. Putative binding sites for miR-10b were found in the 3′-UTRs of the mouse and human ABAC1 and ABCG1 genes, and reporter gene transfection studies demonstrate that these 3′-UTRs confer miR-10b repression that is sensitive to mutations in the miRNA seed or mRNA target sequence. Over expression and knockdown of miR-10b in, macrophages lead to the expected concomitant effects on the expression of ABCA1 and ABCG1 as well as cholesterol efflux. Furthermore,

over expression of miR-10b could overcome the effect of PCA on ABCA1 expression and cholesterol efflux, showing that repression of miR-10b is responsible for the effects of PCA on macrophage cholesterol metabolism. In addition, these authors show that dietary Cy-3-G or PCA increases macrophage RCT to the feces in apoE-deficient mice using the in vivo RCT model developed by Rader and colleagues[20]. This finding was not accompanied by increased RCT to the plasma and hepatic compartments, and was not associated with changes in plasma HDL-C or apoA-I levels. Although the authors speculate that trans intestinal cholesterol efflux may be involved, apoE-deficient mice with their large pool of plasma cholesterol in βVLDL may not be the best model to study the fine points of in vivo RCT. Finally, they show that 4 weeks of dietary Cy-3-G (only in the absence of antibiotics) or PCA leads to apparent aortic root lesion regression in apoE-deficient mice. This finding suggests that increased macrophage efflux can actually reverse atherosclerosis in the face of continued hyperlipidemia, a finding that may be worthy or replication to determine how robust this effect is. What is still a mystery is how PCA acts to repress miR-10b expression, information that will close the loop on this novel signaling cascade that regulates RCT.

The discovery of miR-10b as a regulator of cholesterol efflux adds to our knowledge of miRNA regulation of ABCA1 and HDL metabolism. Three groups independently discovered that miR-33a/b represses ABCA1 expression by interacting with conserved target sequences in the ABCA1 3′-UTR (for review see Rayner et al. 2012; Ref 21). Human miR-33a and miR-33b are intronic to the sterol response element binding proteins SREBP1 and SREBP2, respectively, and are coordinately regulated with their host genes. Thus, macrophage mir-33a levels are decreased upon cholesterol loading. Similarly the intergenic miR-758 was also found to be repressed by cholesterol loading and to regulate ABCA1 expression and cholesterol efflux[22]. This knowledge has opened up a new avenue of therapeutic investigation, as miR-33 knockdown in vivo in both mice and African green monkeys leads to increased hepatic ABCA1 and plasma HDL levels, and in mice this translates into increased RCT and regression of atherosclerosis[23–25]. Thus, it is reasonable to suggest that both miR-758 and miR-10b are also excellent targets for the development of novel therapeutics to increase HDL and RCT, and thus inhibit atherosclerosis development and progression.

In conclusion, Wang et al. have described a novel pathway in which a dietary flavonoid is converted by gut flora into a circulating metabolite that regulates cellular cholesterol metabolism and RCT through a miRNA mediated mechanism[19]. This study reinforces the importance of the microflora as an important participant at the nutrient-host interface. Thus, modulation of microflora (by probiotics or other dietary intervention), or direct targeting of microflora enzymes (by pharmacological inhibitors or activators) may be a burgeoning area for pharmaceutical and functional food efforts with the goal of reducing the epidemic growth of obesity, insulin resistance, and cardiovascular disease. Such an approach has been described in cancer therapy, where a bacterial enzyme inhibitor has been shown to alleviate toxicity of a chemotherapeutic drug[26].

Acknowledgments

Sources of Funding:

The authors acknowledge grant support from the National Institutes of Health P01HL098055, R01 HL103866, P20HL113452.

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