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Microbe-mitochondrion crosstalk and health: An emerging paradigm

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ABSTRACT

Human mitochondria are descendants of microbes and altered mitochondrial function has been implicated in processes ranging from ageing to diabetes. Recent work has highlighted the importance of gut microbial communities in human health and disease. While the spotlight has been on the influence of such communities on the human immune system and the extraction of calories from otherwise indigestible food, an important but less investigated link between the microbes and mitochondria remains unexplored. Microbial metabolites including short chain fatty acids as well as other molecules such as pyrroloquinoline quinone, fermentation gases, and modified fatty acids influence mitochondrial function. This review focuses on the known direct and indirect effects of microbes upon mitochondria and speculates regarding additional links for which there is circumstantial evidence. Overall, while there is compelling evidence that a microbiota-mitochondria link exists, explicit and holistic mechanistic studies are warranted to advance this nascent field.

1. Introduction

The human body contains more than fifty trillion microbes that outnumber human cells and collectively have many more genes and metabolic processes (Zhao, 2013; Cho and Blaser, 2012). The human gut, in particular, is densely colonized with a diverse collection of microbial species, such that 50% of fecal matter biomass is composed of bacteria (Nicholson et al., 2005). The gut microbiome represents the largest and most intimate connection between man and microbe and strongly influences human health (Tilg and Kaser, 2011; Turnbaugh et al., 2006; Everard et al., 2013; Greenblum et al., 2012; Zhang et al., 2009; Flier and Mekalanos, 2009; Ridaura et al., 2013). So far, mechanistic understanding of the relationship between gut microbiota and human health or disease has proceeded along three distinct lines. First, it has been found that gut microbial communities can harvest additional energy from food by metabolizing indigestible carbohydrates into calorie containing nutrients (Turnbaugh et al., 2006). Second, the interaction of microbes and microbial products with gut epithelium has been shown to modulate host genes like fasting-induced adipose factor (*fiaf*), which regulate nutrient metabolism (Backhed et al., 2007). These have been well studied in the context of obesity. Third, human-microbe interactions are critical in programming the immune system. This may be beneficial by promoting maturation and reducing inappropriate or exaggerated inflammatory responses to innocuous triggers like allergens (Cernadas, 2011). However, this may also promote low grade inflammation in the liver and gut. These aspects are well studied in many contexts including allergy, asthma, non-alcoholic hepatic steatosis, cardiovascular disease, amongst others. Other than these three major directions, others and we have proposed a functional link between microbes and their distant cousins, the mitochondria (Agrawal and Mabalirajan, 2016; Saint-Georges-Chaumet and Edeas, 2016). This review will focus on the microbe-mitochondrion connection, emphasizing common metabolic links, and provide an overview of how this connection can give rise to human disease.

2. Evolutionary and functional links between microbes and mitochondria

The origin of mitochondrion, around 2 billion years ago, is thought to be a strategy adopted by eukaryotic cell to mitigate oxidative toxicity, via endosymbiosis between a mitochondrial ancestor organism and the eukaryotic cell. The endosymbiotic theory, which is well-accepted for mitochondria, broadly states that key organelles of the modern eukaryotic cell arose from fusion between separate single celled organisms (Andersson et al., 2003). Initial phylogenetic studies based on genes encoding, cytochrome c oxidases, cytochrome b (Gray et al., 1999), universally conserved rRNA sequences (Karlberg et al., 2000) and heat shock proteins (Kurland and Andersson, 2000) found the mitochondrial ancestor to be derived from alpha-proteobacteria. Later, with newer phylogenetic tools and availability of sequences of bacterial genomes, the position of mitochondria on the tree of life was reconstructed based on the respiratory chain complex proteins,

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substantiating the origin of mitochondria from alpha-proto-mitochondrion (Gabaldón and Huynen, 2007, 2003). The gradual loss and gain of genes, as exemplified by genes encoding amino-acid synthesis and ATP/ADP translocase respectively, further maps the journey of proto-mitochondrion to modern mitochondria. Interestingly the mostly lost capacity for bacteria-like respiration in human mitochondria, may manifest under specific conditions. For example, bacterial respiration is not dependent on oxygen due to the use of nitrates or fumarates as terminal acceptors of electrons in the Electron Transport Chain (ETC). During adaptive responses to hypoxia, mammalian cardiomyocytes also use fumarate as an alternative electron acceptor at the mitochondrial complex II (Sridharan et al., 2008). This allows an outlet for the ETC during hypoxia, protects the mitochondrial membrane potential and permits Nicotinamide adenine dinucleotide (NADH) oxidation, thereby maintaining ATP synthesis. This represents partial retention of the ancestral capacity for energy metabolism in human mitochondria, bringing forth the possibility of mitochondrial metabolism being influenced by products of microbial fermentation. The potential impact of these products on human energy metabolism is discussed in subsequent sections. Effects on mitochondrial oxidative phosphorylation capacity (OxPhos) or reactive oxygen species (ROS) generation are particularly focused upon, with decline in OxPhos capacity or increased mitochondrial ROS generation taken to be markers of mitochondrial dysfunction.

3. Gut derived short chain fatty acids and mitochondria

Depending on the diet, the total concentration of short chain fatty acids (SCFAs) synthesized by gut microbiota ranges from 70 to 140 mM in the proximal colon to 20 to 70 mM in the distal colon (Topping and Clifton, 2001). Acetate, propionate, and butyrate are the principal SCFA produced by gut microbes, roughly in a 3:1:1 ratio. Around 95% of SCFAs are absorbed and rest 5% is excreted in feces. The study of Cummings et al. showed decrease of butyrate from 22% to 8% in portal vein, suggesting absorption of its main fraction in colon itself as colonocytes obtain ~60–70% of their energy derived from SCFA; preferentially butyrate. This observation is corroborated in an in-vitro model of T84 cell-line derived from human colon, where Lewis et al. (Lewis et al., 2010) have shown that butyrate is preferentially metabolized by colonocytes, compared to acetate and propionate. Germ-free mice have a colon-specific decrease in NADH/NAD + ratio and ATP levels, when compared to conventionally raised mice (CONV-R). This colon specific effect is attributed to lack of microbiota-derived, butyrate. Lack of butyrate leads to markedly diminished production of NAD +, leading to induction of autophagy in colonocytes of germ free mice (Donohoe et al., 2011).

The liver further metabolizes SCFA, that cross the gut and enter the portal vein; mainly acetate and propionate. Propionate is efficiently consumed as a substrate for gluconeogenesis, resulting in a fall in propionate fraction from 21% (portal vein) to 12% (hepatic vein); leading to acetate being the dominant SCFA systemically with smaller amounts of propionate or butyrate being available (fasting venous blood concentrations of about 0.25, 0.01 and 0.002 mM respectively). However, despite acetate being the dominant SCFA in terms of quantity, butyrate and propionate have important effects on systemic metabolism through hormone like actions, via free fatty acid receptor (FFAR) signaling and G protein coupled receptors, that may regulate appetite and protect against diet-induced obesity (Lin et al., 2012). FFAR signaling can also importantly modulate inflammation (Tan et al., 2014; Smith et al., 2013). SCFA seem to have far reaching effects, with apparent bioactivity in multiple aspects of physiology, such as neuroendocrine system (Topping and Clifton, 2001), epigenetics, and cancer (Blouin et al., 2011). Here, we will focus on the effect of SCFA on mitochondrial bioenergetics and the importance of microbiome composition on SCFA flux.

Synthesis of SCFA in gut is dictated by the dietary fibre intake, bacterial content, cross feeding between bacteria, transit time of food, and colonic pH. In simple metabolic models of the gut microbiome, bacteria from the phylum *Bacteroides* consume most of the polysaccharides and secrete propionate and acetate, which in turn is consumed by *Firmicutes* that produce much of the butyrate (Shoaie et al., 2013). Acetate is the most abundant SCFA and can be used by mitochondria as a source of energy (Lumeng and Davis, 1973). However, in isolated perfused rat hearts and its purified mitochondrial fraction, use of acetate as a primary fuel (perfused heart, 10 mM; purified mitochondria, 0.1–10 mM) impaired fatty acid oxidation, depleted ATP and had cardio-depressant effects (Jacob et al., 1997). Such levels may be reached in the hepato-portal system after meals, and in blood after consumption of alcohol. Alcohol consumption elevates blood acetate from < 0.1 mM to 1 to 2 mM within minutes (Mascord et al., 1992). Liver mitochondria are critical in converting ethanol to acetate, with acetaldehyde as an intermediary product that is toxic to mitochondria (Manzo-Avalos and Saavedra-Molina, 2010). High levels of acetate released into the blood by liver, after ethanol consumption, are used as fuel by organs such as heart and brain, leading to major changes in redox balance, glucose consumption and mitochondrial function (Kiviluoma et al., 1989; Jiang et al., 2013). In yeast, acetate increases mitochondrial respiration but reduces functionality and accelerates ageing (Orlandi et al., 2013).

In contrast to acetate, butyrate stimulates mitochondrial biogenesis, leading to increased energy expenditure and weight loss in male C57BL/6J mice fed a high fat diet (Gao et al., 2009). This is thought to relate to inhibition of histone deacetylases, which unveils a mitochondrial biogenesis signature (Galmozzi et al., 2013; Sealy and Chalkley, 1978). The positive effect of butyrate on bioenergetic function is consistent with its fall in obesogenic high fat diets and increase in weight-loss inducing high fibre diets (Gao et al., 2009; Brahe et al., 2013; Jakobsdottir et al., 2013). Further, butyrate may also play an important role in improving barrier function of gut epithelial cells by improving their metabolic function (Lewis et al., 2010). Improved barrier function appears to be a critical aspect of reducing the inflammatory effects of bacterial colonization. A preferential mitochondrial metabolism of butyrate compared to other SCFA or long chain fatty acids has previously been reported in vivo during ischemia and in vitro during competitive experiments, confirming its ability to stimulate mitochondrial metabolism (Gordon and Crabtree, 1992). However, in tightly controlled in vitro experiments, it has also been seen that the positive effects of butyrate are only seen at low concentrations and higher concentrations are associated with uncoupling of OxPhos and progressive degradation of mitochondrial function, possibly via effects on ETC (Hird and Weidemann, 1966). This dichotomy in the effects of butyrate on mitochondrial function is echoed in the often-contradictory data from obese and germ-free mice experiments. While SCFA administration makes germ free mice resistant to obesity, obese mice typically have higher SCFA levels. Human data is similarly contradictory with higher level of *Firmicutes* relative to *Bacteroides* (F/B ratio) being associated with higher butyrate production as well as protection against obesity in some studies while others find highest levels of SCFA in obese subjects and an opposing effect of F/B ratio (Lin et al., 2012; Fernandes et al., 2014; Schwiertz et al., 2010; Finucane et al., 2014). The association of SCFA with human health and disease is likely to extend well beyond typical energy metabolism disorders like obesity or diabetes and may be specific to the different fatty acids. The clinical association of autism spectrum disorders (ASD) and SCFA production in gut has been linked specifically to propionate, via possible effects on brain mitochondria (MacFabe, 2015; Li et al., 2017).

4. Hydrogen economy of the gut and mitochondria

Efficient microbial fermentation of indigestible carbohydrates, such as fibre, requires disposition of hydrogen, since buildup of reaction products inhibits the forward reaction. This makes small microbial