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DOCTORAL THESIS

Increased bile acid-metabolizing bacteria contributes to enhanced gastrointestinal motility in irritable bowel syndrome

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ABSTRACT

Irritable bowel syndrome (IBS), majorly characterized by irregular bowel movements and abdominal pain, is one of the most prevalent functional gastrointestinal disorders (FGIDs) in the world. Disturbance of gut microbiota, closely linking with gut dysfunction, has been regarded as one of important pathogenetic factors for IBS. However, gut microbiota-driven mechanism underlying IBS remains unclear, which leads to inefficient and non-specific effects of current microbiota-oriented therapy. In this thesis, function-based microbiota investigation with combination of metagenomic and metabolomic analyses was separately performed in IBS cohort and model to precisely link pathogenic species with disordered GI motor function. A series of microbiota manipulation studies in rodents were conducted to explore bacteria-driven molecular mechanism. **Firstly**, a pilot study with 'omics' analyses revealed fecal microbial structure significantly varied in IBS patients with disorder GI motility relative to healthy controls (HC). Such changed IBS enterotype was functionally characterized by disturbed metabolism of bile acids (BAs) that are previously proved to regulate GI motor function. It indicates microbiota-driven GI dysmotility relevant to disturbance of BA metabolism in IBS. **Secondly**, a systematic review with meta-analysis was performed to comprehensively understand existing findings related to BA metabolism and its linkage with IBS. Results showed that abnormal BA excretion, previously reported in at least one IBS subtype, is associated with dysregulation of BA synthesis, marked with abnormalities of circulating indices 7α-hydroxy-4-cholesten-3-one (C4) and fibroblast growth factor 19 (FGF19). However, what's the role of gut microbiota in abnormal BA excretion is undetermined. **Thirdly,** to explore possible role of gut microbiota in abnormal BA excretion in IBS, BA metabolites and BA-related microbiome were simultaneously analyzed in stools of recruited subjects. Results found

that total BA and microbiota-derived BAs were remarkably elevated in a quarter of IBS-D patients (BA^+IBS-D) who exhibited more frequent defecation, higher level of serum C4 but lower level of serum FGF19 than those with normal BA excretion (BA^- IBS-D). In line with metabolic results, abundances of BA-metabolizing bacteria, particularly *Clostridium scindens* (*C. scindens*) simultaneously expressed *hdhA* and *bais* that are responsible for BA 7α oxidation and dehydroxylation, were highly enriched in fecal metagenomes of such particular IBS-D population. These findings suggest the increased BA-metabolizing microbiome is associated with the dysregulated host BA synthesis in the subgroup of BA^+IBS-D patients. **Fourthly**, by analyzing metabolites and bacteria related to BA metabolism, a neonatal maternal separation (NMS)-induced IBS-D rat model characterized by accelerated GI motility and excessive BA excretion were found to largely mimic gut microbial BA metabolism in BA^+IBS-D patients. Specifically, intraluminal total and secondary BAs were significantly elevated in the large intestinal lumens (cecum, proximal colon and feces) of NMS rats, together with increased abundances of *hdhA*- and *bais*-expressing *Clostridium* species, including *C. scindens*. Moreover, quantitative polymerase chain reaction (PCR) analysis showed upregulated mRNA expression of cholesterol 7 α-hydroxylase (CYP7A1) whereas downregulated mRNA expression of small heterodimer partner (SHP) in the liver of NMS rats, indicating enhanced hepatic BA synthetic level. These observations based on such IBS-D model suggest the association of excessive BA-metabolizing microbiome and increased hepatic BA synthesis. **Fifthly,** to further clarify whether excessive BA-metabolizing bacteria contribute to enhanced hepatic BA synthesis and to explore the underlying molecular mechanism, we performed bacterial intervention in pseudo germ-free (GF) or/and specific pathogen free (SPF) mice by transplantation of human fecal microbiota and the signal strain *C.*

scindens. Compared with GF mouse recipients of HC and BA^-IBS-D fecal microbiota, BA^+IBS-D fecal microbial recipients displayed shorter GI transit and increased subsistence of *C. scindens* in the cecal contents. In line with higher level of serum C4, taurine-conjugated BA contents and mRNA expressions of BA synthetase CYP7A1 and sterol 12α-hydroxylase (CYP8B1) were significantly elevated in the liver of BA^+IBS-D recipients. These findings showed bioactive effects of BA^+IBS-D fecal microbiota with enrichment of *C. scindens* on hepatic BA synthesis. **Next**, to further confirm the effects of the species *C. scindens* on host BA synthesis, we individually colonized *C. scindens* strains (ATCC 37504) to pseudo GF and SPF mice. Results showed both mice models with single strain colonization exhibited accelerated GI transit and higher contents of hepatic total and taurine-conjugated BAs compared with individual vehicles treated with PBS. Combining metabolic changes, the upregulated expressions of hepatic CYP7A1 mRNA in colonized mice indicate that *C. scindens* substantially promote hepatic BA synthesis in colonized mice. Furthermore, contents of taurine-conjugated BAs, served as natural antagonists of farnesoid X receptor (FXR) that negatively control of new BA synthesis, were elevated in ileal lumens of colonized mice. Expressions of FXR-targeted genes SHP and fibroblast growth factor 15 (FGF15) were consistently reduced in the liver and ileum tissues of colonized mice, respectively. Results suggest that suppression of FXR-mediated feedback signaling is involved in *Clostridium*-driven hepatic BA oversynthesis, which deserve the further investigation. **Collectively**, the works of this thesis integrating clinical and animal studies indicate that BA-metabolizing bacteria, particularly *C. scindens*, enhance hepatic BA synthesis and consequently leads to BA overexcretion. It provides novel bacteria-driven mechanism for enhanced GI motility, and supply a direction in precise microbiota-related pathogenesis and medication for IBS-D population in future.

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