DOCTORAL THESIS

Akkermansia muciniphila ameliorates depressive symptoms in irritable bowel syndrome via improving neuroinflammation

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ABSTRACT

Irritable bowel syndrome (IBS) is characterized by chronic abdominal pain/ discomfort along with altered bowel habits, which accounts for a large proportion of gastrointestinal (GI) disorders worldwide. While psychiatric distress like depression is one of the most frequent comorbidities in IBS patients, which not only influences the quality of life, it also leads to a substantial economic burden and inefficient treatment in IBS patients. Inflammation, altered activities of the HPA axis, aberrant central neuroplasticity and neurotransmission have been highly regarded as pathogenic factors of the depression. Whereas, in recent years, dysfunctions in the gut microbial community has been increasingly discovered to provoke depression disorder. Considering that gut dysbiosis plays causal role in IBS progression, dysfunction of the gut microbiota has been speculated for contributing to the depressive symptoms in IBS (IBS-DP) patients. However, whether and how gut dysbiosis affecting IBS-DP patients remain unclear. We hypothesized that gut microbiota changes contribute to the development of IBS-DP and the change of gut microbiota-driven metabolites induces the structural and/or functional changes of the central nervous system (CNS), thus resulting in the development of IBS-DP.

In this thesis, IBS patients with and without depression and animal models of IBS have been systematically studied, to investigate whether gut dysbiosis mediates depressive disorder in IBS. **Firstly**, we conducted a cross-sectional study involving the distribution of depressive disorder in the IBS population of Hong Kong. According to this survey, we found that there is 36.6% (135/369) of IBS patients

showed symptoms of depression. The severity of depressive symptoms was positively associated with the harshness of visceral pain and bloating signatures in IBS patients. **Secondly**, in comparison to the non-depression of IBS (IBS-ND) group, faecal metagenomic results unveiled the disrupted gut microbiota in IBS-DP patients, mainly with the deficiency of several beneficial bacterial groups, including *Akkermansia, Bifidobacterium* and *Eubacterium*, whereas the gut microbiota profile between IBS-ND patients and healthy controls (HCs) showed no significant changes. Compared with HCs, enzyme-linked immunosorbent Assay (ELISA) results showed higher levels of serum IL-1β, IL-6, and TNF-a in IBS-DP patients, indicating a low-grade peripheral inflammation in IBS-DP patients. Moreover, the abundance of *Akkermansia muciniphila* (*A. muciniphila*), was negatively correlated with Hamilton Rating Scale for Depression (HAMD) score and Zung Self-Rating Depression Scale (SDS) score, IL-1β, TGF-β, and TNF-a in IBS-DP patients. These findings indicate that gut dysbiosis, especially deficiency in *A. muciniphila*, is related to the depressive symptoms and inflammation in IBS-DP patients. **Thirdly**, in a neonatal maternal separation (NMS) rat model, behavioural tests such as colorectal distention (CRD) test, open field test (OFT), forced swimming test (FST), and sucrose preference test (SPT) results showed visceral hypersensitivity and depression symptoms in rats. These results indicate that the model can successfully mimic the visceral hyperalgeisa and the depression-like behaviour of IBS-DP. Immunohistochemical analysis showed an altered morphology and decreased the quantity of astrocytes in the hippocampus of NMS rats when compared with that of controls. More importantly, the mRNA expressions of genes related to Astroglial glutamate transmission including glutamate transporters (GLTs), glutamate receptors, and also glutamate-related exchangers, as

well as astrocyte biomarker glial fibrillary acidic protein (GFAP), which are mediated with chronic inflammation and/or stress, were decreased in NMS rats when compared with the control group. These results indicate that impaired astroglial glutamate neurotransmission in NMS rats. Furthermore, pseudo-GF rats with faecal microbiota transplantation (FMT) of NMS microbiota were also conducted, and results showed that the association of *A. muciniphila* deficiency, depressive-like behaviours and impaired astroglial glutamate neurotransmission were repeated in the rat recipients. These results indicate a causal relationship between NMS microbiota and depressive phenotype, involving dysfunction of the astrocyte- glutamate pathway. **Fourthly**, to further verify the role of *A. muciniphila* in NMS microbiota-induced depressive phenotype and impaired astrocytic glutamate pathway, we orally administered live and heat-killed *A. muciniphila* bacteria in NMS adult rats. *A. muciniphila* (10^8 CFU in 1mL PBS) was administered once-daily for four consecutive weeks. Besides, rifaximin and fluoxetine were also separately treated in NMS rats as control groups. Rifaximin is a broad-spectrum GI-specific antibiotic that is commonly used for IBS treatment, and fluoxetine, a selective serotonin re-uptake inhibitor, is one of the most frequently prescribed anti-depressants. The results showed that *A. muciniphila* efficiently improved depressive-like behaviours, attenuated the impaired astrocytic glutamate neurotransmission, as well as restored the normal morphology and number of astrocytes in the hippocampus of NMS rats. While rifaximin-treated rats only exhibited amelioration of visceral pain, and fluoxetine group mainly performed antidepressant effect, without any significant change in astrocytic glutamate neurotransmission impairment. These results demonstrate that *A. muciniphila* improves depressive symptoms in IBS phenotype and ameliorate astroglial-

glutamatergic pathway dysfunction. Whether and how *A. muciniphila* modulates astroglial glutamate transmission, therefore leading to the improvement of depressive symptom in IBS, remains to be further investigated.

Taken together, the works of this thesis, combining both clinical and animal studies reveal that gut dysbiosis, particularly deficiency of *A. muciniphila*, contributes to the development of IBS-DP via regulating the astroglial glutamatergic pathway. This study gives a different direction to microbial-guided therapy for the IBS-DP patients in the future.

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