PROGRESS REPORT for Mizutani Foundation Research Grant

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Principal Investigator: Hai-Bin Ruan, PhD
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Grant Title: Gut microbiota-sensitive O-GlcNAc signaling protects intestinal inflammation
Progress Report:

(a) Abstract:

Inflammatory bowel disease (IBD) is a result of interactions between genes and environment. It is well-recognized that commensal microbiota-derived signals help maintain barrier function and restrain inflammation in the intestinal epithelium. In this project, we aimed to defining protein O-linked N-acetylglucosamine (O-GlcNAc) as a novel mechanism by which intestinal epithelium senses and integrates microbial signals in health and disease. We demonstrated that protein O-GlcNAcylation levels in intestinal epithelial cells (IECs) was dependent on gut microbiota-derived short-chain fatty acids (SCFAs). In IECs of human IBD patients, levels of protein O-GlcNAcylation and the expression of O-GlcNAc transferase (OGT), the enzyme adding the O-GlcNAc moiety, were reduced and negatively correlated with disease severity. Deletion of OGT specifically in IECs in mice resulted in disrupted epithelial barrier, microbial dysbiosis, Paneth cell dysfunction, and intestinal inflammation. Using fecal microbiota transplantation in mice, we found that microbial dysbiosis although was insufficient to induce spontaneous inflammation but exacerbated chemical-induced colitis. Paneth cell-specific deletion of OGT leaded to Paneth cell dysfunction, which might predispose mice to chemical-induced colitis. On the other hand, the augmentation of O-GlcNAc signaling by inhibiting O-GlcNAcase, the enzyme removing O-GlcNAcylation, alleviated chemical-induced colitis. Our data reveals that protein O-GlcNAcylation in IECs controls key regulatory mechanisms to maintain mucosal homeostasis.

(b) Objectives:

We hypothesized that, in response to microbiota-derived SCFAs, epithelial O-GlcNAc maintains intestinal homeostasis by strengthening the barrier function.

We proposed the following specific aims: Aim 1, determine the role of O-GlcNAc in microbiota-host interactions; Aim 2, assess the effects of elevated intestinal O-GlcNAc levels on inflammation.

(c) Methods used:

In Aim 1, we treated cultured Caco-2 cells and wildtype mice with SCFAs to determine if O-GlcNAcylation in IECs is a metabolic sensor for microbial SCFAs. In Aim 2, we pharmacologically elevated protein O-GlcNAcylation by TMG and genetically overexpressed OGT specifically in IECs in mice to test if mice were protected from chemical-induced colitis.

(d) Results obtained, including negative data:

In Caco-2 cells, we found that SCFAs could not directly increase OGT expression or global O-GlcNAcylation, suggesting an indirect effect. It is known that SCFAs are important for immune development and function in the intestinal mucosa, and we tested several immune cytokines and found that IL-4 could activate OGT and increase protein O-GlcNAcylation, indicating that microbiota control epithelial O-GlcNAcylation by indirectly activate the type 2 immune responses.

To determine whether pharmacologically elevated protein O-GlcNAcylation strengthens barrier function and protects mice from chemical-induced acute colitis, we orally administered water or TMG, an OGA inhibitor, to C57Bl/6 mice, followed by 2.5% DSS in drinking water. TMG-treated mice lost less body weight and had lower colitis score. In vivo barrier functional assay showed that TMG treatment reduced intestinal permeability before and after DSS induction. TMG-treated mice also had longer colon length, accelerated mucosal recovery, and lower levels of inflammatory genes. These data demonstrated that augmenting global protein O-GlcNAcylation by TMG pretreatment ameliorates chemical-induced acute colitis. We also obtained IEC-specific OGT overexpression mice; however, we could not observe any significant increase in protein O-GlcNAcylation, thus prevented us using the model to test our hypothesis.

In addition, we proposed microbial dysbiosis and Paneth dysfunction observed in *IEC-OGT* KO mice precipitated intestinal inflammation cooperatively. Using fecal microbiota transplantation in mice, we demonstrated that microbial dysbiosis although was insufficient to induce spontaneous inflammation but exacerbated chemical-induced colitis. Paneth cell-specific deletion of OGT leaded to Paneth cell dysfunction, which might predispose mice to chemical-induced colitis.

(e) Discussion:

Here we found that protein posttranslational O-GlcNAc modification in IECs was sensitive to microbiota-derived SCFAs and was compromised in human IBD. IEC-specific knockout of OGT leaded to intestinal damage and inflammation in mice. O-GlcNAcylation was a regulator of multiple homeostatic modules in the epithelium. Microbial dysbiosis and Paneth cell dysfunction together potentiated chemical-induced inflammation. Our study will shed light on the future design of novel preventions and therapeutics for IBD.

(f) List of publications:

- Zhao M*, Xiong XW*, Ren K, Xu B, Cheng M, Sahu C, Wu K, Nie Y, Huang Z, Blumberg RS, Han X, **Ruan HB**. (2018). Deficiency in intestinal epithelial O-GlcNAcylation predisposes to gut inflammation. *EMBO Molecular Medicine*. In press. (*, Equal contribution).

- Huang Y, Lia SC, Hu J, **Ruan HB**, Guo HM, Zhang HH, Wang X, Pei YF, Pan Y, Fang C. (2018). Gut Microbiota Profiling in Han Chinese with Type 1 Diabetes. *Diabetes Research and Clinical Practice*. doi: 10.1016/j.diabres.2018.04.032.

(g) Name (signed) & Date:

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