学 位 論 文 の 要 旨

論文題目　Studies on novel roles of dietary fibers for intestinal homeostasis

（消化管の恒常性維持における食物繊維の新たな役割に関する研究）

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博士課程後期　生物機能開発学　専攻

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1. General Introduction (written in Chapter 1)

Human health is critically dependent on the maintenance of intestinal homeostasis. Inflammatory boIl diseases (IBDs) are a group of gastrointestinal disorders including Crohn's disease (CD) and ulcerative colitis (UC) and characterized by chronic inflammation. The number of individuals diagnosed with both UC and CD has steadily increased in the past several decades. IBD patients experience chronic and relapsing inflammation in intestines and suffer from diarrhea, abdominal pain and rectal bleeding. Although the etiology for IBD is unknown, it is believed that the impaired intestinal barrier resulting in hyperpermeability to luminal noxious molecules and robust chronic activation of immune system contribute to the development of intestinal inflammation. Accordingly, it is meaningful for us to develop the novel preventive and/or therapeutic approaches in the maintenance of intestinal homeostasis.

Dietary fiber (DF) is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Accumulating evidence shows that supplemental feeding with DFs provides various beneficial effects for our health. Inflammatory status of intestines also seems to be regulated by feeding DFs and subsequent modification of intestinal microbiota. Intestinal fermentation of DFs produces different metabolites including short chain fatty acids (SCFAs). However, precise roles of DFs for regulation of intestinal inflammation are still unclear.

The objective of the present study was to understand novel roles of DFs for the maintenance of intestinal homeostasis. I used the murine models of experimental colitis and CKD and the intestinal epithelial models under inflammatory conditions.

2. Fermentable and viscous DFs reduce intestinal barrier defects and inflammation in colitic mice

In Chapter 2, I aimed to investigate the preventive effect of guar gum (GG) fiber on colonic inflammation and barrier defects in dextran sodium sulfate (DSS)-induced colitis mice. GG fiber, a soluble DF, is characterized by high fermentability and high viscosity. DSS administration caused severe colon damage and inflammation, as indicated by body Iight loss, increased clinical scores, colon shortening, increased plasma lipopolysaccharide binding protein (LBP), elevated myeloperoxidase activity, and decreased TJ protein expression in the colon. Supplemental feeding with GG fiber partially or totally reversed

these symptoms, suggesting that GG fiber ameliorates the DSS-induced colitis at least partially through protection of the TJ barrier.

3. Fermentable DFs reduce intestinal barrier defects and inflammation in colitic mice

In Chapter 3, I aimed to examine the physicochemical properties of DFs contributed to protection of colitis against DSS. Along with GG, mice Ire fed with partially enzymatic hydrolyzed GG (PHGG), which shares high fermentability with GG, but presents low viscosity due to the low molecular mass. The results found that feeding PHGG as Ill as GG reversed the colitic symptoms, suggesting that high fermentability, rather than viscosity, is important for the DF-mediated protection of colons against DSS. In addition, feeding GG and PHGG suppressed the increased colonic cytokine expression by DSS and increased the production of SCFAs through the microbial fermentation. These observations suggest that SCFAs at least in part contribute to the anti-inflammatory effects of PHGG and GG through the suppression of inflammatory cytokines.

4. SCFAs suppress inflammatory reactions in Caco-2 cells and mouse colons

In Chapter 4, I aimed to examine the roles of SCFAs on the regulation of inflammatory reactions in the colonic epithelium using human intestinal Caco-2 cells and mouse colons. Stimulation of Caco-2 cells with tumor necrosis factor (TNF)-α increased interleukin (IL)-8 and IL-6 expression through the inflammatory cellular signaling, whereas pre-treatment of cells with acetate, propionate and butyrate suppressed these inflammatory reactions by TNF-α. Pharmacological inhibition of monocarboxylate transporter (MCT)-1 attenuated

the SCFAs-mediated suppression of the TNF-α-induced inflammatory responses. Administration of DSS to mice increased the CXC motif chemokine ligand 2 (an IL-8 homologue) and IL-6 expression in the colonic organ culture, whereas treatments with SCFAs mixtures composed of acetate, propionate and butyrate decreased them. These results indicate that the SCFAs acetate, propionate, and butyrate suppressed up-regulation of cellular signaling and expression of IL-8 and IL-6 in TNF-α-stimulated Caco-2 cells and in colons of colitic mice. Activity of MCT-1, located on the apical membranes, was essential for SCFA effects.

5. GG fiber suppresses inflammatory response in small intestinal epithelial cells

In Chapter 5, I examined the anti-inflammatory effect of intact GG fiber in small intestinal epithelium. Because ingested DFs such as GG pass through the small intestines without any degradation, they directly interact with small intestinal epithelium. Although the SCFAs, microbial metabolites of DFs, shoId the anti-inflammatory regulation in colons, the intact DFs may also present the biological functions. I hypothesized that the intact GG has a role for the regulation of inflammatory responses in small intestinal epithelium based on the observation that GG suppressed the inflammatory cytokine expressions in the small intestines of DSS-administered mice. Pre-treatment of cells with GG suppressed the production of the IL-8 in intestinal Caco-2 cells stimulated by TNF-α. Interestingly, the pre-incubation of cells with anti-TLR-2 or anti-dectin-1 reduced the suppressive effects of GG fiber. In addition, the

reporter cells confirmed the direct interaction and stimulation of TLR-2 and dectin-1 with GG fiber. Taken together, GG suppresses the inflammatory response in intestinal Caco-2 cells through the activation of TLR-2 and dectin-1.

6. General Discussion (written in Chapter 7)

The present results demonstrated that fermentable DFs, such as GG and PHGG, had ameliorative effects on intestinal barrier defects and inflammation in a murine model of colitis and CKD. Fermentable DFs reach the colons and are metabolized to SCFAs by microbial activity without any degradation in small intestines. My results show that both the intact DF and the SCFAs have roles for the regulation of intestinal inflammation with the distinct molecular mechanisms. The present study suggested that supplemental feeding with fermentable DFs might be beneficial for prevention and/or management of different disorders associated with intestinal inflammation and barrier defect.