学 位 論 文 の 要 旨

論文題目　　Studies on intestinal barrier regulation by dietary polyphenols

(ポリフェノールによる腸管バリア調節作用に関する研究)

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1. Introduction and Literature Review (written in Chapters 1 and 2)

Intestinal tight junction (TJ) structures provide a physical barrier to the permeation of luminal pro-inflammatory molecules such as pathogens, toxins, and dietary antigen. The TJ structure consists of different proteins, including both transmembrane and cytosolic proteins such as occludin, claudins, and zonula occludens. Some basic scientific and clinical studies have shown that there is a close association between intestinal barrier defect and the pathogenesis of different inflammatory diseases. Intestinal barrier defects that result in the penetration of pro-inflammatory molecules induce abnormally robust inflammatory responses, as in the case of inflammatory bowel disease (IBD). Due to the crucial roles of intestinal barrier regulation in health, much attention is being paid to the involvement of TJ in therapeutic and preventive approaches against some disorders. A growing body of evidence suggests that IBDs are initiated and developed through the interaction between excess immune reaction and barrier defects in intestines. Previous studies by our research group have demonstrated that dietary polyphenols such as quercetin, kaempferol, and naringenin strengthen and protect the intestinal TJ barrier and reduce intestinal inflammation. However, information on the roles of polyphenols in intestinal barrier function is still limited and protection of the TJ structure could be effective approaches to preventing intestinal inflammation and treating IBDs.

The purposes of this study were to survey and select polyphenols having potentials to promote intestinal TJ barrier integrity, to examine alleviative effects of selected polyphenol on intestinal barrier defects and inflammation in both *in vitro* and *in vivo* studies, and to unveil molecular mechanisms underlying the polyphenol-mediated protection in intestinal TJ barrier.

2. Promotion of Intestinal Barrier Function by Dietary Polyphenols

In Chapter 3, the study aimed to survey and to select polyphenols including

resveratrol, EGCG, and delphinidin having potentials to promote intestinal TJ barrier integrity, and to investigate the promotive effect of selected polyphenol (resveratrol) in the TJ protein expression and localization in Caco-2 cell monolayers. Among three polyphenols (resveratrol, EGCG and delphinidin), only resveratrol showed to increase the intestinal barrier integrity in Caco-2 cell monolayers, evidenced by TER measurement. Therefore, the following studies focused on the effect of resveratrol on the intestinal barrier function *in vitro* (Chapters 5, 6 and 7) and *in vivo* (Chapter 4). This chapter shows that resveratrol has the potential to promote TJ barrier integrity in intestinal Caco-2 cells. In the absence of harmful substances, resveratrol-mediated promotion of TJ barrier integrity occurs through increased expression and assembly of TJ proteins in intestinal epithelial cells.

3. Resveratrol Prevents Intestinal Barrier Defect and Inflammation in Colitic Mice

In Chapter 4, the study aimed to examine the ameliorative effect of dietary resveratrol in a murine model of colitis induced by dextran sodium sulfate (DSS) administration, focusing on neutrophil infiltration and TJ structure. DSS administration resulted in dramatic body weight loss, watery and bloody diarrhea, and colon length shortening, but supplemental resveratrol ameliorated these symptoms. DSS administration severely impaired the localization and expression of these TJ proteins, whereas resveratrol supplementation clearly attenuated these impairments induced by DSS administration. In the molecular level, gene expression of inflammatory molecules—including TNF-α, IL-6, IL-17A, CXCL-2, IL-1β, and MCP-1—was markedly increased by DSS, and significantly mitigated by resveratrol supplementation. The DSS-administered mice showed a higher population of neutrophil in the colonic mucosa, whereas the increase was effectively mitigated by resveratrol supplementation. In conclusion, this study suggests that supplemental resveratrol prevents the intestinal inflammation in DSS-induced colitic mice through the protection of TJ structure and prevention of neutrophil infiltration into inflamed tissues.

4. Resveratrol Restores TNF-α-Induced-IL-8 Production in Intestinal Caco-2 Cell Monolayers

In Chapter 5, the study aimed to examine the resveratrol-mediated suppression of neutrophil infiltration using Caco-2 cell monolayers. This study suggests that supplemental resveratrol prevents barrier defects and inflammation through the inhibition of IL-8 production in inflamed tissue, in turn resulting in the prevention of neutrophil infiltration into inflamed tissues. Resveratrol suppresses TNF-α-induced-inflammatory signaling and IL-8 production in Caco-2 cell monolayers. This study, therefore, elucidates the novel molecular mechanisms underlying the beneficial role of resveratrol for maintaining intestinal homeostasis.

5. Resveratrol Ameliorates Hydrogen Peroxide-Induced Intestinal Barrier Disruption in Caco-2 Cell Monolayers

In Chapter 6, the study aimed to investigate the protective effect of resveratrol in H_2O_2-induced impaired barrier model in Caco-2 cell monolayers. Occludin protein expression in Caco-2 cell monolayers was reduced by hydrogen peroxide (H_2O_2), which reduced TJ integrity; however, resveratrol restored the decrease. Treatment with H_2O_2 resulted in increased Tyr phosphorylation of occludin in the Caco-2 cells;

in contrast, resveratrol suppressed the Tyr phosphorylation. Taken together these results demonstrate that resveratrol restored the H_2O_2-induced intestinal barrier integrity by upregulating occludin expression through mitigation of Tyr phosphorylation. In conclusion, this study shows that resveratrol inhibits inflammatory or harmful intracellular signaling to protect intestinal barrier against H_2O_2-induced barrier disruption.

6. Resveratrol Ameliorates IL-6-Induced Intestinal Barrier Disruption in Caco-2 Cell Monolayers

In Chapter 7, the study aimed to investigate the protective effect of resveratrol in IL-6-induced impaired barrier model in Caco-2 cell monolayers. In pathological states, excessive IL-6 production and activation of IL-6 signaling pathways play key roles in acute and chronic inflammation. IL-6 increased claudin-2 protein expression in the Caco-2 cells; however, this increase was suppressed by resveratrol in a dose-dependent manner. Immunoblot analysis reveals that resveratrol reduces the IL-6-induced phosphorylation of ERK. The results of the present study show that resveratrol has the potential to inhibit the IL-6-induced inflammatory signaling and intestinal hyperpermeability. Resveratrol-mediated regulation of intestinal barrier integrity may be associated with prevention or alleviation of inflammatory diseases.

7. General conclusion (written in Chapter 8)

In the *in vitro* study using intestinal Caco-2 cells, resveratrol shows the potential to protect the TJ barrier and promote TJ barrier integrity. In the absence of harmful substances, resveratrol-mediated promotion of TJ barrier integrity occurs through increased expression and assembly of TJ proteins in intestinal epithelial cells. In addition, resveratrol inhibits inflammatory or harmful intracellular signaling induced by IL-6 and H_2O_2 and suppresses the barrier disruption. Resveratrol-mediated regulation of intestinal barrier integrity may be associated with prevention or alleviation of inflammatory diseases. The *in vivo* study using a murine model of colitis suggests that supplemental resveratrol prevents barrier defects and inflammation through the protection of TJ barrier structure and the inhibition of neutrophil infiltration into inflamed tissues. In addition, the study using Caco-2 cell monolayers demonstrated that resveratrol directly suppresses the TNF-α-induced-inflammatory signaling and IL-8 production. This study, therefore, elucidates the novel molecular mechanisms underlying the beneficial role of resveratrol for maintaining intestinal homeostasis.