学 位 論 文 の 要 旨

論文題目　Regulation of heat shock proteins in the intestine by dietary fibers
（食物繊維による腸管ヒートショックタンパク質発現の調節に関する研究）

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1. Introduction

Disrupted intestinal barrier can lead to entry of noxious luminal contents into the circulation, triggering the immune response and causing chronic inflammatory diseases such as chronic kidney disease, cardiovascular disease, and diabetes. This highlights the importance of maintaining the intestinal epithelial integrity and human health.

Studies have identified the crucial role of heat shock proteins (HSP) in maintaining and protecting the integrity of intestinal epithelial cells. HSPs restore damaged intracellular proteins to protect the function, barrier integrity, and survivability of epithelial cells under stressful conditions. For instance, the induction of HSP25 and HSP70 expression was shown to promote cytoprotection in intestinal epithelial cells and linked to the suppression of experimental colitis in mice. Thus, the regulation of HSP expression by dietary components could be a potential therapeutic strategy to promote intestinal health.

Several studies have highlighted that dietary fibers (DFs) promote intestinal homeostasis. Intestinal microbiota ferments DFs to generate different metabolites including short chain fatty acids (SCFAs) such as acetate, propionate, and butyrate. Previous studies in our laboratory demonstrated that fermentable DFs, guar gum (GG) and partially hydrolyzed GG (PHGG), reduced intestinal inflammation and barrier defects in a murine model of colitis, suggesting that the microbial metabolites such as SCFAs have a crucial role in maintaining the intestinal homeostasis. Whereas intact GG is shown to upregulate the suppressor of cytokine signaling1 (SOCS1) through activation of toll-like receptor (TLR)-2 and dectin-1 signaling pathways to reduce inflammation in the small intestine of mice.

Since both HSP25 and HSP70 are attributed to intestinal cytoprotection, the purpose of this study was to examine the effect of GG and PHGG on intestinal HSP25 and HSP70 expression in mice. The study also highlights the importance DF fermentation by microbiota to confer the physiological benefits of DFs in the colon. Furthermore,

the regulation of HSP70 expression by SCFA was examined using human intestinal Caco-2 cells in *in vitro* studies.

2. Regulation of intestinal HSP25 and HSP70 expression by GG in mice

The study examined if GG had a promotive effect on HSP25 and HSP70 expression in the intestine of mice. The results showed that feeding GG increased the HSP70 protein expression in epithelial cells of both the small intestine and colon but did not affect HSP25. These findings suggest that the structure of intact GG directly stimulate the epithelial cells to increase the HSP70 in the small intestines. Whereas the microbial metabolites of GG such as SCFAs have a role in the HSP70 expression in the colon. Feeding GG fiber diet increased SCFA production, such as acetate, propionate, and n-butyrate.

3. The effects of GG in comparison with PHGG on HSP regulation in mice

PHGG is produced through the controlled enzymatic hydrolysis of GG fiber and has low viscosity, unlike GG. To determine whether the viscosity of GG plays a role in the increased HSP70 expression in mouse intestine, PHGG was administered to the mice. Like GG, feeding PHGG increased the HSP70 expression in both small intestine and colon of mice. However, no effect on HSP25 expression was observed. Supplemental GG and PHGG fibers increased the cecal acetate, propionate, and n-butyrate in mice. These results suggest that the HSP70 expression by GG and PHGG is independent of their difference in viscosities. Whereas the increased SCFAs production may be responsible for the upregulation of colonic HSP70 in mice fed GG and PHGG fibers. To clarify if the bacterial generated SCFAs are important in the HSP70 expression, the HSP70 expression in the colon of germ-free (GF) mice was examined in the next study.

4. Influence of microbiota activity on colonic HSP70 expression

This study utilized specific pathogen free (SPF) and GF mice and the HSP70 expression in the colon and cecal SCFA profile was determined. As a result, HSP70 expression in the colon of SPF mice was significantly higher compared to GF mice. Acetate, propionate, and butyrate were highly generated in the cecum of SPF mice, whereas their production was barely observed in GF mice. Considering these two observations, we suggest that colonic bacteria activity: namely fermentation and generation of SCFAs is closely involved in the HSP70 expression in the colon of SPF mice. This result supports the notion that HSP70 expression in the colon is dependent on microbial signals or metabolites. Hence, the next study investigated the effect of SCFAs on HSP70 regulation and the underlying mechanisms.

5. Regulation of HSP70 expression in Caco-2 Cells by SCFA

In this section, *in vitro* studies were carried out to elucidate roles of SCFAs on HSP70 expression using human intestinal Caco-2 cells. Propionate, butyrate but not acetate, increased the HSP70 protein expression in Caco-2 cells. Phosphorylation (activation) of heat shock factor1 (pHSF1), a well-known transcriptional factor of HSP70, was also increased by propionate and butyrate, but not acetate. The qRT-PCR analysis demonstrated the increase

in *Hspa1a* (HSP70) mRNA levels by propionate and butyrate in a dose-dependent manner. Propionate and butyrate increased *Hspa1a* promoter activity in a dose-dependent manner indicating that the HSP70 expression induced by propionate and butyrate occurs at transcriptional level. The pharmacological inhibition of MEK and mTOR kinases downregulated HSP70 expression by propionate and butyrate. The result also reveals that at least these 2 kinases are important in the activation of HSF1. Propionate and butyrate can modulate cellular functions via their ability to inhibit histone deacetylases (HDAC). Trichostatin A (TSA), a well-known HDAC inhibitor, increased HSP70, phosphorylated HSF1 and *Hspa1a* promoter activity in Caco-2 cells. This suggests that propionate and butyrate-induced upregulation of HSP70 is partly mediated by HDAC inhibition.

6. General conclusion

Fermentable DFs such as GG and PHGG increased the colonic HSP70, possibly through the SCFA production by intestinal microorganisms. In particular, propionate and butyrate, but not acetate activate the transcriptional regulation of HSP70 via HSF1 phosphorylation. At least, two kinases, MEK and mTOR, are responsible for the HSF1 phosphorylation. In addition, HDAC inhibition also seems to be involved in the HSP70 expression. HSP70 expression possibly increases the integrity and viability of intestinal epithelial cells, contributing to the symbiosis with intestinal microorganisms and maintaining intestinal homeostasis. The increased colonic HSP70 could be involved in the fermentable DFs-mediated benefits to intestinal health.