Doctoral Thesis

Study on a novel function of non-digestible saccharides in the intestines

(Summary)

DINA MUSTIKA RINI

Graduate School of Integrated Sciences for Life

Hiroshima University

September 2023

1. General Introduction (written in Chapter 1)

Apart from the main function of the intestinal epithelium to digest food and absorbs food-derived nutrients, it also serves as a physical and biochemical barrier to protect the body from potentially harmful substances such as food antigens, pathogens, and microbial toxins. The pathogenesis of various diseases, such as metabolic diseases, cardiovascular diseases, chronic kidney diseases, and inflammatory diseases, is closely associated with impaired barrier function as a result of systemic and intestinal inflammation. The intestinal epithelial barrier is composed of the interaction of several barrier components, including antimicrobial peptides and intercellular tight junctions (TJs). In addition, heat shock protein (HSP) is crucial to maintain intestinal barrier function in response to environmental stress and cytotoxic agents. Thus, understanding the mechanisms regulating these intestinal barrier components is critical for preventing infections and maintaining human health.

Non-digestible saccharides (NDS) are known to reduce the incidence of chronic diseases. NDS are carbohydrate polymers that escape digestion and absorption in the upper gastrointestinal tract, remain intact, and enter the colon. In the colon, NDS are fermented by the colonic microbiota to produce short-chain fatty acids (SCFAs), which are responsible for most of the beneficial effects of NDS. However, NDS can also suppress inflammation in the small intestine, where there is less microbial activity, suggesting that the direct interaction of NDS with intestinal epithelium may affect the cellular functions. Sensory mechanisms may exist in the intestinal epithelium to detect NDS and activate various pathways to regulate intestinal barrier function, such as chemical sensing by specific receptors and mechanosensing by mechanically activated ion channels. Recent studies revealed that some NDS are directly recognized by intestinal epithelial and immune cells through pattern

recognition receptors such as toll-like receptors and C-type lectin receptors. Meanwhile, in response to mechanical stimuli, the Piezo1, a non-selective ion channel, converts the mechanical stimuli into a biochemical signal, that triggers the downstream cellular response. However, the direct effects of NDS on intestinal barrier function remain unknown.

The aim of this study was to investigate the direct effect of NDS on intestinal barrier function and the underlying mechanisms in mouse and human intestinal Caco-2 cells. We used 3 different NDS such as partially hydrolyzed guar gum (PHGG), xylobiose, and psyllium.

2. Effects of PHGG on the regulation of HSP27 in the small intestine of mice and Caco-2 cells

In Chapter 3, we investigated the direct effect of PHGG on maintaining intestinal barrier function by regulating heat shock proteins. This study found that PHGG upregulates HSP27 expression, a cytoprotective protein, in intestinal Caco-2 cells. PHGG possibly exerts this effect through translational modulation by increasing the activation of mechanistic targets of rapamycin (mTOR). In addition, the deactivation of extracellular signal-regulated kinase 1/2 (ERK) is likely involved in the PHGG-mediated HSP27 expression. Our study also demonstrated that mice fed with PHGG fiber increased HSP25 expression in the small intestinal epithelial cells. Taken together, supplemental PHGG enhances the intestinal epithelial integrity through the translational regulation of HSP27.

3. Effects of xylobiose on the regulation of TJs and HSPs in Caco-2 cells

In Chapter 4, we investigated the direct effect of a non-digestible disaccharide, xylobiose, on the maintenance of intestinal barrier function. This study showed that

xylobiose suppressed claudin-2 and increased HSP27 expression in intestinal Caco-2 cells. Different signaling pathways appear to regulate the claudin-2 and HSP27 by xylobiose at the post-transcriptional level. Phosphatidyl 3-inositol kinase (PI3K) signaling may be involved in xylobiose-mediated claudin-2 expression. Meanwhile, PI3K, mitogen-activated protein kinase kinase (MEK), and Src were possibly involved in xylobiose-mediated HSP27 expression. However, the precise underlying mechanisms are still unclear and require further investigations.

4. Role of Piezo1 on psyllium-mediated antimicrobial peptide Reg3β in the small intestine of mice

In Chapter 5, we investigated the mechanistic link between the mechanosensitive Piezo1 channel and the physiological function of psyllium fiber in mice lacking Piezo1 specifically in the intestinal epithelium. In this study, we found that mechanical forces induced by psyllium fiber activate Piezo1 and further upregulate the antimicrobial peptide Reg3β in the small intestine of mice. Activation of STAT3 seems to be involved in the Piezo1-mediated Reg3β expression by psyllium fiber. An *in vitro* study using intestinal Caco-2 cells confirmed that Piezo1 activation increased *REG3G* mRNA, a human homolog of murine *Reg3b*. Although psyllium fiber modulates the diversity and composition of the cecal microbiota, the contribution of the psyllium-mediated Reg3β expression remains unclear.

5. General conclusions (written in Chapter 6)

The present studies demonstrated that NDS, such as PHGG, xylobiose, and psyllium fiber regulate the intestinal barrier function in the small intestine of mice and Caco-2 cells. Our studies showed that intact PHGG, xylobiose, and psyllium fiber play a role in regulating intestinal barrier components by directly interacting with intestinal epithelial cells via different molecular mechanisms. Although the precise mechanism is still unclear, the present study provides Sa new insight into the molecular mechanism of NDS in protecting and maintaining intestinal homeostasis.