学 位 論 文 の 要 旨

論文題目　Regulation of intestinal homeostasis by gut microbial metabolites
腸内細菌代謝物による腸管恒常性の制御

広島大学大学院統合生命科学研究科
Food and AgriLife Science　プログラム
学生番号　D190241
氏　　名　徐聞曦 Wenxi Xu

Introduction (Written in Chapter 1)

Trillions of microorganisms reside in the human gut, and they are collectively known as the intestinal microbiota. At homeostasis, gut microbes and humans are mutualistic. Gut microbes obtain energy mainly via metabolizing undigested carbohydrates and proteins, whereas the human host benefits from some microbial metabolites, such as short chain fatty acids (SCFAs) and vitamins. On the other hand, the bacterial metabolism of some nutrients may also create potentially harmful metabolic products such as hydrogen sulfide (H_2S) and ammonia. The accumulation of these substances has been associated with an increase in diseases.

Desmosomes, adherent junctions, and tight junctions (TJs) connect intestinal epithelial cells to form the epithelial layer, which, in conjunction with the adhesive mucous gel layer and immune regulators, constitutes a complex intestinal barrier system. As one of the greatest physical and immunological epithelial barriers, the intestinal barrier protects human health by separating the internal host milieu from the external environment and immune sensing. The integrity of the intestinal physical barrier is determined mainly by the TJs.

In this study, we focused on the microbial metabolites, especially H_2S and SCFAs, with the objectives of investigating their roles on the regulation of intestinal barrier and homeostasis.

Hydrogen sulfide suppresses the proliferation of intestinal epithelial cells through cell cycle arrest (Written in Chapter 2)

The pathogenesis of chronic kidney disease (CKD) is closely related to the changes in the intestinal microbiota and integrity. Our previous studies have shown the accumulation of hydrogen sulfide (H_2S)-producing bacterial family, *Desulfovibrionacea*, in the colon of a murine model of CKD, suggesting that the increased H_2S contributes to the impaired intestinal integrity in CKD. Here, we investigated the anti-proliferative effect of H_2S in the intestinal epithelial cells. A slow H_2S releasing molecule GYY4137 ((p-methoxyphenyl)morpholino-phosphinodithioic acid) reduced the proliferation of Caco-2 and IEC-6 cells. Flow cytometric analysis demonstrated that GYY4137 accumulated Caco-2 cells in the S phase fraction, suggesting that H_2S arrested the cell cycle at G2 and/or M phases. The RNA sequencing analysis demonstrated that GYY4137 modulated the mRNA expression of the genes involved in the G2/M and the spindle assembly checkpoints; increased mRNA levels of *Cdkn1a*, *Gadd45a*, and *Sfn* and decreased mRNA levels of *Cdc20*, *Pttg1*, and *Ccnb1* were observed. These alterations were confirmed by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and Western blot analyses. Besides, studies exploring the MEK inhibitor indicated that MEK activation is involved in the GYY4137-mediated increase in the *Sfn*

expression. Altogether, our data showed that H_2S reduced the proliferation of intestinal epithelial cells through transcriptional regulation in G2/M and the spindle assembly checkpoints. This may be one of the underlying mechanisms for the observed impaired intestinal integrity in CKD.

Butyrate regulates intestinal homeostasis through regulation of *Cldn23* (Written in Chapter 3)

The intestinal microbiota and its metabolites would positively and negatively regulate the intestinal barrier. As a result, they may either safeguard human health or be implicated in the development of certain disorders. In this study, we aimed to investigate the roles of intestinal microbiota on the TJs. The RNA-seq found that the expression of *Cldn23* is significantly lower in the germ-free mice compared to the specific pathogen-free mice. The qRT-PCR analysis confirmed this result. In addition, claudin-23 is expressed higher in the lower part of the gastrointestinal tract, which has higher diversity and abundance of microbiota than the upper intestinal part. These findings suggest that the intestinal claudin-23 expression, at least in part, depends on the intestinal microbiota, and specific components and/or metabolites of intestinal microbiota possibly have a role in the claudin-23 expression. The qRT-PCR, promoter assay, and western blot analyses revealed that butyrate transcriptionally upregulated claudin-23 expression. Furthermore, butyrate treatment enhanced the TJ barrier integrity in Caco-2 cells. Studies exploring a siRNA targeting SP1 and the AMPK inhibitor indicated that the SP1 transcription factor and AMPK pathway are involved in the butyrate-mediated transcription of *Cldn23*.

General conclusion (Written in Chapter 4)

In this study, we focused on two microbial metabolites, H_2S and butyrate, and investigated their roles in regulating intestinal barrier and homeostasis. Our findings would help to understand the physiological and pathological interactions between intestinal microflora and the intestinal barrier.