論文 / 著書情報
Article / Book Information

題目(和文)	腸内細菌代謝産物による腸上皮免疫の昼夜リズム調節
Title(English)	Circadian rhythm regulation in intestinal epithelial immunity by intestinal bacterial metabolites
著者(和文)	DUYAO
Author(English)	Yao Du
出典(和文)	学位:博士(学術), 学位授与機関:東京工業大学, 報告番号:甲第12825号, 授与年月日:2024年6月30日, 学位の種別:課程博士, 審査員:折原 芳波,梶原 将,小畠 英理,黒田 公美,小倉 俊一郎,柘植 丈治
Citation(English)	Degree:Doctor (Academic), Conferring organization: Tokyo Institute of Technology, Report number:甲第12825号, Conferred date:2024/6/30, Degree Type:Course doctor, Examiner:,,,,,
学位種別(和文)	博士論文
Category(English)	Doctoral Thesis
種別(和文)	論文要旨
Type(English)	Summary

（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 ライフエンジニアリング	系 コース	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	(Philosophy)
学生氏名： Student's Name	DU YAO		審査員主査： Chief Examiner	折原　芳波	

要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

Circadian rhythm is a natural and internal process that regulates the most physiology in life from bacteria to mammals and repeats roughly every 24 hours and play an important role in the intestinal homeostasis and intestinal immune function. Circadian rhythm dysregulation was reported to induce intestinal microbiota dysbiosis, which further led to intestinal barrier broken and triggering intestinal inflammation. However, the relationship between intestinal microbiota metabolites and the circadian rhythm of the intestinal epithelial immune system was still unclear. This study aimed to explore the effect of intestinal microbial metabolites on the circadian rhythm of the intestinal epithelial immune system. This study also attempts to determine the impact of gut bacterial metabolites on the disruption of the circadian rhythm of the intestinal immune system caused by inflammation and explore potential molecular mechanisms or signaling pathways. This research will investigate the feasibility and efficacy of using intestinal bacterial metabolites to prevent or treat Inflammatory Bowel Disease (IBD) by improving the circadian rhythm of the intestinal immune system.

By examining the effects of cecal digesta, it was confirmed that intestinal microbial metabolites can ameliorate the circadian rhythm of the intestinal epithelial immune system. Expression rhythms of *CXCL8, TNFA, MUC2,* and *CLDN1* in HT-29 and Caco-2 co-culture system were changed after cecal digesta treatment. In addition, *CXCL8* and *MUC2* mRNA expression rhythms were improved after inulin digesta treatment which can improve intestinal homeostasis.

To determine the relationship between microbiota metabolites and clock genes, UA was selected as a representative microbial metabolite. Through comparing clock genes expression rhythm in different intestinal epithelia cell models, the ability of UA on influencing the expression of clock genes in intestinal epithelial cells was confirmed. In HT-29 cells as a goblet cell model, UA increased the expression level of *BMAL1*. In Caco-2 cells, as a absorptive cell model, UA downregulated the expression level of *BMAL1* but increased the curve fitting of *BMAL1* expression rhythm and tend to increase the *PER2* expression rhythm. These results suggested that UA, as one kind of microbial metabolite, can show a direct impact on epithelial cell clock genes expression rhythms.

Furthermore, by inducing inflammation in intestinal epithelial cell model and mouse model, the effects of UA on circadian rhythm disruptions caused by inflammation were examined. UA improved the expression rhythm of *BMAL1, PER2,* and *OCLN* which downregulated by proinflammatory cytokines treatment in HT-29 cells and Caco-2 cells. UA also downregulated the overexpression rhythm of *CLDN1* induced by proinflammatory cytokines treatment in Caco-2 cells and showed similar ability in mice model which downregulated the overexpression rhythm of *Cldn1* and *Cldn4* in C57BL/6J mice. In addition, UA treatment enhanced the fecal IgA concentration especially in dark phase and increase the expression level of *Bmal1* and *Per2* in mice SCN. These findings suggest that UA may cross the blood-brain barrier and affect the SCN, thereby modulating the central circadian clock. These data suggest that chronic UA treatment can form the disrupted circadian rhythms in intestinal epithelial cells caused by inflammation.

Finally, the potential mechanisms that UA affects the circadian rhythms of intestinal epithelial immunity were elucidated. The use of antagonists reveals that AHR and Nrf2 signal pathways are involved in UA management on circadian rhythm. Expression rhythms of *PER2, MUC2, MUC3A, CLDN1,* and *IL33* in HT-29 and Caco-2 co-culture system were affected after AHR antagonist CH223191 treatment. In addition, Expression rhythms of *BMAL1, PER2, MUC2, MUC3A, CLDN1, OCLN* and *IL33* in HT-29 and Caco-2 co-culture system were affected after Nrf2 antagonist ML385 treatment. Results also showed that the effect of CH223191 and ML385 on *CLDN1, MUC2,* and *MUC3A* were different. Molecular docking simulation data indicate a high possibility of UA binding to the BMAL1-CLOCK protein complex, with the binding site located in

the PAS-A domain. These results suggested that UA may employ multiple molecular mechanisms to manage the circadian rhythms of intestinal epithelial cells.

To date, relatively few papers have discussed the direct impact of intestinal bacterial metabolites on the circadian rhythm of the intestinal epithelial immune system, particularly regarding clock genes. The findings of this study could be used to reveal the relationship between these two factors.

Overall, the influence of intestinal microbial metabolites on the expression rhythm of the intestinal epithelial immune system holds not only potential for treating IBD but may also be significant for metabolic diseases caused by modern lifestyle factors such as shift work and jet lag.