様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　６ 年　 ６ 月 １８ 日現在

機関番号：３２６７６
研究種目：若手研究
研究期間：2020～2023
課題番号：２０Ｋ１６３４７

研究課題名（和文）Single-cell transcriptomic and epigenomic analysis of young and elder mouse gastric mucosa infected by Helicobacter pylori

研究課題名（英文）Single-cell transcriptomic and epigenomic analysis of young and elder mouse gastric mucosa infected by Helicobacter pylori

研究代表者
Ｌｉｕ　Ｙｕｙｕ（Liu, Yuyu）

星薬科大学・先端生命科学研究所・特任助教

研究者番号：１０８７０４６２

交付決定額（研究期間全体）：（直接経費）　　2,800,000円

研究成果の概要（和文）：3と20週齢の非感染又はピロリ菌感染マウスの胃粘膜と線維芽細胞てDNAメチル化解析を行った結果、炎症は3週マウスでは胃粘膜、20週マウスでは胃線維芽細胞のメチル化異常を誘導した。RNA-seq解析は3と20週齢マウスでの発現変動遺伝子が異なることを示した。
正常マウスの胃組織のscRNA-seqとscATAC-seq解析では、上皮細胞での加齢関連クロマチンアクセシビリティが同定され、間質細胞ではクロマチンアクセシビリティ変化を伴わない発現変動遺伝子が特定された。3週の幹細胞一部でのみオープンクロマチンになった領域が20週マウスでは完全に閉じていることから、幼少期でのエピゲノム可塑性を示唆された。

研究成果の学術的意義や社会的意義
This research revealed epigenetic plasticity unique to infant gastric stem cells.
The mechanism of epigenetic plasticity could manifest as: 1) diversity in chromatin accessibility, or 2) the ability to rapidly modify chromatin accessibility.
Further research will aim to elucidate this hypothesis.

研究成果の概要（英文）：DNA methylation analysis was conducted on gastric mucosa and fibroblasts from 3- and 20-week-old mice, both non-infected and infected with H. pylori (PMSS1) by oral gavage for 2 or 12 weeks. Results showed that the infection induced more aberrant methylation in the gastric mucosa of 3-week-old mice but more aberrant methylation in the fibroblasts of 20-week-old mice after 12 weeks of infection. RNA-seq analysis revealed distinct sets of up-regulated genes in the gastric mucosa and fibroblasts of 3- and 20-week-old mice post-infection.
Additionally, scRNA-seq and scATAC-seq of uninfected mice stomach tissue indicated age-related differential accessible regions enriched in epithelial cells. Moreover, age-related differentially expressed genes were identified in stromal cells with unmodified chromatin accessibility.
Furthermore, chromatin regions accessible in a small proportion of 3-week-old stem cells but wholly closed in 20-week-old mice likely reflect youthful epigenetic plasticity.

研究分野：Epigenetics

キーワード：Epigenetics　Epigenetic plasticity　gastric stem cell

１．研究開始当初の背景

(1)　Our latest results revealed a differential sensitivity for inflammation-induced aberrant DNA methylation in *H. pylori* infected gastric mucosa between youngster and. This reflects that different groups of genes are affected by *H. pylori*-triggered inflammation between youngster and elderly. Specifically, known cancer driver genes (e.g. *CDKN2A*, *CDH1*) and cancer risk marker genes (e.g. *BHLHE22*, *RIMS1*) are more extensively methylated in youngster. Non-biased analysis shows that youngster presents a preferential hypermethylation in genes with high expression, including cancer driver genes. These findings might be the key to explain the aggressive phenotypes of gastric cancer in youngster if ever a cancer develops.

(2)　The results mentioned above raised the following essential scientific question: what is the mechanism causing differential sensitivity and targeting for *H. pylori*-induced aberrant DNA methylation between youngster and elderly?
As explanation, we assume that 1) the cell population is different in the gastric unit of youngster and elderly, and that 2) the basal epigenetic (e.g. DNA methylation, H3K27me3 histone modification) is different due to aging.

２．研究の目的

(1)　We aim to validate our hypotheses: 1) old gastric mucosa contains less progenitor cells than youngster, and 2) the basal DNA methylation of gastric mucosa stem cells in elder is different from that in youngster due to aging.

(2)　This research project brings explanation for the connection between the age of an individual infected by *H. pylori* and the characteristics of the disease. This knowledge will contribute to the development of gastric cancer prevention methods applicable to youngster as well as to elderly, or may even provide novel therapeutic approaches to aggressive cancers in youngster. The results will as well bring an answer to the debate on the subject of *H. pylori* eradication for middle school students.

３．研究の方法

(1)　To mimic the data obtained with human samples, we will prepare two groups of mice: the healthy ones and the ones infected with *H. pylori* at 2-week-old. Each group will then be divided again into two groups: the young mice group will be kept until 10-week-old and the old ones 18-week-old. The gastric tissue of all groups of mice will be extracted by dissection and will be subjected for further analysis.

(2)　Single-cell transcriptome analysis involves the following steps: isolation of single cell and RNA, reverse transcription, amplification, library generation and sequencing. Then, the t-SNE analysis method will be applied to identify the different type of cells present in the tissue using known cell type markers. For instance, *Lgr5* and *Aqp5* are marker genes for stomach epithelial progenitor cells and stem cells, respectively. Our hypothesis will be validated by comparing the cell fraction for progenitor and stem cells in the gastric mucosa between young and old mice.

(3)　Single-cell assay for transposase-accessible chromatin (ATAC-seq) will be applied on mice as described previously to obtain single-cell epigenomic data with the ATAC-seq method. Gastric tissues will be collected from mouse dissection, followed by the isolation of single cell and of the nucleus to be subjected to ATAC-seq. This analysis is able to detect accessible chromatin that indicate an open chromatin structure with low DNA methylation level and histone modifications favoring transcription activity. The results will show intercellular differential aging- related DNA methylation to confirm our hypothesis.

(4)　The two methods mentioned above allow the obtainment of 1) the gene expression profile of each cell type and 2) the epigenomic profile of all cells. Based on 1) the gene expression profile of each cell type, we will match back 2) the chromatin accessibility data (marker CpG islands) to the corresponding cell type by conjecture. In this manner, we will be able to identify the chromatin accessibility profile in each cell type of the gastric mucosa of youngster or elderly, and to compare the epigenetic profile of gastric epithelial stem cells. The results will lead to the identification of genes with expression affected by chromatin remodeling due to the following aberrant DNA methylation: aging-specific, inflammation-specific, and inflammation-accelerated.

４．研究成果

(1) After documentation on correspondence between the age of mouse and human, we modified our model by using 3-week-old and 20-week-old mice, which represent infant and adult individuals, respectively. Each group is either non-infected or infected by the *H. pylori* PMSS1 strain by oral gavage at 3-week-old or 20-week-old. The mice are then sacrificed 2 weeks or 12 weeks after infection, and the following samples were collected for further analysis: stomach (mucosa and fibroblasts), thymus, blood (plasma and buffy coat), liver, bone marrow, and heart.

(2) DNA methylation analysis was performed using the Mouse Infinium BeadChip on gastric mucosa, fibroblasts, PBMCs and bone marrow samples. *H. pylori* infection induced more aberrant methylations in the gastric mucosa of infants compared to adults, but caused more aberrant methylations in the fibroblasts of adults compared to infants (Figure 1). Moreover, the inflammation-induced aberrant methylations identified in the gastric mucosa of adults overlapped partially with aging-induced DNA methylations. Overall, aging-induced DNA methylations involved only a few TSS regions, but inflammation-induced aberrant methylations overlapped more CpG islands in gene bodies and TSS regions.

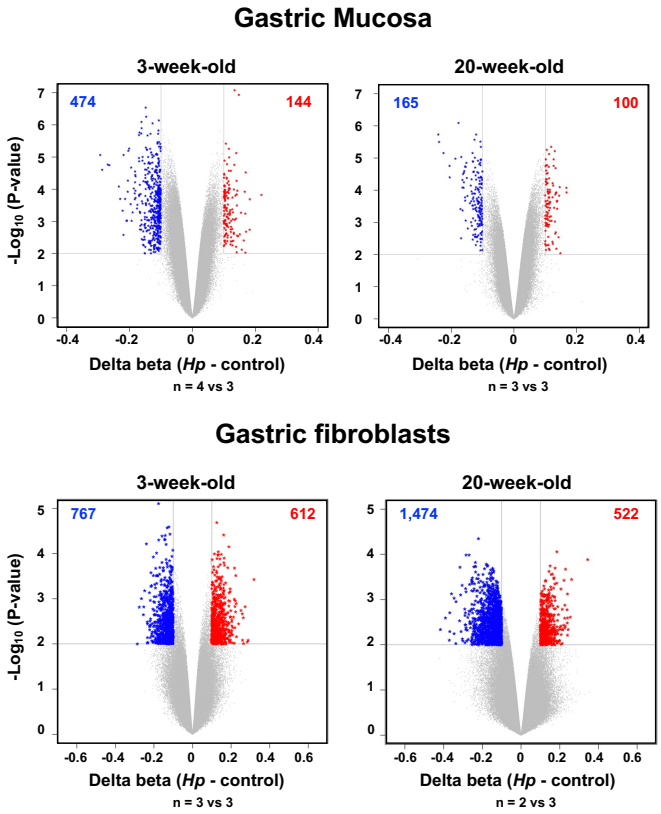

Figure 1. DNA methylation analysis of gastric mucosa (top) or fibroblasts (bottom) of 3-week-old and 20-week-old *H. pylori*-infected mice compared to control mice.

(3) RNA-seq analysis of control and *H. pylori*-infected mice gastric mucosa and fibroblasts revealed that inflammation induced distinct differentially expressed genes in infant mice compared to adult mice. This suggests a different response to environmental stimuli between the age groups.

(4) Single-cell RNA-seq analysis of healthy 3-week-old and 20-week-old mice stomach (antrum) tissue revealed an identical proportion of *Lgr5*-expressing gastric epithelial stem cells, but a larger population of fibroblasts in infant mice.

(5) Single-cell ATAC-seq analysis of healthy 3-week-old and 20-week-old mice stomach (antrum) tissue revealed age-related differential accessible regions enriched in epithelial cells, but not in stromal cells. Conversely, age-related differentially expressed genes were identified in stromal cells with unmodified chromatin accessibility regions.
Chromatin accessibility regions that are wholly close in 3-week-old stem cells but open in a small amount of stem cells in adult and aged mice are considered age-associated detrimental chromatin regions that should remain closed to avoid pathogenic risks. Approximately 5,000 such regions were identified in the stem cell cluster.
On the contrary, chromatin regions open in only a small proportion of stem cells in 3-week-old mice but completely closed in adult and aged mice reflect diversity in 3-week-old stem cells, possibly representing the epigenetic plasticity in youth and providing a new perspective on our hypothesis.

５．主な発表論文等

〔雑誌論文〕　計0件

〔学会発表〕　計9件（うち招待講演　0件／うち国際学会　1件）

１．発表者名 Yu-Yu Liu
２．発表標題 High plasticity of infant gastric epithelial cells; a potential basis for aggressive phenotypes of AYA cancers
３．学会等名 第82回日本癌学会学術総会
４．発表年 2023年

１．発表者名 Yu-Yu Liu
２．発表標題 Comparative epigenomic profiling of gastric antral tissues in infant and adult mice
３．学会等名 第16回日本エピジェネティクス研究会年会
４．発表年 2023年

１．発表者名 Yu-Yu Liu
２．発表標題 Stromal-epithelial crosstalk dependency at early life stage: implications for aggressive AYA cancers
３．学会等名 第46回日本分子生物学会年会
４．発表年 2023年

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２．発表標題 Plastic epigenome in youth: Insights from open chromatin in stem cells
３．学会等名 American Association for Cancer Research（国際学会）
４．発表年 2024年

１．発表者名
Yuyu Liu, Naoko Hattori, Chihiro Takeuchi, Satoshi Yamashita and Toshikazu Ushijima.

２．発表標題
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第81回日本癌学会学術総会

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１．発表者名
YuYu Liu, Naoko Hattori, Chihiro Takeuchi, Satoshi Yamashita and Toshikazu Ushijima.

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第35回発癌病理研究会

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2022年

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２．発表標題
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シングルセルゲノミクス研究会2022

４．発表年
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１．発表者名
Yu-Yu Liu

２．発表標題
Comparative analysis of epithelial and stromal responses to H. pylori infection in infant and young adult mice

３．学会等名
第80回日本癌学会学術総会

４．発表年
2021年～2022年

１．発表者名 Yu-Yu Liu
２．発表標題 CUT&RUN method identifies histone modifications and bivalent chromatin at single nucleosome resolution
３．学会等名 第44回日本分子生物学会年会
４．発表年 2021年～2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関