論　文　概　要 (Thesis Abstract)

○ 論 文 題 目 in silico analysis of RNA expression profiles as potential biomarkers under spaceflight
（宇宙飛行中のバイオマーカー候補を探索するための RNA 発現プロファイルのインシリコ分析）

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目　　的:

(Purpose)

Recently, space development has dramatically increased opportunities and prolonged the period for humans to stay in space. With increasing opportunities for human spaceflight, understanding of the effects of spaceflight on the human body at the molecular level is essential.

microRNA (miRNA) has been detected by less invasive methods from biological fluids, such as plasma, saliva, urine, and feces, and are promising biomarkers for various diseases, including many cardiovascular and neurological disorders and cancer. Indeed, miRNA is attracting significant attention as a research avenue for monitoring health risks associated with spaceflight. However, the underlying mechanism of circulating miRNAs during spaceflight conditions remains unknown.

The final goal of this study was the search for biomarkers to assess tissue response in spaceflight. During the four years of my Ph.D. study, I investigated gene-level effects across mice tissues in response to spaceflight and identified miRNA biomarker candidates using ground-based preliminary experiments.

対象と方法:

(Material and method)

In Chapter 1, since NASA GeneLab database was launched in 2015, this open-access database has been available for research community to provide novel hypotheses. In addition, GeneLab Analysis Working Groups (AWGs) regularly update data standardization workflows in the database to meet the latest recommendations from the bioinformatics discipline. As such, multi-omics data from a platform like NASA GeneLab can be continuously re-investigated with newer and deeper analyses to potentially extract novel insights and hypotheses that assess the biological risks of space missions. Therefore, I sought to analyze RNA-seq data across various tissues from mice under spaceflight as a pilot study by using an open-access database, NASA GeneLab.

In Chapter 2, since there are limited opportunities for spaceflight experiments, various experimental systems on Earth have been generated as simulated space environments. Simulated microgravity experiments are generally performed using two methods: an in vivo method using the mice hindlimb unloading (HU) model or an in vitro method using a clinostat or rotary cell culture system. This study performed nanoscale sample manipulation techniques and miRNA-seq using plasma samples from mice exposed to HU for 14 days.

結　　果:

(Result)

In Chapter 1, my main findings indicated that the common ontologies and critical regulators

were identified the circadian rhythm-related terms in the peripheral tissues. Furthermore, I observed asynchrony in the expression of the clock genes between certain peripheral tissues.
In Chapter 2, I identified 66 miRNA profiles in response to hindlimb unloading, which is a spaceflight model on Earth. Some tissue-specific miRNAs support the utility of plasma miRNA for minimally-invasive evaluation of internal tissues. I further discovered a previously-uncharacterized miRNA network related to the nervous system and found a potential novel-functional miRNA as a conserved stress marker related to retrograde endocannabinoid signaling and cyclic adenosine monophosphate (cAMP) signaling pathways.

考　　察:

(Discussion)

In Chapter 1, the central clock localized in the suprachiasmatic nucleus (SCN) unifies circadian rhythm between tissues through neurotransmission and hormonal signals. After the SCN receives light input from the eye, the resulting signal from the SCN synchronizes the peripheral clocks of peripheral tissues. At the same time, this light-induced mechanism can be altered by external stimuli such as food intake, which can cause peripheral tissues to perform their own phase of circadian rhythm. Therefore, result of this study is an unexpected finding given that biological mechanisms are in place to maintain synchrony in clock genes throughout the body. Given that circadian clock disruption could compromise astronaut physiology and performance, further studies on how spaceflight affects tissue functions should be an essential agenda item in order to better understand and potentially create countermeasures for such implications of human spaceflight.
In Chapter 2, my results suggested that HU-induced, stress-related TFs cause plasma miRNA expression changes. HU-DEMs appear to mainly target nervous system pathways. I also identified tissue-specific miRNA biomarker candidates that could reflect tissue status. Among HU-DEMs, I found one particular miRNA that could pose important implications for nervous system disorders by targeting nervous system pathways. Given that miRNAs are attracting a great deal of attention in the space biology field, my work could serve as an important first step by using a ground-based experimental approach. However, my pilot study remains inconclusive as to whether the change in the miRNA expressions in mice during spaceflight is due to microgravity, radiation, or both. Therefore, by studying mouse and human samples derived from actual spaceflight, future studies may more conclusively determine causes and countermeasures to health risks in space using miRNAs.

結　　論:

(Conclusion)

My studies could function as a step towards a better understanding of tissue status under spaceflight through liquid biopsy assessment. During the four years of my Ph.D. course, I

conducted an integrated RNA-seq analysis to estimate the effects across mice tissues in response to spaceflight, and identified miRNA biomarker candidates using ground-based preliminary experiments. The new line of research that arises from these achievements is to elucidate the biological mechanisms between tissue and blood during spaceflight at a molecular level. In addition, my study could not determine whether genes were primarily affected by spaceflight factors (such as microgravity and radiation) and/or extraneous factors (such as rearing environment and sample processing). I reiterate the importance of elucidating the causes of disease mechanisms using space biology research to potentially develop countermeasures that benefit humans on Earth and in space. As human and mice samples derived from liquid biopsies under spaceflight continue to be investigated, future studies may more conclusively determine the cause of and develop countermeasures for aberrant tissue status in space.