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| 学位の種類 | 博士（　人間生物学　） |
| 学位記番号 | 博 甲第　９２５９　号 |
| 学位授与年月 | 令和元年８月３１日 |
| 学位授与の要件 | 学位規程　第４条第１項該当（昭和２８年４月１日文部省令第９号） |
| 審査組織 | グローバル教育院 |
| 学位論文題目 | Study on the Association between Arginine Methylation and Cardiac Diseases<br>(心臓におけるアルギニンメチル化の機能解析) |

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# 論文の要旨
## Abstract of thesis

Arginine methylation is one of the most prevalent post-translational regulations catalyzed by a group of enzymes, protein arginine methyltransferases (PRMTs). There are three forms of methylated arginine residues in eukaryotes: ω-NG, monomethylarginine (MMA); ω-NG,NG-asymmetric dimethylarginine (ADMA); ω-NG,N'G-symmetric dimethylarginine (SDMA). In mammalian cells, 10 PRMTs are classified into three types, according to their catalytic functions. Type-I PRMTs catalyze the introduction of two methyl groups into an identical terminal nitrogen of arginine residue, generating ADMA. In contrast, to generate SDMA, type-II PRMTs add two methyl groups to different terminal nitrogens of arginine residue. Type-III PRMTs only synthesize MMA. Among PRMTs, PRMT1 and PRMT5 represent the most common enzymes of type-I and type-II PRMTs, respectively. Recently, protein arginine methylation has been reported to be closely related to the heart diseases. The mRNA and protein levels of PRMT1 both increase in the heart of patients who suffer from coronary artery disease, and in heart-failure rats previously induced by isoproterenol injection. However, the physiological and pathological roles of arginine methylation in the heart remain obscure. In this thesis, the author produced cardiomyocyte-specific PRMT1-deficient

(PRMT1-cKO) mice to explore the functions of PRMT1 in the heart, and to evaluate the enzyme activities of each splice variant (v) of PRMT5.

The author indicated that PRMT1-cKO mice exhibit dilated cardiomyopathy (DCM) and die within 3 months, owing to the lethal heart failure at the early stages of life. Cardiac histological analysis revealed the hypertrophy of heart in PRMT1-cKO mice. Also, heart contractions were reduced by the loss of PRMT1 4 weeks after birth, while ANP and BNP as heart-failure markers were significantly increased. On the other hand, the author has established a novel ribosomal profiling (Ribo-seq) to monitor global on-going translation of mRNAs in mammalian tissues, and then carried out RNA-seq, Ribo-seq, and metabolic analysis of the heart of PRMT1-cKO mice. The gene expression patterns obtained by RNA-seq and Ribo-seq analysis were different not only from each other but also between the wild-type (WT) and PRMT1-cKO mice. A total of 3,080 and 740 genes in the RNA-seq and Ribo-seq data, respectively, were altered by the PRMT1 loss. However, the genes found by RNA-seq analysis were partially overlapping with those found by Ribo-seq analysis. Most importantly, gene ontology analysis of altered genes in these three groups (only RNA-seq, overlapping, and only Ribo-seq data) strongly suggest the mitochondria dysfunction and glutathione (GSH) metabolic changes in the heart of PRMT1-cKO mice. Indeed, the mitochondria from the PRMT1-cKO mice exhibited a decrease in ATP production compared with those from the WT mice.

The author showed that the loss of PRMT1 in the heart results in an increased level of SDMA mainly produced by PRMT5, and that the mRNA level of *Prmt5-v2* is significantly decreased in the heart of PRMT1-cKO mice. In the NCBI sequence database, mouse PRMT5-v1 and -v2 only contain 33 and 16 different amino acids in the N-terminal sequences, respectively. The author found that although *Prmt5-v1* and *-v2* mRNAs are both present in a variety of mouse tissues, PRMT5-v2 is an unstable protein that is constantly degraded through the ubiquitin proteasome system (UPS) and the autophagic-lysosomal pathway (ALP) in an N-terminal sequence-dependent manner. The author also revealed that the inhibition of UPS and ALP elevates the stability of PRMT5-v2 localized in the nucleus and the cytoplasm, and that PRMT5-v2 exhibits the enzyme activity to catalyze histone H2A and H4 methylation. These findings suggest that the stability of PRMT5-v2 may be regulated by the symmetrical arginine dimethylation of histones and several non-histone proteins. It is also suggested that heat shock protein (Hsp) 70 recognizes the N-terminal sequence of PRMT5-v2, and the carboxyl terminus of Hsp70-interacting protein CHIP is required for poly-ubiquitination and degradation of PRMT5-v2. On the basis of these results obtained, the author propose a model indicating that the turnover of PRMT5-v2 may be regulated by the Hsp70/CHIP chaperon system in the N-terminal region-dependent manner.

To elucidate the physiological role of PRMT1 *in vivo*, tissue-specific PRMT1-deficient mice have been widely generated. In the present thesis, the author demonstrates that PRMT1 is crucial to maintain normal cardiac function. In the heart of PRMT1-cKO mice, the mRNA level of *Prmt5-v2* is significantly reduced, while SDMA mainly produced by PRMT5 is remarkably raised. The imbalanced arginine methylation may be the cause of dilated cardiomyopathy (DCM) and heart failure. PRMT1-cKO would be a novel model for DCM and heart failure to understand the pathogenesis of these diseases in human.

# 審査の要旨
# Abstract of assessment result

【批評 Review】

The author demonstrates several lines of evidence that the loss of PRMT1 in the mouse heart affects both transcription and translation systems that cause mitochondria dysfunction and glutathione metabolism, leading to the conclusion that PRMT1 is crucial to maintain normal cardiac functions. The author also reveals that PRMT1 has a large impact on the enzyme activity of PRMT5 by regulation of the *Prmt5* mRNA splicing. Moreover, PRMT5-v2 has been shown to be constantly degraded through both UPS and ALP, suggesting the importance of an inner balance between ADMA and SDMA in cardiac cells. These findings are expected to provide clues for understanding the arginine methylation in cardiac diseases, which would contribute to offering a potential therapy in the human disorders. However, it is important to note that to elucidate the underlying molecular mechanism of the heart failure in PRMT1-cKO mice, further studies are necessary to identify cardiac substrates for PRMTs *in vivo*.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 30th May, 2019. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Human Biology.