School of Integrative and Global Majors
Ph.D. Program in Human Biology (HBP)

論文概要

Dissertation Abstract

Title of Doctor Dissertation:

Study on the Association between Arginine Methylation and Cardiac Diseases
(心臓におけるアルギニンメチル化の機能解析)

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Abstract

Purpose

Arginine methylation is catalyzed by protein arginine methyltransferases (PRMTs) and participates in a variety of cellular processes such as signal transduction, transcription, and translation. PRMTs are divided into three main types according to their catalytic activity. PRMT1 is a type I PRMT that produces monomethyl arginine (MMA) and asymmetric dimethylarginine (ADMA), while PRMT5 is a type II PRMT that generates MMA and symmetric dimethylarginine (SDMA). Recently, it was reported that arginine methylation is closely related to heart diseases. Arginine methylation and PRMT1 levels are markedly disturbed in the diseased heart. However, the physiologic and pathophysiologic role of arginine methylation and PRMT1 is poorly understood and difficult to study in vivo due to early embryonic lethality in PRMT1 null mice. The aims of this study were to clarify the role of protein arginine methylation and its major enzyme PRMT1 in heart disease, and to evaluate the properties and molecular stability of each variant of murine PRMT5.

Materials and Methods

Cardiomyocyte-specific PRMT1 deficient (PRMT1-cKO) mice were generated using the Cre-loxP gene recombination system and Myh6-Cre mice. As we reported previously, PRMT1-cKO mice exhibited dilated cardiomyopathy (DCM) which finally led to lethal heart failure at an early

stage of life. In my study, I established a ribosome profiling (Ribo-seq) strategy for monitoring the on-going translation of expressed mRNA in mammalian tissue. RNA-seq, Ribo-seq, and metabolic analysis were performed to monitor the global transcription, translation, and metabolite profiles in the hearts of PRMT1-cKO mice from the onset of DCM to heart failure.

Protein stability of PRMT5 in HEK293T cells was studied. Biotin tagged N-terminal sequence peptides of PRMT5 variant (v) 1 and v2 were synthesized and used to look for binding partners with the aid of the biotin-tagged pull-down assay and MALDI-TOF-MS.

Results

One month after birth, PRMT1-cKO mice displayed respiratory abnormality and died suddenly. The heart-to-body weight ratio showed that the heart mass was significantly increased in PRMT1-cKO mice compared with the wildtype group. The decline in cardiac systolic function of PRMT1-cKO mice began within 4 weeks after birth and was worse at 6 weeks. The PRMT1-cKO mice had elevated levels of the heart failure markers, ANP and BNP, indicating they died from heart failure, and decreased level of Prmt5-v2 due to the absence of PRMT1, which affected the splicing of Prmt5. PRMT5-v2 is constantly degraded through both the ubiquitin proteasome system (UPS) and the autophagic-lysosomal pathway (ALP) in an N-terminal sequence-dependent manner. Many proteins such as SFPQ and eEf1A1 were identified as the binding partners with the N-terminal sequence of PRMT5-v2.

Both RNA-seq and Ribo-seq were performed on the hearts of wildtype (WT) and PRMT1-cKO mice. In all, 3,080 genes and 740 genes were altered in RNA-seq and Ribo-seq, respectively. A gene ontology (GO) analysis of altered genes in three groups (RNA-seq only profile, RNA-seq-Ribo-seq overlapping profile, Ribo-seq only profile) suggested the presence of mitochondrial dysfunction and glutathione (GSH) metabolism disorder in the hearts of PRMT1-cKO mice. Metabolic analysis revealed the presence of abnormal GSH metabolism as well.

Discussion

Currently, many research groups have generated tissue-specific PRMT1-deficient mice to elucidate the physiologic role of PRMT1 in vivo. In the present study, the PRMT1-cKO revealed that arginine methylation and PRMT1 are crucial to maintaining normal cardiac function. In the heart of PRMT1-cKO mice, the mRNA level of Prmt5-v2 was significantly reduced and the protein level of PRMT5 tended to increase while the level of SDMA, mainly produced by PRMT5, was markedly elevated. Proteins like eEf1A1 which binds to N-terminal of PRMT5-v2 might engage in PRMT5-v2 protein degradation. The imbalanced arginine methylation status might be the cause of DCM and heart failure as well. The pathogenesis of DCM and heart failure are complicated and not well understood. Global transcription, translation, and metabolic analysis may provide new insights into the molecular mechanism at the onset of heart diseases. PRMT1-cKO could be a novel DCM

and heart failure model for studying the pathogenesis of these diseases in humans.