Cardiac-specific deletion of myosin phosphatase targeting subunit 2 alleviates cardiac fibrosis in mineralocorticoid receptor-related hypertension

Z. Ye1, R. Okamoto1, H. Ito1, K. Moriwaki1, R. Ito1, M. Ida1, M. Ito1, K. Dohi1

1Mie University Graduate School of Medicine, Tsu, Japan

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Introduction: Myosin phosphatase targeting subunit 2 (MYPT2) is a crucial subunit of cardiac myosin light chain (MLC) phosphatase. The phosphorylation of MLC in the ventricle plays a pivotal role in cardiac hypertrophy and function. Our previous study demonstrated that overexpression of MYPT2 activated MLC phosphatase leading to cardiac dysfunction. Mineralocorticoid receptor (MR)-related hypertension is associated with extensive cardiac fibrosis. It remains unknown whether MYPT2 may play a role in the development of cardiac fibrosis in MR-related hypertension.

Purpose: Our purpose is to investigate the effect of MYPT2 on cardiac fibrosis in MR-related hypertension.

Methods: After the knockdown of MYPT2 using MYPT2-siRNA, HL-1 cells (murine cardiomyocytes) were incubated with aldosterone (ALDO) for 24h. Cardiac-specific MYPT2 knockout (c-MYPT2-/-) mice were generated using the Cre-lox system (αMHC-Cre; MYPT2f/f). MR-related hypertension mice were induced in c-MYPT2-/- and wild-type (MYPT2+/+) mice with the subcutaneous infusion of ALDO and 8% NaCl food for 4 weeks after uninephrectomy. Systolic blood pressure (SBP) was measured by tail cuffs every week. WB, qPCR, cardiac echo, and histological examinations were performed.

Results: In HL-1 cells, ALDO increased the expression of connective tissue growth factor (CTGF), MYPT2, and the catalytic subunit of the type-1 phosphatase δ isoform (PP1cδ) in a concentration-dependent manner. MYPT2 protein levels significantly decreased by 80% in HL-1 cells transfected with MYPT2 siRNA compared with cells transfected with unspecific siRNA. Knockdown of MYPT2 significantly decreased the protein level of CTGF. In the mice model, the MYPT2 expression in the heart from MYPT2-/- mice was decreased to less than 30% compared with MYPT2+/+ mice. Heart rate, blood pressure, and cardiac systolic function were normal in c-MYPT2-/- mice. PP1cδ was decreased in c-MYPT2-/- mice compared with MYPT2+/+ mice. The expression of cardiac MLC kinase was not changed. The level of phosphorylation of MLC in c-MYPT2-/- mice was significantly higher than MYPT2+/+. Blood pressure elevation and left ventricular hypertrophy were observed in both MR-related hypertension mice. No significant difference in heart size and the nuclear localization of MR in cardiomyocytes. However, ejection fraction and fractional shortening in echocardiography were higher in c-MYPT2-/- than in MYPT2+/+ in MR-related hypertension mice. Histopathological examinations revealed the degree of fibrosis in c-MYPT2-/- mice was lower than in MYPT2+/+ mice with the decreased expression of CTGF.

Conclusions: Cardiac-specific deletion of MYPT2 resulted in a decrease of MLC phosphatase and an increase of phosphorylation in MLC in vivo. MYPT2 deletion can inhibit cardiac fibrosis in a MR-related hypertension model.