論文の内容の要旨

論文題目

Sphingosine 1-phosphate (S1P) signaling is involved in the pathogenesis of cardiac hypertrophy in response to pressure overload and angiotensin II

スフィンゴシン 1-リン酸（S1P）シグナル伝達は圧負荷およびアンジオテンシンIIによる心肥大の病態に関与する

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Cardiac hypertrophy occurs in response to physiologic stimuli such as exercise, and in response to diverse pathophysiological stimuli such as hypertension, ischemic heart disease, valvular insufficiency, or mutations in sarcomeric genes. Although hypertrophic growth of the myocardium is thought to temporarily preserve pump function, prolongation of the hypertrophic state inevitably leads to the structural remodeling of the myocardium, which is a key determinant of the clinical course of heart failure. However, molecular mechanisms underlying cardiac remodeling and heart failure are insufficiently understood and effective pharmacological means to prevent those are not currently

available. Sphingosine 1-phosphate (S1P), a biologically active lysophopholipid, is involved in many critical cellular processes including the cardiovascular system. Recent reports suggest that activation of S1P signaling might lead to cardiac hypertrophy and fibrosis. However, the role of S1P signaling in the pathogenesis of cardiac hypertrophy is insufficiently understood. I investigated the potential involvement of S1P signaling in cardiac hypertrophy in response to pressure overload and angiotensin II.

Transverse aortic constriction (TAC) and angiotensin II infusion induced cardiac hypertrophy, and significantly increased cardiac expression levels of S1P1, the major cardiac receptor subtype for S1P, and sphingosine kinase 1 (SphK1), the key enzyme catalyzing the formation of S1P. Immunohistochemical analysis demonstrated that both S1P1 and SphK1 were expressed in cardiomyocytes and endothelial cells. There was little expression in the other cells including cardiac fibroblasts. In cultured cardiomyocytes, expression levels of S1P1 and SphK1 were upregulated by angiotensin II and were much higher than those in cardiac fibroblasts, suggesting S1P signaling is primarily activated in cardiomyocytes. Angiotensin II increased S1P1 promoter activity. Pretreatment with p38 MAPK inhibitor reduced angiotensin II-induced transactivation of S1P1

promoter. Results so far suggested that angiotensin II induces cardiac hypertrophy at least partly by activating the S1P1 signaling. To test that idea, I analyzed if S1P might induce cardiomyocyte hypertrophy. Both S1P and SEW2871, a selective S1P1 agonist, induced cardiomyocyte hypertrophy and activated extracellular signal-regulated kinase 1/2 (ERK1/2) of the mitogen-activated protein kinase (MAPK) family in cultured cardiomyocytes. Pharmacological inhibition of the ERK pathway using U0126 reduced S1P-induced cardiomyocyte hypertrophy. Moreover, knocking down S1P1 inhibited S1P-induced phosphorylation of ERK1/2. Taken together, S1P induces hypertrophic responses in cardiomyocytes via S1P1-mediated activation of ERK pathway. To further assess the involvement of S1P signaling in cardiac hypertrophy, I next analyzed the contribution of SphK1/S1P/S1P1 pathway to the angiotensin II-induced cardiomyocyte hypertrophy. When S1P1 was knocked down by a specific siRNA, the hypertrophic response to angiotensin II was significantly suppressed. Similarly knocking down SphK1 inhibited cardiomyocyte hypertrophy in response to angiotensin II. Taken together angiotensin II appears to induce hypertrophic responses by activating S1P signaling. Next I searched for small molecular weight compounds that can potentially inhibit S1P signaling for therapeutic intervention in the treatment of cardiac hypertrophy and

remodeling. I found LE135, a synthetic retinoid, inhibited angiotensin II-induced S1P1 upregulation and S1P-mediated phosphorylation of ERK1/2. To analyze the effect of LE135 on the heart in vivo, LE135 was administered to mice subjected to TAC. LE135 inhibited TAC-induced S1P1 upregulation in the heart and reduced cardiac hypertrophy and fibrosis. Increased mRNA expressions levels of atrial natriuretic factor (ANF), brain natriuretic peptide (BNP) and β-myosin heavy chain (β-MHC) in TAC hearts were significantly suppressed by LE135. Upregulation of profibrotic genes, such as collagen types I/III and fibronectin, was also significantly inhibited by LE135 4 week after TAC. LE135 also ameliorated cardiac hypertrophy and fibrosis induced by angiotensin II infusion.

These results demonstrate that S1P/S1P1 signaling is involved in the pathogenesis of cardiac hypertrophy in response to pressure overload and angiotensin II, and is an attractive therapeutic target for cardiac hypertrophy.