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Involvement of peptidyl-prolyl isomerase Pin1 in the inhibitory effect of fluvastatin on endothelin-1-induced cardiomyocyte hypertrophy ☆

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ABSTRACT

Aims: Cardiac hypertrophy is elicited by endothelin (ET)-1 as well as other neurohumoral factors, hemodynamic overload, and oxidative stress; HMG-CoA reductase inhibitors (statins) were shown to inhibit cardiac hypertrophy partly via the anti-oxidative stress. One of their common intracellular pathways is the phosphorylation cascade of MEK signaling. Pin1 specifically isomerizes the phosphorylated protein with Ser/Thr-Pro bonds and regulates their activity through conformational changes. There is no report whether the Pin1 activation contributes to ET-1-induced cardiomyocyte hypertrophy and whether the Pin1 inactivation contributes to the inhibitory effect of statins. The aim of this study was to reveal these questions.
Main methods: We assessed neonatal rat cardiomyocyte hypertrophy using ET-1 and fluvastatin by the cell surface area, ANP mRNA expression, JNK and c-Jun phosphorylation, and [^{3}H]-leucine incorporation.
Key findings: Fluvastatin inhibited ET-1-induced increase in the cell surface area, ANP expression, and [^{3}H]-leucine incorporation; and it suppressed the signaling cascade from JNK to c-Jun. The phosphorylated Pin1 level, an inactive form, was decreased by ET-1; however, it reached basal level by fluvastatin. Furthermore, Pin1 overexpression clearly elicited cardiomyocyte hypertrophy, which was inhibited by fluvastatin.
Significance: This is the first report that ET-1-induced cardiomyocyte hypertrophy is mediated through the Pin1 activation and that the inhibitory effect of fluvastatin on cardiomyocyte hypertrophy would partly be attributed to the suppression of the Pin1 function. This study firstly suggests that Pin1 determines the size of hypertrophied cardiomyocyte by regulating the activity of phosphorylated molecules and that statins exert their pleiotropic effects partly via Pin1 inactivation.

Introduction

Cardiac hypertrophy is elicited by endothelin (ET)-1 (Suzuki et al., 1990; Yorikane et al., 1993; Ito, 1997; Kolettis et al., 2013; Miyauchi and Goto, 2013) as well as other neurohumoral factors, hemodynamic overload, and oxidative stress. One of their common intracellular pathways is the phosphorylation cascade of MEK signaling. Peptidyl-prolyl cis-trans isomerase 1 (Pin1) is a highly conserved enzyme that isomerizes specific phosphorylated Ser/Thr-Pro bonds in certain proteins, inducing conformational changes (Lu et al., 1996). It has been reported that Pin1 has a binding activity to the pSer/Thr-Pro pocket of the target protein and that Pin1 catalyzes such portion. Both of these activities are exerted when the Ser-16 residue of Pin1 is dephosphorylated; on the other hand, these activities are inhibited when the residue is phosphorylated (Lu and Zhou, 2007). The family of proline-directed protein kinases containing a major regulatory phosphorylation motif (pSer/Thr-Pro) includes c-Jun, Akt, cyclin dependent kinases, Raf-1, SMAD2/SMAD3, etc., which play an important role in the regulation of cell proliferation, tumorigenesis, neurological disorders, and autoimmune and inflammatory diseases (Lee et al., 2011a,b).

The endothelin (ET) system consists of two G protein coupled-receptors, ET type A receptor and ET type B receptor, and three endogenous ligands, ET-1, ET-2, and ET-3 (Davenport, 2002; Horinouchi et al., 2013; Kolettis et al., 2013; Miyauchi and Goto, 2013). ET-1, a potent vasoconstrictive peptide produced by endothelial cells (Yanagisawa et al., 1988), is also produced by cardiomyocytes and contributes to the development of cardiac hypertrophy (Ito et al.,

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1991; Yorikane et al., 1993; Kolettis et al., 2013; Miyauchi and Goto, 2013). We reported that the production of ET-1 is markedly increased in the failing hearts of rats with chronic heart failure (Sakai et al., 1996a,b) and that the enhancement of myocardial ET-1 contributes to the modulation of the cardiac function (Sakai et al., 1996a) and cardiac hypertrophy at the molecular level in the failing hearts (Sakai et al., 2000); moreover, chronic administration of the ET_A receptor antagonist BQ-123 inhibits the cardiac remodeling and ameliorates the cardiac function (Sakai et al., 1996b). In addition, the signaling cascades of the mitogen activated protein kinase (MEK) family including extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) are augmented by ET-1 in cardiomyocyte hypertrophy (Yue et al., 2000; Irukayama-Tomobe et al., 2004; Shimojo et al., 2006). Therefore, in the present study, we hypothesized that Pin1 contributes to the development of cardiomyocyte hypertrophy through the activation of phosphorylated kinases of the MEK family by exerting its catalytic activity.

3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) are widely employed classes of cholesterol-lowering drugs that work through the inhibition of HMG-CoA reductase. Much evidence has demonstrated that statins reduce the cardiovascular risk to a greater extent than that expected based on the blood cholesterol-lowering effect alone; those additional activities of statins are known as pleiotropic effects including the suppression of inflammation and improvement of endothelial dysfunction (Wierzbicki et al., 2003). We have reported that pitavastatin ameliorates the severity of experimental autoimmune myocarditis through the inhibition of T-cell mediated autoimmunity (Tajiri et al., 2013). It was reported that statins interfere with the protein (iso)prenylation processes (Wierzbicki et al., 2003), which may be involved in the regulation of several cellular mechanisms such as signal transduction and cell proliferation and differentiation. Previous studies have shown that statins inhibit cardiomyocyte hypertrophy provoked by angiotensin II by blocking the Rho kinase-mediated cyclin D1 activation (Morikawa-Futamatsu et al., 2006) and by an antioxidant mechanism involving Rac1 inhibition (Takemoto et al., 2001). Such reports suggest that another unresolved mechanism for the pleiotropic effects of statins remains.

However, there is no report whether the Pin1 activation is involved in cardiomyocyte hypertrophy provoked by ET-1; therefore, we hypothesized that the activation of Pin1 activity would contribute to the ET-1-induced cardiomyocyte hypertrophy. In addition, there is no report whether the inhibitory effect of statins on the ET-1-induced cardiomyocyte hypertrophy is mediated through the modulation of the Pin1 activity. The aim of this study was to reveal these questions.

Materials and methods

Adenovirus vectors

Human Pin1 cDNA cloned from 293A cells by PCR using the following primers, (forward) 5′-CACCATGGCGGACGAGGAGAAGCT-3′ and (reverse) 5′-CTCAGTGCGGAGGATGATGTGGATG-3′, was ligated to the pENTR-TOPO plasmid as an entry clone; cDNA was transferred to the pAd/CMV/V5-DEST Gateway vector by an LR recombination (Life Technologies, Carlsbad, CA). The cDNA for human Pin1 was designed to add the V5 tag at C-termianl of the entire Pin1 fusion protein. The adenovirus vector plasmid was digested by Pac I, transfected to 293A cells and amplified, and finally purified by a Vivaspin column system (Sartorius Stadium Biotechnology, Goettingen, Germany). As a control, an adenovirus vector of LacZ was used. Adenovirus for an enhanced green fluorescent protein (EGFP) was used to visualize the morphology of the cardiomyocytes and to take photographs.

Cardiomyocyte culture

Neonatal rat cardiomyocytes were isolated from 2- to 3-day-old Sprague–Dawley rats, as described previously (Shimojo et al., 2007; Sakai et al., 2012), and were incubated on fibronectin-coated dishes in DMEM-Ham's F-12 medium (Wako) supplemented with 0.1% fatty acid-free bovine serum albumin (BSA) (Sigma, St. Louis, MO) in 95% air–5% CO_2. The cells were cultured for 2 days after the differential adhesion and then used for further experiments. The animal experiment for cardiomyocyte isolation was carried out in a humane manner after we received approval from the Institutional Animal Experiment Committee of University of Tsukuba and were in accordance with the Regulation for Animal Experiments in our university.

Study protocol

Cardiomyocytes were pretreated with a vehicle or fluvastatin (0.1–10 μM) (Wako Pure Chemical Industries, Osaka, Japan) and 12 h later, human/rat ET-1 (10 nM) (Peptide Institute Inc., Osaka, Japan) was applied and incubated for 48 h; the cell surface area was evaluated as describing below; and the sampling for the [^{3}H]-leucine uptake, real-time PCR, and Pin1 expression was done in an individual experiment. A Western blot was performed for ERK, JNK, and c-Jun, and the samples were collected 30 min after the ET-1 stimulation. In the transient transfection experiments, cardiomyocytes were transfected with an adenovirus for Pin1 or LacZ (control) at a titer with 20 multiplicity of an infection (MOI); in the experiment of taking photographs of cardiomyocytes, the adenovirus for EGFP (10 MOI) was transfected additionally; 12 h later after adenoviral transfection, cardiomyocytes were treated with a vehicle or fluvastatin and harvested for 48 h. There are some reasons why we selected fluvastatin in this experiments; (1) as fluvastatin is known as one of the lipophilic statins contrasted to a hydrophilic statin, pravastatin, and is suspected to be easily incorporated into the muscle cells, it is suggested that using fluvastatin leads to an easier evaluation of determining the inhibitory effect of statin on cardiomyocyte hypertrophy; (2) other researchers previously reported that fluvastatin has the inhibitory effect on cardimyocyte hypertrophy (Morikawa-Futamatsu et al., 2006); and (3) other lipophilic statins, simvastatin and atorvastatin, were reported to prevent cardiac hypertrophy (Liu et al., 2008; Indolfi et al., 2002; Planavila et al., 2005).

Cardiomyocyte size measurement

Cardiomyocytes were observed by microscope (DM1L, Leica Japan, Tokyo, Japan) and captured by a charge-coupled device camera (Olympus, Tokyo, Japan). The surface area of the attached cardiomyocytes on the dish (the area of cardiomyocytes in 2D image) for 10 cells/field with 5 fields in each group was evaluated by the software NIH ImageJ ver 1.47 (National Institute of Health, Bethesda, MD) and repeated for 3 times.

Protein synthetic rate

The rates of protein synthesis in cultured neonatal rat cardiomyocytes were assessed by measuring the [^{3}H]-leucine uptake into acid-insoluble cellular materials as described previously (Shimojo et al., 2007). The cells were plated on 24-well dishes at a density of 1×10^5 cells/well and pretreated with fluvastatin or vehicle 12 h before the ET-1 stimulation. Twenty-four hours after the ET-1 treatment, 0.1 mCi/ml [^{3}H]-leucine (GE Healthcare, Piscataway, NJ) was added and the cells were incubated for 24 h. The cells were finally fixed by 5% trichloroacetic acid and detached by 0.25% trypsin, and the cell residues were solubilized in 0.5 M NaOH. Aliquots were counted with a scintillation counter (LS-6500 scintillation counter; Beckman Coulter, Fullerton, CA). In the transient transfection assay, cardiomyocytes were transfected with an adenovirus for Pin1 or LacZ; 12 h later, fluvastatin or vehicle was added and cardiomyocytes were incubated for 48 h; [^{3}H]-leucine was added and incubated for the last 24 h.