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Estrogen-related receptor gamma induces cardiac hypertrophy by activating GATA4

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ABSTRACT

Estrogen-related receptor gamma (ERRγ) is an orphan nuclear receptor that has biological roles mainly in metabolism and that controls metabolic switching in perinatal heart. In adult heart diseases, however, the functional roles of ERRγ have not yet been elucidated. In the present study, we aimed to characterize the role of ERRγ in cardiac hypertrophy. The functional roles of ERRγ in the development of cardiac hypertrophy were examined in primary cultured cardiomyocytes and in animal models. ERRγ expression was increased in hearts from human hypertrophic cardiomyopathy patients and in both cellular and animal models of cardiac hypertrophy. Transgenic overexpression in mouse heart as well as forced expression of ERRγ in cardiomyocytes induced hypertrophic phenotypes. Knock-down of ERRγ blocked agonist-induced hypertrophic phenotypes. ERRγ bound directly to the proximal ERR-responsive element in the GATA4 promoter in a sequence-specific manner and thereby induced transcription. ERRγ-induced hypertrophy was blocked by inhibition of GATA4. GSK-5182, an inverse agonist of ERRγ, completely blocked cardiac hypertrophy in cardiomyocytes. It also prevented aortic banding-induced cardiac hypertrophy and fibrosis in mouse heart. These findings demonstrate a novel ERRγ/GATA4 signal cascade in the development of cardiac hypertrophy and suggest GSK-5182 as a possible therapeutic.

1. Introduction

The heart usually adapts to exogenous stresses by increasing individual cardiomyocyte volume [1]. This initial hypertrophic response, however, is also an important cause of cardiomyopathy and heart failure [2]. Cardiomyocyte hypertrophy is accompanied by enhanced protein synthesis and rearrangement of the sarcomere, which are tightly regulated by heart-specific transcription factors such as GATA4, myocyte-specific enhancer factor 2 (MEF2), serum response factor (SRF), c-jun, and c-fos [3]. The increase in transcription factor activity reactivates the fetal gene program.

Nuclear receptors orchestrate diverse biological actions, including development, immune responses, cell growth, and metabolism. Among the nuclear receptors, the estrogen-related receptor (ERR) subfamily has three members, ERRα, ERRβ, and ERRγ [4]. The ERRs were originally discovered through a screen to identify steroid hormone receptors related to the estrogen receptor [5]. Although they can bind to the estrogen response element in target gene promoters, ERRs are not activated by estrogen-like ligands and are currently classified as orphans with no known endogenous ligands. ERRs are highly expressed in tissues with metabolic demands [6–9], and ERRα/γ regulates the mitochondrial genetic network that triggers postnatal metabolic transition [6].

In heart diseases, ERRα works to switch bioenergetic responses to hypertrophic stresses [10]. In contrast, genetic disruption of the ERRγ gene in mice causes early death within a week after birth owing to failure of switching to lipid-based oxidative metabolism [11]. Thus, ERRγ is

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an essential regulator of metabolic switch in highly energy-consuming organs in perinatal periods. Other than in metabolic switching in neonates, however, the functional roles of ERRγ, especially in heart diseases in adults, have not yet been elucidated.

In the present study, we aimed to characterize the role of ERRγ in cardiac hypertrophy. We studied the transcriptional activation of GATA4 signal pathways by ERRγ in hypertrophic phenotypes in response to exogenous stresses. In addition, we examined the role of GSK-5182, an inverse agonist of ERRγ, in the inhibition of cardiac hypertrophy.

2. Materials and methods

2.1. Human samples

Autopsied left ventricle specimens were obtained from 6 individuals: 3 hypertrophied and 3 age-matched males. Hypertrophic cardiomyopathy was diagnosed according to total heart weight, left ventricular free wall thickness, and microscopic findings. This work was performed according to the regulations of the institutional review board of Chonnam National University Hospital (CNUH-2011-129).

2.2. Animal model and transgenic mice

Partial aortic constriction (aortic banding; AB) was performed as described previously [12–14]. α-Myosin heavy chain (U71441) promoter-driven ERRγ transgenic mice were generated by a commercial company (Macrogen, Seoul, Korea) on the C57BL/6 background in accordance with National Institutes of Health guidelines. All experimental procedures were approved by the Chonnam National University Medical School Research Institutional Animal Care and Use Committee.

2.3. Reagents and experimental procedures

Reagents, experimental methods, and statistical analysis were described previously [12–16] and are presented in the Supplementary Methods.

3. Results

3.1. ERRγ induces cardiac hypertrophy

To investigate the functional roles of ERRγ in cardiac hypertrophy, we first investigated ERRγ expression in hypertrophic cardiomyopathy patients (Fig. 1A). Compared with that in age-matched normal human hearts, the expression of ERRγ was significantly increased in all three patients investigated. We also examined the expression profiles of ERRγ in mouse heart, and this expression was gradually reduced with aging (Supplementary Fig. 1A). Next, we investigated ERRγ expression in animal model of cardiac hypertrophy. AB increased the expression of ERRγ at 5 days and the protein amounts were dramatically increased at 14 days after the operation (Fig. 1B). ET-1 significantly increased the protein amount of ERRγ in rat neonatal cardiomyocytes (Fig. 1C). By use of quantitative real-time polymerase chain reaction (qRT-PCR), we observed that both ET-1 and PE significantly increased ERRγ mRNA in cardiomyocytes (Supplementary Fig. 1B). Minimal ERRγ promoter (−0.5 kb from transcription start site) was sufficient to be activated by either PE or ET-1 (Supplementary Fig. 1C).

Next, we investigated whether ERRγ itself provokes hypertrophy. We transfected *pcDNA3-Flag-ERR*γ to cardiomyocytes and examined the hypertrophic phenotype by use of immunocytochemistry techniques. ERRγ was localized mainly in the nucleus (Supplementary Fig. 2A, second left in lower panels), and forced expression of ERRγ induced the formation of stress fibers (Supplementary Fig. 2A, lower panels). Protein level of exogenous ERRγ by transfection of *pcDNA3-Flag-ERR*γ (4 μg) into cardiomyocytes was 4.5-fold higher than endogenous ERRγ (Supplementary Fig. 2B).

We infected cardiomyocytes with adenoviral *ERR*γ and measured cardiomyocyte size; after cells were visualized by fluorescent immunocytochemistry staining with sarcomeric α-actin, the sizes of multiple cells in one field (3–5 cells per field) were averaged and counted as one case. Three independent sets of immunocytochemistry staining were performed. Compared with *adeno-GFP*-infected control, *adeno-ERR*γ significantly increased cardiomyocyte size (Fig. 1D). Cardiac hypertrophy is accompanied by an increase in protein synthesis [17], which can be quantified by measuring [^{3}H]-leucine incorporation. Adenoviral *ERR*γ dramatically increased [^{3}H]-leucine incorporation (Fig. 1E). Protein level of exogenous ERRγ by infection of adeno *ERR*γ (20 MOI) into cardiomyocytes was 3.8-fold higher than endogenous ERRγ (Supplementary Fig. 2C). The transcript amount of *Myh7*, one of the fetal gene programs [18], was increased by adenoviral *ERR*γ (Fig. 1F), which further supports the previous report [6]. Transient overexpression of *ERR*γ dramatically increased −3003 *natriuretic peptide precursor type A* (*Nppa*) promoter activity in cardiomyocytes (Fig. 1G), in H9c2, a rat cardiomyoblast cell line (Supplementary Fig. 3A), and even in 293 T (Supplementary Fig. 3B). *ERR*α, an isoform of ERRγ, did not increase the promoter activity (Supplementary Figs. 3C and D). Adenoviral *ERR*γ infection did not alter mitochondrial genes of electron transport/oxidative metabolism or energy transfer except uncoupling protein 3 and creatine kinase (Supplementary Table).

We further investigated the prohypertrophic effects of ERRγ in α-myosin heavy chain promoter (MHC)-driven transgenic mice that overexpresses Flag-tagged ERRγ (Fig. 1H). Out of 5 transgenic lines with germ-line transmission, male mice in two independent lines of #3 and #6 have hypertrophic phenotypes. In the following studies, we used line #3 in which *ERR*γ expression level was 9-fold higher than endogenous amount in wild type (Supplementary Fig. 4A). Transgenic heart showed approximately 8% increase, as evaluated by the ratio of heart weight to body weight (Fig. 1I). The increase in heart size was further confirmed by evaluation of heart weight to tibia length (Supplementary Fig. 4B). Overexpression of *ERR*γ resulted in a 4-fold increase in *Nppa* transcript amount (Fig. 1J).

3.2. ERRγ is required for hypertrophic phenotypes

Next, we knocked down ERRγ and examined the responses to hypertrophic stresses. *ERR*γ siRNA successfully reduced the endogenous ERRγ level (Fig. 2A). Diverse hypertrophic stresses such as PE (Fig. 2B), ET-1 (Fig. 2C), or even fetal bovine serum (FBS, Supplementary Fig. 5) increased protein synthesis, which was significantly attenuated by *ERR*γ siRNA. *Nppa* promoter activity was also increased by either PE (Fig. 2D) or ET-1 (Fig. 2E), which was completely blocked by treatment with *ERR*γ siRNA. −3500 *Myh7* promoter activity was also inhibited by *ERR*γ siRNA (Fig. 2F).

3.3. ERRγ binds to ER response element in GATA4 promoter

It is known that ERRγ works as a transcriptional modulator [19]. The almost complete attenuation of hypertrophic phenotypes by knock-down of ERRγ suggests that its action is mediated by modulation of key transcription factors. Thus, we examined the expression of several transcription factors after infection with adenoviral *ERR*γ in cardiomyocytes. Among the transcription factors tested, the expression of Gata4 and Srf was increased by adenoviral infection of ERRγ in cardiomyocytes (Fig. 3A). We further examined changes in transcript levels. *Gata4* mRNA was significantly increased by *ERR*γ (Fig. 3B), whereas the transcript levels of the other transcription factors including *Srf* were unchanged (Fig. 3C and Supplementary Figs. 6A and B). It is noteworthy that adenoviral infection of *ERR*α did not alter those transcription factors (Fig. 3A). Alteration of Gata4 protein amounts by overexpression of ERRγ was further examined in mice;