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Differential effects of and mechanisms underlying the protection of cardiomyocytes by liver-X-receptor subtypes against high glucose stress-induced injury

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ABSTRACT

Liver-X-receptors (LXRs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. The two popular homologous receptor subtypes, LXRα and LXRβ, exhibit differential expression patterns, thereby probably playing different roles in different contexts. This study aimed to evaluate the different roles of the two LXR subtypes and the mechanisms underlying their protection of cardiomyocytes against high-glucose stress. Silencing of *LXRα*, but not *LXRβ* impaired normal LXR-mediated cardioprotective effects against high glucose-induced oxidative stress, apoptosis, and inflammation. Mechanistically, silencing of *small ubiquitin-like modifier (SUMO)1* or *SUMO2/3* did not affect LXR-mediated cardioprotective effects; however, these were impaired in response to *nuclear receptor corepressor (NCoR)* silencing. Together, these findings indicate that LXRα, but not LXRβ, protects against high glucose-induced cardiomyocyte injury, probably via the NCoR-dependent transrepression of downstream target genes.

1. Introduction

According to the World Health Organization, the global prevalence of diabetes mellitus (DM) among adults has increased considerably in recent decades and is estimated to cause approximately 1.5 million deaths annually, largely via DM-induced cardiovascular disease, particularly diabetic cardiomyopathy (DCM). In this condition, DM-induced hyperglycemia disrupts normal metabolism and directly affects cardiomyocytes, endothelial cells, and microcirculation, thereby inducing structural and functional changes eventually resulting in cardiac failure and mortality [1]. In particular, the induction of cardiomyocyte apoptosis in response to high-glucose stress is a key feature of DCM pathogenesis and is the primary cause of diabetic heart failure and mortality [2]. Hyperglycemia also induces the accumulation of reactive oxygen species (ROS), which result in oxidative protein modifications leading to further tissue injury and dysfunction. ROS also react with nitric oxide (overproduced by activated inducible nitric oxide synthase [iNOS]) to generate significant amounts of the reactive nitrogen species (RNS), especially the peroxynitrite anion ($ONOO^-$). Moreover, increasing evidence from experimental and clinical studies suggest that ROS/RNS critically mediate the pathogenesis of diabetic cardiovascular diseases. Furthermore, they also trigger cardiomyocytic apoptosis, and damage mitochondrial membranes via endogenous or exogenous signaling pathways, thereby initiating the harmful "ROS-induced ROS release" positive-feedback loop [3,4]. Thus, studies on novel DCM therapies have long focused on the identification of therapeutic targets and

Abbreviations: LXR, liver-X-receptor; SUMO, small ubiquitin-like modifier; NCoR, nuclear receptor corepressor; DM, diabetes mellitus; DCM, diabetic cardiomyopathy; ROS, reactive oxygen species; iNOS, inducible nitric oxide synthase; RNS, reactive nitrogen species; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; Cyt-c, cytochrome c; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HG, high glucose; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; RT-qPCR, reverse transcription quantitative polymerase chain reaction; PI, propidium iodide; PBS, phosphate-buffered saline; SEM, standard error of the mean; ANOVA, analysis of variance; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cell.

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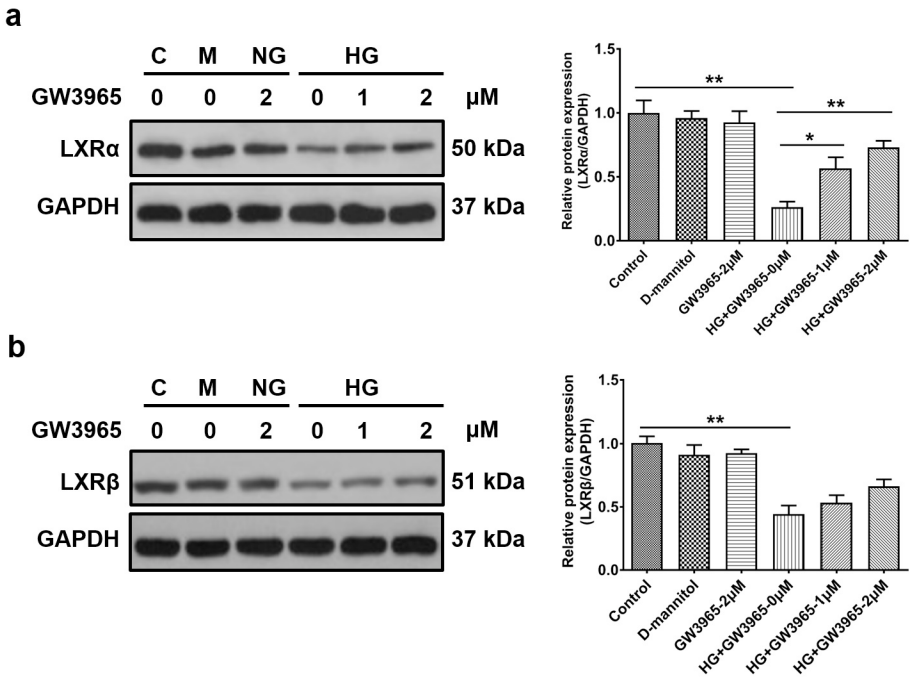

Fig. 1. Changes in liver-X-receptor (LXR) subtype expression under high glucose conditions. Cardiomyocytes were treated with either the physiological concentration of glucose (5.5 mM), high osmotic (33 mM mannitol), or high glucose (33 mM glucose) medium for 48 h, before the expression of the LXRα and LXRβ subtypes was analyzed via western blotting. The presented LXRα (~50 kDa) and LXRβ (~51 kDa) bands represent three independent experiments. GAPDH served as a loading control for total protein expression. *P < 0.05, **P < 0.01 vs. the HG + GW3965-0 μM group. C, control; M, mannitol; NG, normal glucose; HG, high glucose; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

development of new methods to attenuate high glucose stress-induced injury. Figs. 1–4.

Liver-X-receptors (LXRs), comprising two different but highly homologous LXR isoforms (LXRα and LXRβ), are ligand-activated transcriptional factors belonging to the nuclear receptor superfamily. LXRα (NR1H3) is highly expressed in metabolically active tissue and cell types, including those of the liver, intestine, adipose tissue, and macrophages, whereas LXRβ (NR1H2) is ubiquitously expressed throughout the body [5]. Endogenous LXRs are also expressed in the cardiovascular system and play important roles in several cardiac diseases [6–8]. Recent evidence suggests that LXRs serve as functional nuclear receptors during cellular responses to high glucose stress-induced injury. For example, LXR activation protects against high glucose-induced apoptosis in H9C2 cardiac muscle cells *in vitro*, via inhibition of ROS production, mitochondrial death, and nuclear factor (NF)-κB activation [9]. Similarly, our previous *in vivo* study reported that LXR activation protects against DCM by attenuating insulin resistance and reducing both the induced oxidative/nitrative stress and the inflammatory response [10]. However, the specific roles of the two LXR subtypes in the regulation of cardiomyocyte function under high-glucose stress and the mechanisms underlying LXR-mediated protection of cardiomyocytes against DCM are presently unknown. Interestingly, different types of SUMOylation reportedly affect the two LXR subtypes and downregulate downstream inflammatory genes in macrophages and neuroglial cells [11,12]. Furthermore, LXRs direct the ligand-dependent transrepression of numerous endogenous inflammatory-response genes in primary macrophages in a SUMOylation- and/or NCoR-dependent manner [13]. Together, these findings led us to hypothesize that the SUMOylation and NCoR-dependent pathways may underlie the LXR-induced inhibition of target gene expression in cardiomyocytes. Thus, the present study aimed to investigate the specific role and the mechanisms underlying the protection of cardiomyocytes by the two LXR subtypes in DCM.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin/streptomycin (pen/strep, 10,000 U/ml each) were purchased from Gibco (Carlsbad, CA, USA). Synthetic LXRα/β dual agonist 3-[3-[N-(2-Chloro-3-trifluoromethylbenzyl)-(2,2-diphenylethyl) amino] propyloxy] phenylacetic acid hydrochloride (GW3965) was kindly provided by Jon Collins (GlaxoSmithKline, Research Triangle Park, NC, USA). TRIzol Reagent was purchased from Life Technologies (Carlsbad, CA, USA). A mouse monoclonal anti-LXRα antibody and rabbit polyclonal anti-LXRβ and anti-iNOS antibodies were obtained from Abcam (Cambridge, UK). Rabbit anti-nuclear factor kappa-light-chain-enhancer of activated B cell p65 (NF-κB p65), rabbit anti-caspase-3, rabbit anti-cytochrome c (Cyt-c), and rabbit anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were purchased from Cell Signaling Technology (Beverly, MA, USA). IRDye 800CW goat anti-mouse and anti-rabbit IgG secondary antibodies were obtained from LI-COR Biosciences (Lincoln, NE, USA). All other chemicals were sourced commercially.

2.2. Cell culture and lentiviral shRNA transfection

H9C2 rat ventricular myocardial cells (American Type Culture Collection [ATCC]; Rockville, MD, USA) were maintained (37 °C, 5% CO_2 in air) in DMEM supplemented with 10% FBS, and 1% penicillin/streptomycin. High glucose (HG) stress was induced by replacing the culture medium with 33 mM D-glucose, as described previously [14]. Control cells were treated with 33 mM D-mannitol (M) to exclude the potential effects of osmolarity. The shRNAs targeting *LXRα* (5′-GGATAGGGTTGGAGTCATC-3′), *LXRβ* (5′–CCTGCCAGATGGATGCCTT-3′), *NCoR* (5′-GCCTCGGACAAGGATGCAA-3′), *SUMO1* (5′-GGAAGAA-GACGTGATTGAA-3′), *SUMO2/3* (5′-CGACGAGAAACCCAAGGAA-3′),

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