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Downregulation of adipose triglyceride lipase promotes cardiomyocyte hypertrophy by triggering the accumulation of ceramides

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ABSTRACT

Adipose triglyceride lipase (ATGL), the rate-limiting enzyme of triglyceride (TG) hydrolysis, plays an important role in TG metabolism. ATGL knockout mice suffer from TG accumulation and die from heart failure. However, the mechanisms underlying cardiac hypertrophy caused by ATGL dysfunction remain unknown. In this study, we found that ATGL expression declined in pressure overload-induced cardiac hypertrophy *in vivo* and phenylephrine (PE)-induced cardiomyocyte hypertrophy *in vitro*. ATGL knockdown led to cardiomyocyte hypertrophy, while ATGL overexpression prevented PE-induced hypertrophy. In addition, ATGL downregulation increased but ATGL overexpression reduced the contents of ceramide, which has been proved to be closely associated with cardiac hypertrophy. Moreover, the accumulation of ceramide was due to elevation of free fatty acids in ATGL-knockdown cardiomyocytes, which could be explained by the reduced activity of peroxisome proliferator-activated receptor (PPAR) α leading to imbalance of fatty acid uptake and oxidation. These observations suggest that downregulation of ATGL causes the decreased PPARα activity which results in the imbalance of FA uptake and oxidation, elevating intracellular FFA contents to promote the accumulation of ceramides, and finally inducing cardiac hypertrophy. Upregulation of ATGL could be a strategy for ameliorating lipotoxic damage in cardiac hypertrophy.

Introduction

Homeostasis of myocardial lipid metabolism plays a vital role in maintaining the normal cardiac function. Myocardial content of triglyceride (TG)[2], the major form of fatty acids (FAs) storage, is elevated in cardiac hypertrophy [1,2]. Although TG itself is not lipotoxic [3,4], the accumulation of TG reflects lipid metabolic dysfunction in the heart and is associated with impaired cardiac function [5–7].

In cardiomyocytes, the synthesis of TG is dependent on the uptake of FAs, while TG is hydrolysed by lipase to release free FAs (FFAs) [8]. Part of the FFAs released from TG hydrolysis is transported to the mitochondria for beta-oxidation to provide energy, while a majority of FFAs are recycled to synthesize TG [9,10]. Since the rate of FA turnover within TG is 3.75-fold faster than FA oxidation [10], the amount of FAs for TG re-synthesis is more than that for oxidation. The TG/FFA cycling thus forms. Blockage of this cycling by interfering TG synthesis or hydrolysis may result in cardiac lipid metabolic disorder.

Adipose triglyceride lipase (ATGL), a rate-limiting enzyme mediating TG hydrolysis, converts TG to a molecule of FFA and a molecule of diacylglycerol (DG) [8]. Recent studies have shown that ATGL plays a key role in cardiac hypertrophy [11,12]. ATGL-knockout mice suffer from bulk deposition of TG in the cardiac muscle, exhibit cardiac hypertrophy and interstitial fibrosis, and eventually die from heart failure [12]. In contrary, ATGL transgenic mice show a protective effect against cardiac hypertrophy induced by pressure overload [13].

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[2] *Abbreviations used:* ATGL, adipose triglyceride lipase; PPARα, peroxisome proliferator-activated receptor α; TG, triglyceride; PE, phenylephrine; FFA, free fatty acid; DG, diacylglycerol; FB1, fumonisin B1; AAC, abdominal aortic constriction; ANF, atrial natriuretic factor; BNP, brain natriuretic polypeptide; FATP1, fatty acid transport protein 1; CPT-1b, carnitine palmitoyltransferase 1b; MCAD, medium-chain acyl-CoA dehydrogenase; LCAD, long-chain acyl-CoA dehydrogenase; PDK4, pyruvate dehydrogenase kinase isoform 4.

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The mechanisms of cardiac hypertrophy caused by ATGL deficiency are not fully understood. Previous studies demonstrate that ATGL deficiency increased signal transducer and activator of transcription 3 (STAT3) [14], enhanced oxidative stress and inflammation [15], and attenuated the activity of peroxisome proliferator-activated receptor (PPAR) α [11]. These observations reveal the molecular mechanisms of ATGL ablation in cardiac hypertrophy. However, it is still unknown whether or not cardiac hypertrophy caused by ATGL dysfunction is attributed to ATGL-induced cardiac lipid metabolic disorders.

Based on the importance of TG hydrolysis in myocardial lipid metabolism, we hypothesise that downregulation of myocardial ATGL may cause myocardial lipid metabolic disorder via blockage of TG hydrolysis, which causes abnormal accumulation of toxic lipid metabolic intermediates in cardiomyocytes, such as ceramides and FFA, subsequently induces cardiac hypertrophy. The present study was performed to determine the role of ATGL in the accumulation of lipotoxic substances during cardiac hypertrophy.

Materials and methods

Chemicals and antibodies

Cay10499 (10007875) and fumonisin B1 (62580, FB1) were purchased from Cayman Chemical (Cayman, Ann Arbor, Michigan, USA). Fenofibrate (Feno, F6020), palmitic acid-d_{31} (366897) and phenylephrine (PE, P6126) were obtained from Sigma–Aldrich (Sigma, St. Louis, MO, USA). C_6-ceramide (sc-3527) and C_{16}-ceramide (sc-201379) were purchased from Santa Cruz Biotechnology (Santa Cruz, Dallas, TX, USA). Rabbit monoclonal antibodies against ATGL (3370-1) were obtained from Epitomics Biotech Company (Epitomics, Burlingame, CA, USA). Mouse monoclonal antibodies against glyceraldehyde-3-phosphate dehydrogenase (GAPDH, G8795) and mouse monoclonal antibodies against PPARα (P0869) were purchased from Sigma–Aldrich (Sigma, St. Louis, MO, USA).

Animal model of cardiac hypertrophy

Sprague–Dawley rats (male, 180–200 g) were obtained from the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). The rats were randomly divided into two groups, the sham-operated control group and the pressure overloaded group. After anaesthetised with sodium pentobarbital (30 mg kg^{-1}, i.p.), the pressure overloaded rats were performed abdominal aortic constriction (AAC) surgery as described previously [16], while the sham-operated group underwent a similar procedure without banding of the aorta. Eight weeks after the operation, all the rats were anaesthetised and then sacrificed for further experiments. All experimental procedures were conducted in conformity with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and approved by the Animal Care and Studies Committee of Sun Yat-sen University.

Primary culture of neonatal rat cardiomyocytes

Primary culture of neonatal rat cardiomyocytes (NRCMs) was prepared as described previously [17]. In brief, the myocardial tissues from 1- to 2-day-old Sprague–Dawley rats were repeatedly digested with 0.08% trypsin solution at 37 °C for 10–12 times. The cells were collected from each digestion and were harvested by centrifugation for 5 min at 800*g*. Afterwards, the cells were re-suspended by Dulbecco's modified Eagle's medium (DMEM) with 15% new born calf serum (NBCS) and were plated in culture flask for 1 h at 37 °C in a humidified atmosphere (5% CO_2 and 95% air) to exclude the non-myocytes. The purified cardiomyocytes were cultured in DMEM supplemented with 15% NBCS and 0.1 mM 5-bromodeoxyuridine for 16–24 h. Finally, the culture medium was replaced with fresh DMEM containing 15% NBCS.

Cardiomyocyte was treated with 100 μM PE for 24 h to induce cardiomyocyte hypertrophy. In order to study the effects of FAs on ceramide synthesis, cardiomyocyte was treated with 150 μM myristate or 150 μM palmitate for 24 h, or 10 μM FB1 for 16 h.

RNA extraction and quantitative RT-PCR

Total RNA was extracted using TRIZOL (Takara, Japan) according to the manufacturer's instructions. 2.0 μg of total RNA was reversely transcribed to first-strand cDNA in 20 μl reactions. The cDNA was amplified using 2× SYBR Green I Master Mix (Takara, Dalian, China) in a Real-time PCR machine (iCycler; Bio-Rad). The thermal cycling profile for the reaction was: 95 °C 30 s; 95 °C 5 s and 60 °C 30 s for 40 cycles. Rat specific primers for atrial natriuretic factor (ANF), brain natriuretic polypeptide (BNP), fatty acid transport protein 1 (FATP1), CD36, carnitine palmitoyltransferase 1b (CPT-1b), medium-chain acyl-CoA dehydrogenase (MCAD), long-chain acyl-CoA dehydrogenase (LCAD), pyruvate dehydrogenase kinase isoform 4 (PDK4) and GAPDH were used. The primer sequences are listed in Supplemental Table S1. Each PCR reaction was done in triplicates and quantification was performed with the efficiency-corrected $2^{-\Delta\Delta CT}$ method using GAPDH as endogenous control [18]. Data were presented as fold change over control group.

RNA interference and plasmid transfection

Cardiomyocytes were transfected either with ATGL siRNA or with non-targeting siRNA (negative control, NC) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. SiRNA sequence targeting ATGL is: 5′-CAGACAACUUGCCACUUUAdTdT-3′ (sense) and 3′-dTdTGUCU GUUGAACGGUGAAAU-5′ (antisense).

Cardiomyocytes were transfected with 4 μg ATGL plasmid using 8 μl Lipofectamine 2000 in 6-well plates according to the manufacturer's instructions. The ATGL plasmid (pEGFP-N1 vector) was a gift from Dr. Guoquan Gao (Zhongshan School of Medicine, Sun Yat-sen University) [19]. The pEGFP-N1 vector was used as a control.

Western blot

Cultured cardiomyocytes or cardiac tissues were homogenised in RIPA lysis buffer containing 150 mM NaCl, 50 mM Tris (pH 7.4), 1% (w/v) sodium deoxycholate, 1% (v/v) Triton X-100, 0.1% (w/v) SDS and 1 mM EDTA (Beyotime, Haimen, Jiangsu, China) supplemented with protease inhibitor cocktail (Sigma). The mixture was incubated on ice for 30 min and then centrifuged at 12,000*g* for 10 min at 4 °C and the supernatant was collected. The protein concentrations were determined using a BCA Protein Assay Kit (Pierce, Rockford, IL, USA). The proteins were separated in 10% SDS–PAGE and then were transferred to PVDF membranes (Millipore, CA, USA). The membranes were blocked with 5% bovine serum albumin and then incubated with primary antibodies overnight at 4 °C and secondary antibodies for 1 h at room temperature. Blots were developed with a super-western sensitivity chemiluminescence detection system (Pierce, Rockford, IL, USA) and detected by the LAS4000 imager (GE Healthcare, Waukesha, WI, USA). The intensities of the blots were quantified with the Image J software. GAPDH was used as a loading control.