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Roles of transcriptional corepressor RIP140 and coactivator PGC-1α in energy state of chronically infarcted rat hearts and mitochondrial function of cardiomyocytes

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ABSTRACT

Transcriptional coactivator PPARγ coactivator-1α (PGC-1α) and corepressor receptor-interacting protein 140 (RIP140) are opposing-functional regulators in maintaining energy balance of most metabolic tissues and cells. However, the relative contributions of both factors to energy metabolism in cardiomyocytes remain largely unknown. Herein, we reported that the relative protein levels of RIP140/PGC-1α were up-regulated in the failing hearts after chronic myocardial infarction (MI), and correlated negatively with the energy state index phosphocreatine (PCr)/ATP ratios. Real-time PCR analysis revealed that mRNA expressions of estrogen related receptor α (ERRα), peroxisome proliferate activated receptor α and β (PPARα, PPARβ), nuclear respiratory factor 1 (NRF1) and their target genes were repressed by RIP140 and induced by PGC-1α in a dose dependent manner in neonatal rat cardiomyocytes. We also observed that overexpression of RIP140 through adenovirus delivery can abrogate the PGC-1α-mediated induction of mitochondrial membrane potential elevation and mitochondrial biogenesis, and activate both autophagy and apoptosis pathways. We conclude that RIP140 and PGC-1α exert antagonistic role in regulating cardiac energy state and mitochondrial biogenesis.

1. Introduction

The heart has an extraordinarily high capacity for ATP resynthesis via mitochondrial oxidative phosphorylation (OXPHOS) to maintain its contractile function (Stanley et al., 2005). Collective evidences show that mitochondrial energy metabolic derangements are associated with the development and progression of heart failure, following pressure- or volume-overload, or regional myocardial infarction (MI). Reduced capacity for ATP production is inextricably associated with the severity of heart failure. The phosphocreatine (PCr)/ATP ratio which reflects the mitochondrial energy reserve state was confirmed to be reduced in animal models of cardiac hypertrophy (Massie et al., 1995) and failure (Ye et al., 2001), as well as in Patients with heart failure (Conway et al., 1991). Furthermore, the ratio of PCr to ATP was considered to be a better predictor of cardiovascular mortality than ejection fraction (EF) in failing heart subjected to energy metabolism alterations (Neubauer et al., 1997).

Transcription factors and nuclear receptors superfamily, such as nuclear respiratory factors (NRFs), peroxisome proliferator activated receptors (PPARs) and estrogen related receptors (ERRs) regulate the transcription of gene networks maintaining energy balance in many metabolic tissues and cells (Huss and Kelly, 2005). These factors activate or repress target genes depending on the recruitment of coactivators or corepressors. PPARγ coactivator-1α (PGC-1α) is a best-studied transcription coactivator that positively regulated the expression of metabolic genes associated with mitochondrial and metabolic adaptations, and consequently influences cardiac substrate selection and mitochondrial ATP production capacity (Finck and Kelly, 2007). Conversely, receptor-interacting protein 140 (RIP140) is a corepressor for nuclear receptors and transcription factors that suppressed catabolic signaling

Abbreviations: ERR, estrogen related receptor; FAO, fatty acid oxidation; FFAs, free fatty acids; GLUT, glucose transporter; HIF-1α, hypoxia inducible factor-1α; LAD, left anterior descending coronary artery; MI, myocardial infarction; MOI, multiplicity of infection; NRFs, nuclear respiration factors; OXPHOS, oxidative phosphorylation; PCr, phosphocreatine; PDK4, pyruvate dehydrogenase kinase-isoform 4; PPAR, peroxisome proliferate activated receptor; PGC-1α, PPARγ coactivator-1α; RIP140, receptor-interacting protein 140; TEM, transmission electron microscopy.

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processes in white adipose tissue (Powelka et al., 2006), muscle (Seth et al., 2007), and heart (Fritah et al., 2010). It was first identified in human cancer extracts (Cavailles et al., 1995) and localized in nuclear as PGC-1α. A set of genes that are negatively regulated by RIP140 are also targets activated by nuclear receptors through recruiting of PGC-1α. Previous studies have reported that the PGC-1α/PPAR and PGC-1α/ERR signaling were deactivated in the failing hearts of animal models and human (Finck and Kelly, 2007), but the expression and precise function of RIP140 under conditions of cardiac stress were incompletely understood. In view of the common characteristics of the colocation in nuclear and common downstream target genes for these two transcriptional cofactors, the regulation of metabolic process in heart may depend on the relative contributions of RIP140 and PGC-1α. In this study, we aim to examine the correlation of relative expressions of RIP140 to PGC-1α with the energy state index PCr/ATP ratios on the progression of heart failure in a rat model of MI, and the influence of different levels of RIP140 and PGC-1α expressions on mitochondrial biogenesis and function in the neonatal rat cardiomyocytes.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighting 220 to 250 g were obtained from Experimental Animal Center of Sun Yat-sen University. Rat MI model was established by coronary artery ligation as previously described (Murray et al., 2008).In brief, following incubation and thoracotomy, a 6/0 silk suture was tied around left anterior descending coronary artery (LAD) approximately 2–5 mm from its origin and tightly ligated to occlude the vessel. Sham-operated animals underwent the same procedure, excepting that the arterial ligation was omitted. All animal experiments were approved by the Animal Care and Studies committee of Sun Yat-sen University.

2.2. Echocardiographic and left ventricular hemodynamic study

Echocardiography was performed at 9 weeks post-surgery to evaluate the left ventricular (LV) function. Animals were anesthetized with sodium pentobarbital (30 mg/kg, Merck). Two-dimensional short-axis and M-mode echocardiographic images were recorded at the papillary muscle levels using MPX DU8 (Technos, Italy) with 8.5 MHz transducer. All ligated hearts were divided into two groups, infarcted-failing group and infarcted-unfailing group, according to the values of left ventricular ejection fraction (LVEF) at 50% (Murray et al., 2008), which reflects the extent of cardiac function impairment.

Following echocardiography, a polyethylene pressure transducer catheter was inserted from the right carotid artery into LV for hemodynamic study (Ding et al., 2009). Blood was obtained and plasma was separated immediately to measure the free fatty acids (FFAs) concentration using a commercially available spectrophotometric kit (Nanjing Jiancheng Bioengineering Institude, China). Hearts were rapidly excised, weighed, and frozen in liquid nitrogen.

2.3. Measurements of PCr and ATP

The apex of LV (200 mg) was homogenized in 0.4 M perchloric acid ($HClO_4$) for measuring PCr and ATP concentrations. 20 μl of filtered extract was assayed by high performance liquid chromatography (HPLC) with a Hypersil BDS C_{18} reversal-phase column (5 μm particle size, 250 × 4.6 mm, Thermo Electron Corp., USA). Samples were separated by a gradient of mobile phase buffer A (0.1 M KH_2PO_4, 5 mM tetrabutylammonium hydroxide, 2.5% (v/v) acetonitrile, pH 6.0) and buffer B (0.1 M KH_2PO_4, 5 mM tetrabutylammonium hydroxide, 25% (v/v) acetonitrile, pH 5.5) at a flow of 1.0 ml/min with detection of PCr at 205 nm and ATP at 260 nm, as previously described (Bernocchi et al., 1994). The retention times of PCr and ATP were approximately 6 and 31 min, respectively.

2.4. Primary cell culture and adenoviral infection

Primary cultures of cardiomyocytes were prepared from LV of neonatal Sprague–Dawley rats as described (Fu et al., 2005). Myocardial tissues were digested with 0.08% trypsin solution and cells were plated in DMEM with 10% FBS for 1 h to reduce the non-myocytes. Non-attached cardiomyocytes were cultured with DMEM supplemented with 10% FBS and 0.1 mM bromodeoxyuridine.

Adenovirus expressing RIP140 (Ad-RIP140), PGC-1α (Ad- PGC-1α) or GFP (Ad-GFP) was constructed and performed with pAd-Easy system (Stratagene) according to the manufacturer's instructions (Lehman et al., 2000). Briefly, the sequence encoding rat full-length of RIP140 or PGC-1α was cloned into pAdTrack-CMV shuttle vector, and then recombined with virus backbone pAdEasy-1 vector in BJ5183 bacteria. Ad-GFP is a recombination between the pAdTrack-CMV and pAdEasy-1 vectors for control. Viruses were propagated in the AD-293 cell line. Cardiomyocytes were exposed to adenovirus at a series of different multiplicity of infection (MOI of 20, 60, or 180), approximately 36 h after initial plating. Cells were harvested for isolation of RNA and protein extracts 36 h after infection.

2.5. Real time RT-PCR and Western blot analysis

Total RNA was isolated from LV tissue or primary cultured cardiomyocytes using RNA isolation reagent (TaKaRa, Japan) and used for cDNA synthesis. Expression levels of genes were determined by specific primers in the presence of Syber green fluorescent dye (TaKaRa, Japan). Primer sequences were listed in Table 1 of Supplementary data. Gene expression was normalized to β-actin and no difference was observed in this housekeeping gene β-actin between groups. LV tissues or primary cultured cardiomyocytes were lysed in RIPA buffer containing 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and protease inhibitor cocktail (Beyotime, China) for proteins extracts. Proteins were separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gel, and transferred to a polyvinylidene difluoride membrane. Membrane was incubated with specific antibodies to PGC-1α (1:1000, Calbiochem), RIP140 (1:1000, Abcam), LC3 (1:2500, Sigma), caspase 3 (1:1000, Cell Signaling Technology), or α-tubulin (1:8000, Sigma). The secondary antibodies were conjugated at the dilution of 1:1000 (Sigma) and bands were visualized with Super-Signal West Pico substrate (Pierce). Bands of interest are quantified densitometrically by the GEL DOC 2000 image analysis system and Quantity One software (Bio-Rad, USA).

2.6. Mitochondrial membrane potential and transmission electron microscopy (TEM)

Cells were plated in circular glass-bottomed dishes and infected with Ad-RIP140 or Ad-PGC-1α for 36 h. Then 10 nM tetramethylrhodamin ethyl esters (TMRE, Invitrogen, Molecular Probes), a mitochondrial potential-indicating fluorophore, was added to cells for 30 min, after which cells were imaged using a laser scanning microscope (LSM710, Zeiss). Images were analyzed as described previously (Hickson-Bick et al., 2008; Nakagawa et al., 2009; Perry et al., 2011). For TEM analysis, cardiomyocytes pellets were pre-