FEBS Letters 589 (2015) 3631–3639

journal homepage: www.FEBSLetters.org

Profiling metabolic remodeling in PP2Acα deficiency and chronic pressure overload mouse hearts

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ARTICLE INFO

Article history:
Received 10 September 2015
Revised 8 October 2015
Accepted 11 October 2015
Available online 21 October 2015

Edited by Laszlo Nagy

Keywords:
Protein phosphatase 2a
Myocardial energetics
Heart failure
Transcriptional profiling

ABSTRACT

Our understanding of how metabolic switches occur in the failing heart is still limited. Here, we report the emblematic pattern of metabolic alternations in two different mouse models. PP2Acα deficient hearts exhibited a dramatic decrease in the levels of mRNA encoding for transporters and enzymes involved in glucose utilization, which compensated by higher expression levels of genes controlling fatty acid utilization. These features were partly reproduced in cultured PP2Acα KD cardiomyocytes. Equivalently, a decrease in the expression of most of the transporters and enzymes controlling both glucose and fatty acid metabolism were observed in TAC model.

1. Introduction

In metazoans, the constant generation of ATP is vital for the heart to maintain its pump function. Therefore, cardiomyocytes must be able to dynamically re-program fuel and energize their metabolic capacity in response to environmental and physiological cues to ensure that energy supply meets demands. During the early stages of heart development, remodeling the fetal into the adult heart is a complicated process, which relies on important transitions that are triggered shortly after birth [1,2]. In the meantime, during early postnatal stages, the heart undergoes a switch in substrate utilization to catabolize fatty acid, and carbohydrates become a secondary energy source. This switching is vital for the heart. There is much evidence emerging that derangements in cardiac energy metabolism and mitochondrial function contribute to the pathological remodeling that leads to congestive heart failure (CHF) [3–8]. The concept of metabolic inflexibility has been proposed to explain the lack of energetic and mechanical reserves in the failing heart. However, the metabolic pattern presented at different disease stages, even using different methodologies, is often contradictory.

Recent reports have identified that cardiomyopathy may associated with imbalanced phosphorylation status [9–12]. Protein phosphatase 2A (PP2A) is a Ser/Thr phosphatase that exists across many eukaryotic cell types. The PP2A holoenzyme is composed of a scaffolding A subunit, a catalytic C subunit and a variable regulatory B subunit [13], which accounts for greater than 90% of protein dephosphorylation activity in the heart together with PP1 [14]. In mammals, there are two almost identical PP2Ac isoforms, PP2Acα and PP2Acβ, of which PP2Acα is the predominant isoform in the myocardium. Several murine models demonstrating gain or loss

Abbreviations: ACADM, acyl-coenzyme A dehydrogenase, middle chain (C4–C12); ACADS, acyl-coenzyme A dehydrogenase, short chain (C4–C8); ACADVL, acyl-coenzyme A dehydrogenase, very long chain; ACSL1, acyl-CoA synthetase long-chain family member 1; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; CD36, fatty acid translocase; CPT, carnitine palmitoyltransferase; CS, citrate synthase; EF, ejection fraction; FABP, fatty acid binding protein; FS, fractional shortening; GLUT, solute carrier family 2 (facilitated glucose transporter); HDL, high-density lipoprotein; HK2, hexokinase-2; IDH, isocitrate dehydrogenase 1; LDL, low-density lipoprotein; LVID, left ventricular internal diameter; LVM, left ventricular mass; PDHb, pyruvate dehydrogenase subunit beta; PDK, pyruvate dehydrogenase kinase; PFKM, phosphofructokinase; PKM2, pyruvate kinase muscle isoform-2; TC, total cholesterol; TG, triglyceride

Author contributions: D.C.D. and L.Y.L. conceived and designed the experiments. P.Y.G. provided the PP2Acα$^{f/f}$ mice strain. D.C.D. conducted most of experiments, L.Y.L. performed the experiments on TAC model. Z.Z. and C.L. (Z.Z. and C.L. are the associate fellows at the Collaborative Innovation Center for Cardiovascular Disease Translational Medicine of Nanjing Medical University) cosupervised the project and provided reagents. Z.Z. supervised the entire project. D.C.D. and L.Y.L. wrote the manuscript with helpful comments from all coauthors.

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http://dx.doi.org/10.1016/j.febslet.2015.10.016
0014-5793/

of PP2A function have been reported using genetic manipulation technology. PP2Acα overexpression in mouse heart severely impaired cardiac function [15,16]. However, PP2Acα deficiency in mice resulted in embryonic lethality by embryonic day 6.5 [17,18]. These data indicate that PP2A plays an essential role in cell function, so altered PP2A expression or activity would cause severe consequences. Herein, we report a mouse model with conditionally inactivated PP2Acα in cardiomyocytes. In this new model, we noted a hypertrophic heart at early postnatal stage, which could be an ideal model to explore the relationships between phosphorylation events and heart function during early development.

Understanding the metabolic consequences of heart failure is important to explore potential mechanisms for disease progression and to find targets for therapies that are designed to improve myocardial metabolism in patients with heart failure. Nevertheless, a comprehensive metabolic remodeling profile of hypertrophy is still lacking. In this study, we delineated how myocardial metabolic substrate utilization influenced heart failure disease progression during different developmental stages. Interestingly, the present results demonstrate that cardiac metabolic adaptations in response to different stresses do not exhibit a unique pattern despite a similar degree of hypertrophy. The disparate challenges will invoke specific gene sets to an appropriate adaptive response.

2. Materials and methods

2.1. Animal breeding and genotyping

Animal experiments in this study were performed in accordance with the Guide for the Care and Use of Laboratory Animals as published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996) using a protocol approved by the Animal Center of Nanjing Normal University, China (Permit No. 2090658). All mice used in this study were C57BL/6J background. Animals were bred in a specific pathogen-free animal facility under standard conditions. Genotyping was performed by PCR analysis of genomic DNA that was extracted from mouse toes.

2.2. Transverse aortic constriction (TAC) surgical procedures

Two-month-old male C57BL mice were anesthetized with an intraperitoneal (IP) injection of Avertin (250 mg/kg) and body temperature was maintained at 37 °C with a heating pad. After opening of chest, a 6–0 silk suture was tied around the transverse aorta against 27G needle, after which the needle was removed to yield a reproducible degree of constriction. Stitching the chest then kept ventilated until recovery of autonomic breathing. Sham-operated mice were subjected to the same surgical protocol but without tying off the suture.

2.3. Echocardiographic measurements

2D and M-mode echocardiography were used to assess the heart function of mice under Avertin (250 mg/kg) anesthesia using the method as Collins et al. described [19], on a Visualsonics Vevo 2100 echocardiography system (Visualsonics, Toronto, ON).

2.4. Cell culture and infection

Neonatal mouse ventricular myocyte (NMVM) cultures were prepared from 1 to 3-day-old PP2Acα$^{f/f}$ mice. The cells were infected with adenovirus (10^9 pfu/ml) for 48 h and were then subjected to measurements.

2.5. Quantification of mRNA expression

Total RNA was extracted from the left ventricle using Trizol reagent (TaKaRa, Japan). cDNA was synthesized using the PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Japan) according to the manufacturer's protocol. Relative mRNA expression levels of genes of interest were quantified by Real-time RT-PCR using the SYBR® Premix Ex Taq™ kit (TaKaRa, Japan). The primer sequences are listed in Table 1. 36B4 was used to normalize gene expression levels. Real-time RT-PCR was performed in a StepOnePlus™ Real-time PCR Detection System (Applied Biosystems, USA).

2.6. Western blot analysis

Proteins were separated by SDS polyacrylamide gels and incubated overnight at 4 °C with appropriate primary antibodies (see Table 2). After washing with TBST, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 h at room temperature. A chemiluminescence reaction

Table 1
Primer sequences used in this study.

Primer	Forward (5′-3′)	Reverse (5′-3′)
36B4 Mus	GAAACTGCTGCCTCACATCCG	GCTGGCACAGTGACCTCACACG
ACADM Mus	AGGTTTCAAGATCGCAATGG	CTCCTTGGTGCTCCACTAGC
ACADS Mus	ATTTGCCAGCACAGACAGGT	CCAAAGGCATTGCGGTTCTC
ACADVL Mus	CTATCTCTGCCCAGCGACTTTAT	GCTCTTTGAGAAATTGTGCCTGT
ACSL1 Mus	GGAGCTTCGCAGTGGCATC	CCCAGGCTCGACTGTATCTTGT
ANP Mus	TCGTCTTGGCCTTTTGGCT	TCCAGGTGGTCTAGCAGGTTCT
BNP Mus	AAGCTGCTGGAGCTG ATAAGA	GTTACAGCCCAAACGACTGAC
CD36 Mus	GAACAGAGGATGACAACT	GGAACATAGAAGACTTGGA
CPT1α Mus	TTGCTGATGACGGCTATG	GGCTAGAGAACTTGGAAGAA
CPT1β Mus	AAGATTCTCATTGCTACA	GATAAGGGTGAAAGATTTG
CPT2 Mus	GAAGTGTTGTGTAAGGATT	TGGTGTGCTTATTCTGTT
Cre Mus	GCCTGCATTACCGGTCGATGC	CAGGGTGTTATAAGCAATCCC
CS Mus	TTTGTTTTGTTTCAGGGGCCTTT	CTGTCCCTGGCGTAGATGAC
ENO1α Mus	TCAAGACTGCAATCGCAAAG	TGGGTAGTTCTGGACGAAGG
FABP Mus	CAAGGTTATCGCTACTATGAC	CACTCTGCTCTGGGATTT
GLUT1Mus	CCTGTCTCTTCCTACCCAACC	GCAGGAGTGTCCGTGTCTTC
GLUT4Mus	TATGTTGCGGATGCTATG	TTAGGAAGGTGAAGATGAAG
HK2 Mus	ATGAGGGGCGGATGTGTATCA	GGTTCAGTGAGCCCATGTCAA
Loxp Mus	TAGCCCATGCCTTTAATCTCAGAGC	CACTCGTCGTAGAACCCATAAACC
PDHb Mus	GGGAAGAAGTTGCCCAGTATGA	AGTCCTTTTGCATCCTCGGAAT
PFKM Mus	ATCTCCAGGTGAATGTTG	AAGTCAGTAGTGTAGTTCTC
PKM2 Mus	AGATGCTGAAGGAGATGATTA	ACGGACATTCTTGATGGT
PP2A Cα Mus	GGCATCATGGACGAGAAGTTG	TTCTCGCAGAGGCTCTTGAC