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Activation of Serine One-Carbon Metabolism by Calcineurin Aβ1 Reduces Myocardial Hypertrophy and Improves Ventricular Function

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ABSTRACT

BACKGROUND In response to pressure overload, the heart develops ventricular hypertrophy that progressively decompensates and leads to heart failure. This pathological hypertrophy is mediated, among others, by the phosphatase calcineurin and is characterized by metabolic changes that impair energy production by mitochondria.

OBJECTIVES The authors aimed to determine the role of the calcineurin splicing variant CnAβ1 in the context of cardiac hypertrophy and its mechanism of action.

METHODS Transgenic mice overexpressing CnAβ1 specifically in cardiomyocytes and mice lacking the unique C-terminal domain in CnAβ1 (*CnAβ1*$^{\Delta i12}$ mice) were used. Pressure overload hypertrophy was induced by transaortic constriction. Cardiac function was measured by echocardiography. Mice were characterized using various molecular analyses.

RESULTS In contrast to other calcineurin isoforms, the authors show here that cardiac-specific overexpression of CnAβ1 in transgenic mice reduces cardiac hypertrophy and improves cardiac function. This effect is mediated by activation of serine and one-carbon metabolism, and the production of antioxidant mediators that prevent mitochondrial protein oxidation and preserve ATP production. The induction of enzymes involved in this metabolic pathway by CnAβ1 is dependent on mTOR activity. Inhibition of serine and one-carbon metabolism blocks the beneficial effects of CnAβ1. *CnAβ1*$^{\Delta i12}$ mice show increased cardiac hypertrophy and declined contractility.

CONCLUSIONS The metabolic reprogramming induced by CnAβ1 redefines the role of calcineurin in the heart and shows for the first time that activation of the serine and one-carbon pathway has beneficial effects on cardiac hypertrophy and function, paving the way for new therapeutic approaches. (J Am Coll Cardiol 2018;71:654–67)

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Cardiac hypertrophy progressively decompensates and becomes maladaptive, leading to pathological cardiac remodeling and heart failure (1). Maladaptive cardiac hypertrophy is accompanied by interstitial and perivascular fibrosis and by changes in cardiac metabolism. The hypertrophic heart progressively reverts to an embryonic metabolic program with reduced fatty acid oxidation and increased reliance on glucose metabolism that result in decreased ATP production (2).

The calcium-regulated phosphatase calcineurin plays a major role in pathological hypertrophy. Calcineurin is composed of a catalytic (CnA) and a regulatory (CnB) subunit. CnA is encoded by 3 different genes (resulting in CnAα, CnAβ, and CnAγ), with CnAβ being the main isoform in the heart. Two splice variants for CnAβ have been described. Although CnAβ2 has a C-terminal autoinhibitory domain and acts like a typical CnA, CnAβ1 has a unique C-terminal domain, not shared by any other known protein, that confers these isoform specific properties (3-6).

Constitutive activation of calcineurin or its main target, the transcription factor nuclear factor of activated T cells (NFATc), leads to massive maladaptive cardiac hypertrophy (7). By contrast, mice lacking CnAβ show reduced ventricular hypertrophy in response to pressure overload (8). However, the role of CnAβ1 in this context is unknown.

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METHODS

Full Methods can be found in the Online Appendix.

MICE. *αMHC-CnAβ1* mice express the human CnAβ1 isoform in a cardiomyocyte-restricted manner under the control of the alpha myosin heavy chain (αMHC) promoter (4). CnAβ1$^{\Delta i12}$ mice were generated by deleting intron 12-13 in the gene that encodes CnAβ (*Ppp3cb*), which encodes the unique C-terminal domain in CnAβ1. Only adult male mice were used in this study. All procedures were approved by the ethics committees of the CNIC and the Regional Government of Madrid.

SURGERIES AND ECHOCARDIOGRAPHIC ANALYSIS. Maladaptive cardiac hypertrophy was induced by transaortic constriction (TAC) trying to reproduce the human condition as much as possible (9). Where indicated, L-buthionine-sulfoximine (BSO) (3 g/l in drinking water) or NCT-503 (0.9 mg/mouse, daily intraperitoneal injection) was administered for 21 days, starting on the day of the surgery. Transthoracic echocardiography was performed blindly using an ultra-high-resolution echocardiography system with a linear 30-MHz transducer. Two-dimensional and M-mode echocardiography in parasternal long- and short-axis views were performed blinded as previously described and recorded for posterior blinded analysis (3).

RESULTS

CnAβ1 OVEREXPRESSION REDUCES CARDIAC HYPERTROPHY. To determine the effect of CnAβ1 overexpression on the heart in the context of maladaptive cardiac hypertrophy, we used *αMHC-CnAβ1* transgenic mice that overexpress CnAβ1 in a cardiac-specific manner (Online Figures 1A and 1B) (4). We induced pressure overload in wild-type (WT) and transgenic mice by TAC, and we analyzed cardiac function 21 days later. Transgenic mice showed a significantly reduced heart weight to body weight ratio after TAC compared with WT mice (**Figure 1A**). Similarly, echocardiographic analysis revealed a reduced left ventricular mass index (LVMi) and thinner posterior wall and interventricular septum in CnAβ1-overexpressing mice after TAC compared with WT mice (**Figures 1B–1D**). In agreement with the echocardiography results, transgenic mice showed a more limited increase in cardiomyocyte size (**Figure 1E**). Importantly, whereas contractility declined in WT mice 21 days after TAC, it was preserved in CnAβ1-overexpressing mice, as shown by improved left ventricular ejection fraction (LVEF) (**Figure 1F**). Transgenic mice also showed a limited increase in the expression of the HF markers atrial natriuretic factor (ANF) and brain natriuretic peptide (BNP), which were strongly induced in WT mice (Online Figures 1C and 1D).

Pressure overload hypertrophy was characterized by both interstitial and perivascular fibrosis in WT mice (**Figures 1G to 1J**). Transgenic mice showed significantly reduced cardiac fibrosis with levels similar to those of sham-operated mice. In agreement

ABBREVIATIONS AND ACRONYMS

ATF4 = activating transcription factor 4

BSO = L-buthionine-sulfoximine

GSH = reduced glutathione

LVEF = left ventricular ejection fraction

LVMi = left ventricular mass index

mTOR = mechanistic target of rapamycin

NFAT = nuclear factor of activated T cells

qRT-PCR = quantitative reverse-transcription real-time polymerase chain reaction

TAC = transaortic constriction

WT = wild-type

All other authors have reported that they have no relationships relevant to the contents of this paper to disclose. Drs. Padrón-Barthe, Villalba-Orero, and Gómez-Salinero contributed equally to this work and are joint first authors. Robyn Shaw, MD, PhD, served as Guest Editor for this paper.

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