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Original article

Cardiac-specific haploinsufficiency of β-catenin attenuates cardiac hypertrophy but enhances fetal gene expression in response to aortic constriction

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Abstract

In addition to its role in cell adhesion, β-catenin is an important signaling molecule in the Wnt/Wingless signaling pathway. Recent studies have indicated that β-catenin is stabilized by hypertrophic stimuli and may regulate cardiac hypertrophic responses. To explore the role and requirement of β-catenin in cardiac development and hypertrophy, we deleted the β-catenin gene specifically in cardiac myocytes by crossing loxP-floxed β-catenin mice with transgenic mice expressing a Cre recombinase under the control of the α-myosin heavy chain promoter. No homozygous β-catenin-deleted mice were born alive and died before embryonic day 14.5, indicating significant and irreplaceable roles of β-catenin in embryonic heart development. Heterozygous β-catenin-deleted mice, however, demonstrated no structural and functional abnormality. The response of heterozygous β-catenin-deleted mice to transverse aortic constriction, however, was significantly attenuated with decreased heart weight and heart weight/body weight ratio compared to controls with intact β-catenin genes. Hemodynamic analysis revealed that there was no difference in cardiac function between wild-type and heterozygous β-catenin-deleted mice. On the other hand, the expression of fetal genes, β-myosin heavy chain, atrial and brain natriuretic peptides was significantly higher in heterozygous β-catenin-deleted mice when compared to wild-type β-catenin mice. These results suggest that the cytoplasmic level of β-catenin modulates hypertrophic response and fetal gene reprogramming after pressure overload.

Keywords: Catenin; Hypertrophy; Heart; Pressure overload; Cardiac remodeling; Aortic constriction

1. Introduction

In cardiac myocytes, actin filaments anchor to adherens junctions through a catenin complex. β-Catenin directly binds to cadherin cytoplasmic domains and indirectly to actin filaments through α-catenin. In addition to this role in initiating and maintaining adherens junctions, β-catenin is also involved in the Wnt/Wingless signal transduction [1]. In the cytoplasm, β-catenin forms a large complex including adenomatous polyposis coli (APC), glycogen synthase kinase-3β (GSK-3β) and axin. GSK-3β phosphorylates β-catenin in the complex resulting in its degradation through the ubiquitin-proteasome system, and thus lowering its cytoplasmic level [2]. In the basal state, GSK-3β is constitutively active and cytoplasmic β-catenin level is low. GSK-3β activity is controlled by several signaling pathways and primarily regulated by inactivation. In cardiac myocytes, GSK-3β is mainly regulated by Akt, but not Wnt/Wingless signaling

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pathway [3]. Akt phosphorylates GSK-3β and inhibits its activity, thereby stabilizing β-catenin and increasing its free cytoplasmic level. The inhibition of GSK-3β activity is required for cardiac hypertrophy induced by pressure overload and calcineurin activation [3,4]. Constitutively active GSK-3β attenuates cardiac hypertrophy by phosphorylating the nuclear factor of activated T cells (NFAT) and inhibiting its nuclear translocation. Similarly, the overexpression of wild-type GSK-3β in the heart also inhibits cardiac hypertrophy [5], most likely through the reduction of cytoplasmic β-catenin levels in cardiac myocyte [6]. Interestingly, wild-type GSK-3β also inhibits postnatal physiological growth in addition to pathological hypertrophy. It is not currently determined whether constitutively active and wild-type GSK-3β have differential effects on NFAT activation or cytoplasmic β-catenin levels.

The cytoplasmic level of β-catenin is increased in cultured neonatal cardiac myocytes by hypertrophic stimuli and in adult animals after transverse aortic constriction (TAC) [3]. More importantly, adenoviral infection of adult myocardium with a stabilized form of β-catenin induces cardiac hypertrophy [3]. Thus, stabilization of β-catenin in cardiac myocytes is sufficient to cause cardiac hypertrophy. More importantly, homozygous deletion of β-catenin in cardiac myocytes of maturing mice inhibits both physiological and pathological hypertrophy [7]. The knockout of β-catenin in mature mice, however, has no apparent effect on the heart weight and myocyte morphology in normal physiological condition [8]. The direct role of β-catenin in early cardiac development and postnatal physiological growth remains to be investigated.

β-Catenin is required for early embryogenesis. Deletion of β-catenin disrupts axis formation. Conditional inactivation of β-catenin in endothelial cells also demonstrates that β-catenin plays a critical role during cardiac cushion development [9]. To investigate the direct role of β-catenin in cardiac development and hypertrophy, we specifically deleted β-catenin in cardiac myocytes by crossing loxP-floxed β-catenin mice [10] with transgenic mice expressing a Cre recombinase under the control of the α-myosin heavy chain promoter. No homozygous loxP-floxed β-catenin mice positive for Cre transgene were born alive, indicating that the β-catenin gene was deleted during embryonic development and required for the survival of embryos. Mice with heterozygous deletion of β-catenin gene, on the other hand, were phenotypically unremarkable under normal physiological condition. The haploinsufficiency of β-catenin, however, attenuated cardiac hypertrophy after TAC. Paradoxically, the expression of fetal genes was enhanced by the loss of one copy of the β-catenin gene. These findings also suggest that the reactivation of the fetal gene program does not always correlate with hypertrophic growth.

2. Materials and methods

2.1. Animals

Mice with two loxP sites inserted in introns 1 and 6 of the β-catenin gene were generously donated to the research community by Dr. Rolf Kemler (Max-Planck Institute of Immunology, Germany) [10] and maintained at The Jackson Laboratory (Bar Harbor, ME). Transgenic mice expressing Cre recombinase under the control of the α-myosin heavy chain promoter (αMyHC-Cre) were created as described previously [11]. Homozygous β-catenin loxP-floxed (β-catenin$^{fl/fl}$) mice were crossed with αMyHC-Cre mice to generate heterozygous β-catenin loxP-floxed (β-catenin$^{fl/wt}$) negative for αMyHC-Cre or heterozygous β-catenin-deleted (β-catenin$^{del/wt}$) positive for αMyHC-Cre. Mice with β-catenin$^{del/wt}$ genotype were bred with β-catenin$^{fl/fl}$ or β-catenin$^{fl/wt}$ to produce homozygous β-catenin loxP-floxed or -deleted mice. DNA was isolated from tail or toe biopsy of neonates following proteinase K digestion as described [10]. αMyHC-Cre transgene, loxP-floxed and wild-type β-catenin genes were amplified by PCR as described previously [10,11]. A pilot study confirmed that recombination of β-catenin gene only occurred in atrial and ventricular tissues. All procedures were performed in accordance with *the Guide for the Care and Use of Laboratory Animals* (US Department of Health, Education, and Welfare, Department of Health and Human Services, NIH Publication 85-23) and were approved by the University of South Dakota Animal Care and Use Committee.

2.2. Transverse aortic constriction (TAC)

Eighty 3- to 4-month-old β-catenin$^{fl/wt}$ and β-catenin$^{del/wt}$ mice were randomly subjected to TAC or sham surgery. Wild-type β-catenin mice with Cre transgene were included in the preliminary study and demonstrated similar change to β-catenin$^{fl/wt}$ mice. Midline thoracotomy was performed with sterile technique under endotracheal intubation and isoflurane anesthesia. The aortic arch was ligated with a 7-0 silk suture against a 27-gauge needle between the origin of the innominate and left common carotid arteries. The needle was quickly removed and the incision was closed with sutures. The sham operation was performed identically except that the aorta was not ligated. Mice were allowed to recover on a warming pad until they were fully awake.

2.3. Echocardiography and hemodynamics

Four weeks after the surgery, transthoracic echocardiography was performed using a high-resolution Vevo660 echocardiogram system with a 30-MHz transducer (Visual Sonics, Toronto, Canada). The animals were lightly anesthetized with 1–2% isoflurane via a nose cone during echocardiography. Two-dimensional parasternal short axis images of the left ventricle (LV) were acquired at mid ventricle between the papillary muscles with guided M-mode recordings. Measurements of diastolic and systolic wall thicknesses and left ventricular end-diastolic and end-systolic chamber dimensions were made from leading edge to leading edge of the tracings. Ejection fraction (EF) and percentage fractional shortening (FS) were calculated with the accompanying software.

After echocardiography, mice were endotracheally intubated and anesthetized with isoflurane. The right carotid artery was