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Absence of synemin in mice causes structural and functional abnormalities in heart

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ABSTRACT

Cardiomyopathies have been linked to changes in structural proteins, including intermediate filament (IF) proteins located in the cytoskeleton. IFs associate with the contractile machinery and costameres of striated muscle and with intercalated disks in the heart. Synemin is a large IF protein that mediates the association of desmin with Z-disks and stabilizes intercalated disks. It also acts as an A-kinase anchoring protein (AKAP). In murine skeletal muscle, the absence of synemin causes a mild myopathy. Here, we report that the genetic silencing of synemin in mice (synm −/−) causes left ventricular systolic dysfunction at 3 months and 12–16 months of age, and left ventricular hypertrophy and dilatation at 12–16 months of age. Isolated cardiomyocytes showed alterations in calcium handling that indicate defects intrinsic to the heart. Although contractile and costameric proteins remained unchanged in the old synm −/− hearts, we identified alterations in several signaling proteins (PKA-RII, ERK and p70S6K) critical to cardiomyocyte function. Our data suggest that synemin plays an important regulatory role in the heart and that the consequences of its absence are profound.

1. Introduction

Heart diseases are the leading cause of death globally, affecting 1 in every 3 deaths [1,2]. Cardiomyopathy (CM) represents a collection of diverse abnormal conditions of the heart muscle having as a common denominator the reduced ability of the heart to pump blood. Dilated and hypertrophic myopathies are the most common forms of CM.

Dilated cardiomyopathy (DCM) is characterized by enlargement of the ventricles, thinning of the ventricular wall, non-specific histological features, increased diastolic and systolic volumes and a low ejection fraction [3,4]. Fifteen genes related to DCM are associate with defects in sarcomeric, sarcolemmal and cytoskeletal proteins, such as dystrophin, desmin, titin and myosin [4,5].

Hypertrophic cardiomyopathy (HCM) is characterized by heart remodeling, increased heart mass and abnormal diastolic function that result in heart failure and premature death [3,6]. Genes related to HCM typically encode contractile proteins which play important roles in force generation [7–9].

All 3 major elements of the cytoskeleton – intermediate filaments (IFs), microfilaments, and microtubules — play important roles in striated muscle, with the IFs being the most resistant to strain and thus most likely to play a key role in stabilizing the myoplasm during

Abbreviations: + dP/dt, maximal rate of left ventricular pressure rise; -dP/dt, maximal rate of left ventricular pressure fall; + dP/dt-EDP, end-diastolic pressure adjusted maximal rate of left ventricular pressure rise; AKAP, A-kinase anchoring protein; AKT, protein kinase B also known as Akt; BP, blood pressure; BW, body weight; CM, cardiomyopathy; CO, cardiac output; CREB, cAMP response element-binding protein; DCM, dilated cardiomyopathy; E/A, the ratio of the early (E) to late (A) filling velocities of the left ventricle; ECM, extracellular matrix; EDP, end-diastolic pressure; EDPVR, the slope of the end-diastolic pressure-volume relationship; EF, ejection fraction; ERK 1/2, extracellular signal-regulated kinase 1/2; ESP, end-systolic pressure; Ea, effective arterial elastance; Ees, the slope of left ventricular end-systolic pressure-volume relationship; FS, fractional shortening; HCM, hypertrophic cardiomyopathy; HR, heart rate; IFs, intermediate filaments; IVC, inferior vena cava; LV, left ventricle or left ventricular; LVDd, LV end-diastolic dimension; LVDs, LV end-systolic dimension; MAPKs, mitogen-activated protein kinases; MBP, mean blood pressure; PV, Pressure-volume; PKA RII, protein kinase A subunit II; PRSW, preload recruitable stroke work; Pes, pressure end-systolic; SL, sarcomere length; SV, stroke volume; SW, stroke work; synm −/−, synemin-null; Tau, isovolumic relaxation time constant; WT, wild type mice

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contraction and relaxation. IFs also influence cell shape and motility, anchor cell structural components, support nuclear architecture, and modulate the propagation of signals within cells [10–13]. The major IF proteins of mature striated muscle are the nuclear lamins, desmin, several keratin subunits, including K8, K18 and K19, and synemin [14–16]. Here we focus on synemin and its role in the heart.

Synemin is a large IF protein that can be expressed in one of two isoforms, α (~210 kDa), and β (~180 kDa) [17,18]. Unlike desmin, and keratins 8 and 18, and 8 and 19, synemin is incapable of forming stable IFs on its own and thus requires other IF proteins to form heterofilaments [19]. In neonatal cardiomyocytes, α-synemin stabilizes junctional complexes at the sarcolemma, whereas β-synemin appears to mediate the association of desmin with Z-disks [20]. In addition to desmin, synemin can associate with vimentin in developing heart muscle [19,21]. Synemin is also an A-kinase anchoring protein (AKAP) involved in regulating the phosphorylation of proteins at the sarcolemma and Z-disks via protein kinase A [22,23]. In its role as an AKAP, synemin may regulate the activities of key signaling cascades in the heart, such as those controlled by Akt, PKA RII, and MAPKs such as ERK 1/2 [23,24].

We have studied mice lacking synemin to determine the consequences of its absence on the structure and function of mature cardiac muscle. Our findings suggest that, although the organization of the myoplasm is not altered in the absence of synemin, cardiac muscle lacking synemin shows a mixed hypertrophic and dilated cardiomyopathic phenotype, with features of left ventricular (LV) systolic dysfunction as early as 3 months old, and LV hypertrophy and dilatation at older ages (12–16 mo old). Year-old synemin-null mice also show an increase of ~30% in the total body weight. Defects linked to the absence of synemin are endogenous to cardiomyocytes, as both Ca^{2+} transients and contractile shortening are altered in cells isolated from the synemin-null heart, compared to controls. The absence of synemin also alters the levels of several proteins in signaling pathways that have been associated with cardiomyopathy. Our results suggest that synemin is essential for cardiac health.

2. Materials and methods

2.1. Animals

Synemin-null (synm −/−) animals were generated by inserting cDNA encoding β-galactosidase into the synemin gene by homologous recombination and have been described elsewhere [25]. We used synm −/− mice at young (3 mo) and old ages (12–16 mo) and age-matched male C57Bl/6 controls (WT) for all studies reported here. Animals subjected to closed chest catheterization were anesthetized with 2.5% isoflurane inhalation (VetEquip, Pleasanton, CA) with oxygen at 0.8 l/min at 14.7 Psi (21 °C) and then euthanized by cervical dislocation. All of our protocols were approved by the Institutional Animal Care Committee of the University of Maryland School of Medicine.

2.2. Echocardiography

Transthoracic M-mode echocardiography using high-resolution ultrasound biomicroscopy was performed using the Vevo 2100 imaging system (Fiji-VisualSonics, Toronto, ON, Canada) equipped with a 40-MHz scanhead. Images of the LV in the parasternal short-axis view were obtained at the level of the papillary muscles under light anesthesia (inhalation of 1.2–1.5% isoflurane in oxygen). A core temperature of 37.5 °C was maintained during the measurement via a thermoregulated platform (THM 150, Indus Instruments, Houston, TX). Data were calculated according to generally accepted formulae, as we have used previously [26].

2.3. Left ventricular pressure-volume (PV) loop analysis

Under 2–2.5% of isoflurane inhalation, animals were orally intubated and ventilated via a rodent ventilator (Model 683, Harvard Instruments, Holliston, MA), at a tidal volume of 10–12 ml/kg BW with a ventilatory rate of 60–100 breaths/min. The right carotid artery was cannulated with a conductance catheter (1.2 French, Transonic-Science, London, ON, Canada), and its tip was positioned into the LV. A P10 silicone tube was cannulated into the left jugular vein for administration of 0.9% sodium chloride (0.1 ml/g BW/h). Midline laparotomy was performed, and the inferior vena cava (IVC) was visualized at the level of the origin of the right renal vein after the abdominal tissues were gently retracted with 37 °C wet (0.9% sodium chloride) gauze. A 4–0 silk suture was placed around the IVC and exteriorized through the abdominal wound. The wound was covered with the warm wet gauze. After 10–15 min stabilization under 1.5% isoflurane, the loop data were recorded via the ADVantage System (ADV500, Transonic-Science), prior to and during temporary IVC occlusion created by pulling of the IVC suture for approximately 10 s. The IVC occlusion was repeated 3 times at intervals of at least 5 min. Following PV loop recordings, the catheter tip was repositioned to the aorta for blood pressure (BP) recordings. The data were analyzed offline with LabChart Pro (Version 8.1.5, ADInstruments, Sydney, Australia). All the indices were defined according to generally accepted formulae [27].

2.4. Contractile and calcium measurements

Ventricular myocytes were enzymatically isolated from WT and synm −/− mice as previously described [28]. Myocytes were maintained in Tyrode's solution (in mM: 140 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 0.33 NaH_2PO_4, 11 glucose, 5 HEPES, and 1.8 $CaCl_2$, pH 7.4), at room temperature and used for experiments up to 4 h after dissociation. Prior to experiments, myocytes were loaded with 5 μM Indo-1 AM (TEFLABS Inc., Austin, TX) for 30 min at room temperature. Myocytes were then washed to remove extracellular dye and left for 30 min for de-esterification. Afterwards, myocytes were placed in a custom imaging chamber on an inverted microscope equipped with a dual PMT fluorescence system (Ionoptix), Sutter DG-4 excitation source, and a high-speed sarcomere length camera system (Aurora), all controlled by Fluorotrack software (Aurora, ON, Canada). Unloaded myocytes were paced at 1 Hz (2 ms, square pulses, 40 V) to elicit contractions while simultaneous measures of sarcomere length (SL) and Indo-1 fluorescent transients were collected.

2.5. Hematoxylin and eosin staining

Frozen cross sections, 5–8 μm thick, were fixed with cold acetone, air dried, immersed in Harris hematoxylin (Sigma-Aldrich, St. Louis, MO) for 5 min, then in Scotts Bluing reagent (ThermoFisher Scientific) for 1 min, followed by 10 rapid dips in Wright Eosin staining, with rinsing steps of tap water between solution changes, and finally through a gradient of increasing concentrations of ethanol, as previously described [25]. Coverslips were mounted with Permount (ThermoFisher Scientific). The sections were observed under light microscopy (Zeiss Axioscope, × 20 objective and × 2 eyepiece), and representative digital images were captured.

2.6. Masson trichrome staining

Frozen cross sections of 5–8 μm were fixed with cold acetone, air dried, immersed in Bouin's solution for 20 min at 56 °C, then in Weigert's Iron Hematoxylin for 7 min. Additional staining was with Biebrich Scarlet-Acid Fuchsin for 6 min, phosphotungstic/phosphomolybdic acid for 3 min, and then with Aniline Blue for 2 min. All solutions were from Sigma-Aldrich and each was followed by rinsing in tap water. Samples were then exposed to 1% acetic acid for 1 min, put