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Review article

Functional implications of mitofusin 2-mediated mitochondrial-SR tethering

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ABSTRACT

Cardiomyocyte mitochondria have an intimate physical and functional relationship with sarcoplasmic reticulum (SR). Under normal conditions mitochondrial ATP is essential to power SR calcium cycling that drives phasic contraction/relaxation, and changes in SR calcium release are sensed by mitochondria and used to modulate oxidative phosphorylation according to metabolic need. When perturbed, mitochondrial-SR calcium crosstalk can evoke programmed cell death. Physical proximity and functional interplay between mitochondria and SR are maintained in part through tethering of these two organelles by the membrane protein mitofusin 2 (Mfn2). Here we review and discuss findings from our two laboratories that derive from genetic manipulation of Mfn2 and closely related Mfn1 in mouse hearts and other experimental systems. By comparing the findings of our two independent research efforts we arrive at several conclusions that appear to be strongly supported, and describe a few areas of incomplete understanding that will require further study. In so doing we hope to clarify some misconceptions regarding the many varied roles of Mfn2 as both physical trans-organelle tether and mitochondrial fusion protein. This article is part of a Special Issue entitled "Mitochondria: From Basic Mitochondrial Biology to Cardiovascular Disease."

Contents

1. Introduction

Calcium cycling and signaling are central to normal cardiac development, metabolic homeostasis and contraction. Perturbations in calcium import, release, or re-uptake cause or contribute to post-ischemic cardiac dysfunction, programmed cardiomyocyte death, and intrinsic contractile depression in heart failure [1]. Calcium influx through sarcolemmal membrane channels is the initiating event in excitation–contraction coupling, but free calcium that drives contraction and modulates cell signaling pathways is largely derived from intracellular stores [2]. The sarcoplasmic reticulum and mitochondria are the most important organelle mediators of intracellular calcium uptake, storage, and release in cardiomyocytes. Ultrastructurally, these two organelles appear to exist in intimate physical association. Whether this represents the coincidental co-distribution of two requisite organelles throughout the cardiomyocyte, or a purposeful structural relationship with functional implications for inter-organelle cross-regulation [3] cannot be determined from electron micrographs. Recent identification of the molecular nature of mitochondrial calcium

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import mechanisms [4–6] and the protein tethers that create protected calcium microdomains between mitochondria and SR [7] have helped to identify context-specific roles played by calcium cross-talk between these two organelles in healthy and diseased hearts. Here, we review recent developments that are revising prior concepts about the nature and extent of cardiac SR-mitochondrial cross-talk [8], focusing mainly on insights derived from in vivo cardiac-specific manipulation of the organelle tethering protein, mitofusin (Mfn) 2.

2. Mfn2 tethers ER/SR to mitochondria

The sarcoplasmic reticulum (SR) is a modified smooth endoplasmic reticulum (ER) that passively releases calcium to promote muscle contraction, and then actively takes up calcium to promote relaxation [9]. Because SR and mitochondria are so closely apposed, and since mitochondria also take up and store calcium, one might reason that mitochondria would readily sense the cyclic changes in free cytosolic calcium evoked by SR calcium release and re-uptake. However, mitochondrial calcium uptake through the so-called calcium uniporter is remarkably inefficient, requiring a >10 μM concentration of free calcium for effect [3,10], which is not normally achieved in the cardiomyocyte sarcoplasm [5,11], at least as measured by standard methods that average free calcium concentration cell-wide. Nevertheless, using sensitive techniques it is possible to detect phasic mitochondrial calcium transients that recapitulate, albeit at greatly depressed amplitude, SR calcium release and reuptake transients [12–14]. Hypothetically, a physical connection between SR and mitochondria could enhance mitochondrial delivery of SR-derived calcium by limiting cytosolic diffusion. This notion became the conceptual underpinning for protected ER/SR-mitochondrial calcium microdomains that have subsequently been directly verified by in loco measurements [3,10].

Strong evidence for the dependence of ER/SR-mitochondrial calcium microdomains on physical trans-organelle linkage was provided by the identification of the mitochondrial fusion protein Mfn2 as a molecular tether that links fibroblast ER and cardiomyocyte SR to mitochondria [7,15]. Although Mfn2 plays a number of different roles in the heart [16,17], this dynamin-family GTPase is most widely recognized for its ability to mediate (in most cells redundantly with closely related Mfn1) mitochondrial tethering and outer membrane fusion during regenerative mitochondrial fusion [18]. The biophysical mechanisms of Mfn-mediated mitochondrial membrane fusion have recently been reviewed in detail [19]. Actual membrane fusion is not, however, known to occur after Mfn2-mediated tethering of SR/ER to mitochondria, and is therefore not discussed further here except to note that both published and unpublished data derived from comparative in vivo cardiomyocyte-specific ablation of Mfn2 and Mfn1 suggest that Mfn1 is more important as a mediator of mitochondrial fusion. In this regard, Mfn1 and Mfn2 cardiac knockout mice developed in the Walsh laboratory revealed that deletion of Mfn2 increased mitochondrial size [20], whereas deletion of Mfn1 decreased mitochondrial size [21] in cardiomyocytes. Additional evidence supporting a dominant role for Mfn1 in cardiomyocytes was derived from tri-allele Mfn1/Mfn2 cardiac knockout mice (embryonic deletion with Nkx2.5-Cre) developed in the Dorn laboratory. In previously unpublished work we found that complete embryonic cardiac ablation of either Mfn1 or Mfn2 had no effect on baseline cardiac function and only modestly impairs the adaptive response to experimental pressure overload evoked by partial surgical ligation of the transverse aorta (TAC) (Fig. 1). Likewise total cardiac knockout of Mfn2 and one Mfn1 allele (leaving one Mfn1 allele intact) was compatible with normal viability and baseline cardiac function, although the adaptive response to TAC was impaired (Fig. 1). Strikingly however, total cardiac knockout of Mfn1 and one Mfn2 allele, leaving only one functional Mfn2 allele, evoked a severe cardiomyopathy at baseline that is similar to that observed after

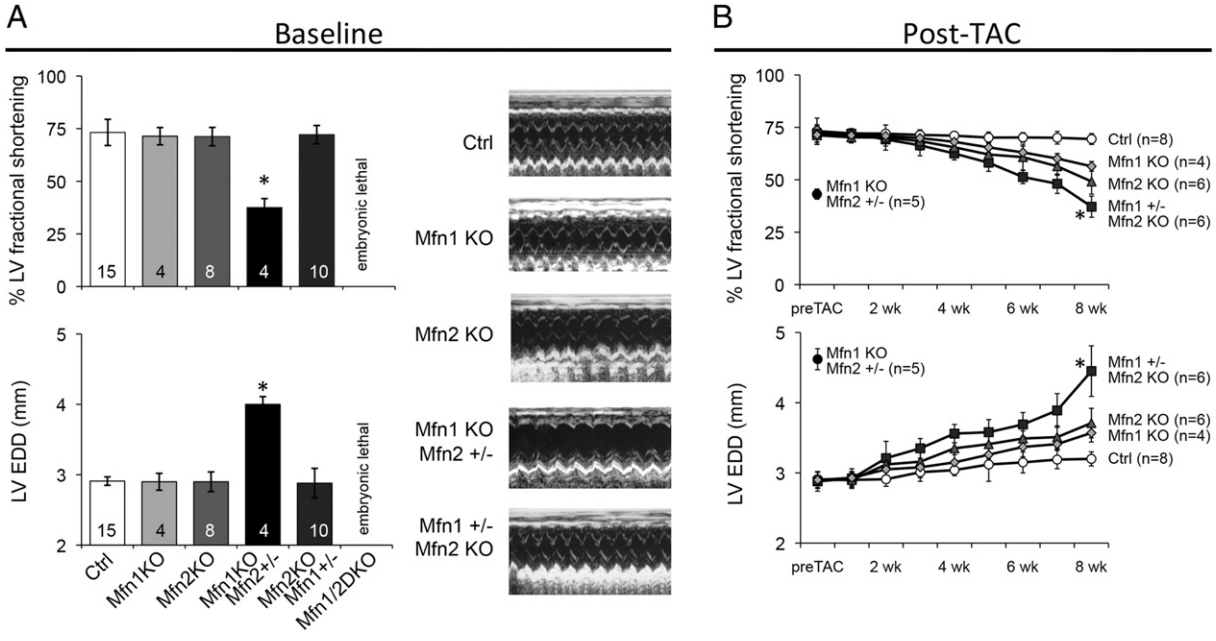

Fig. 1. Preeminence of Mfn1 over Mfn2 for cardiac function. A. Baseline echocardiographic characteristics of 8 week old mice with embryonic heart specific (Nkx2.5-Cre mediated) deletion of Mfn1 and Mfn2 genes in various allelic combinations. Ctrl is *Mfn1*+/+, *Mfn2*+/+; Mfn1 KO is *Mfn1*−/−, *Mfn2*+/+; Mfn2 KO is *Mfn1*+/+, *Mfn2*−/−; Mfn2 hapl (haploinsufficient) is *Mfn1*−/−, *Mfn2*+/−; and Mfn1 hapl is *Mfn1*+/−, *Mfn2*−/−. Mfn1/Mfn2 double cardiac KO is embryonic lethal [15]. A single Mfn1 allele and no Mfn2 expression are sufficient for normal basal cardiac function, whereas mice with a single cardiac Mfn2 allele and no Mfn1 expression develop a spontaneous cardiomyopathy. B. Different responses of cardiac Mfn1 KO, cardiac Mfn2 KO, and cardiac Mfn1 hapl to pressure overload modeling. Time course of cardiac ejection performance and left ventricular remodeling after TAC with ~60 mm Hg transaortic gradient. The absence of either Mfn1 or Mfn2 modestly compromises the late compensatory response, whereas mice having only a single Mfn1 allele show significantly greater decompensation. * = $P < 0.05$ from other groups by ANOVA.