Invited Mini Review

An experimental approach to study the function of mitochondria in cardiomyopathy

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Cardiomyopathy is an inherited or acquired disease of the myocardium, which can result in severe ventricular dysfunction. Mitochondrial dysfunction is involved in the pathological process of cardiomyopathy. Many dysfunctions in cardiac mitochondria are consequences of mutations in nuclear or mitochondrial DNA followed by alterations in transcriptional regulation, mitochondrial protein function, and mitochondrial dynamics and energetics, presenting with associated multisystem mitochondrial disorders. To ensure correct diagnosis and optimal management of mitochondrial dysfunction in cardiomyopathy caused by multiple pathogenesis, multidisciplinary approaches are required, and to integrate between clinical and basic sciences, ideal translational models are needed. In this review, we will focus on experimental models to provide insights into basic mitochondrial physiology and detailed underlying mechanisms of cardiomyopathy and current mitochondria-targeted therapies for cardiomyopathy. [BMB Reports 2015; 48(10): 541-548]

INTRODUCTION

Mitochondrial dysfunction plays a critical role in the underlying pathological process of ischemic heart disease (IHD) and cardiomyopathy (CM) (1). As a leading cause of death worldwide (2), IHD (also known as coronary artery disease) is primarily caused by acute myocardial infarction (AMI). A precursor to heart failure, CM is acutely caused by myocardial ischemia and chronically by hypertension or diabetes mellitus. Mitochondrial damage occurs primarily during the reperfusion phase of ischemia/reperfusion (I/R) (3, 4). In the post-I/R heart, mitochondrial dysfunctions are due to inhibition of electron transport chain (ETC) function and oxygen (O_2) consumption,

mitochondrial calcium (Ca^{2+}) overload, reactive oxygen species (ROS) generation, mitochondrial permeability transition (MPT) pore opening, loss of mitochondrial membrane potential ($\Delta\Psi m$), and increased mitochondrial necrosis and apoptosis (5-7). Alteration of mitochondrial energy metabolism also plays a substantial role in IHD and CM (8). Not surprisingly, mitochondria are a potential therapeutic target for IHD and CM.

CARDIOMYOPATHY

The heart is composed mostly of the cardiac muscle, or myocardium. Severe or long-standing problems with the cardiac muscle can lead to heart failure. Inflammation of the heart is called myocarditis, whereas non-inflammatory cardiac muscle disease is known as CM, in which the myocardium becomes damaged, weakened, or stretched by various causes. Clinically, CM is divided into ischemic CM and non-ischemic CM, and in turn non-ischemic CM includes three types: dilated (DCM), hypertrophic (HCM), and restrictive (RCM) (9, 10). DCM is characterized by enlargement of the heart and dilated ventricle walls, which associated with reduced left ventricle function or systolic function. In contrast, HCM is characterized by a thickened wall of the left ventricle and/or by a thickened septum that separates the left ventricle and the right ventricle. In the condition of RCM, the walls of the ventricles become rigid, which reduces cardiac output due to diastolic dysfunction. Over the past few decades, another type of CM called mitochondrial CM has emerged and has been extensively studied. The clinical and pathological phenotype of mitochondrial CM, which is primarily found in DCM, HCM, arrhythmias and heart failure, results from abnormalities in the cardiac mitochondrial oxidative phosphorylation (OXPHOS) system, due to genetic defects (11).

MITOCHONDRIAL DEFECTS IN EXPERIMENTAL MODELS OF CARDIOMYOPATHY

Mitochondrial dysfunction plays a critical role in the pathogenesis of CM (12, 13). Among various animal models that have been established to study the process and mechanisms

involved in cardiac disease (14-16), many exhibit CM phenotypes associated with mitochondrial defects (Table 1).

Transcriptional regulation
As with nuclear DNA (nDNA), mitochondrial DNA (mtDNA) is subject to epigenetic modifications related to energetic capacity and disease status. When calories are limited, gene expression is suppressed due to decreased phosphorylation and acetylation of chromatin (17). Sirtuins, or the Silent Information Regulator 2 (SIR2) protein family includes 7 (Sirt1-7) nicotinamide adenine

Table 1. Experimental models for cardiomyopathy with mitochondrial dysfunction

	Modification	Species	Phenotype	Mitochondrial abnormalities	Ref.
Transcriptional regulation	CAP^R mtDNA germ-line transmission	Mouse	DCM	Enlarged mitochondria	(22)
	Tfam cKO	Mouse	DCM	Respiration ↓	(52)
	Sirt7 KO	Mouse	CM	Acetylation of GABPβ1 ↑	(19)
	Ncoa6 cKO or DN1 TG	Mouse	DCM	Complex II ↓	(20)
Mitochondrial protein function	*Scgd* deficient (BIO 14.6)	Hamster	Congenital CM	St3 in IFM ↓ Ca^{2+} overload	(31) (95)
	Sod2 KO	Mouse	Neonatal DCM	Complex II ↓	(73, 96)
	Ant1 KO	Mouse	HCM	Respiration ↓, ROS ↑	(57)
	Nix/Bnip3 DKO	Mouse	CM	Accumulation of abnormal mitochondria	(37)
	CryAB^{R120G} mutation	Mouse	Desmin-related CM	Complex I ↓ MPT pore opening ↑ ΔΨm ↓	(62)
	Dystrophin-deficient (*mdx*)	Mouse	CM	Substrate shift O$_2$ consumption ↑	(66)
	LTCC β2a subunit cTG	Mouse	CM	Ca^{2+} overload	(67)
	MutUNG1 TG	Mouse	HCM	mtDNA stability ↓ Respiration ↓	(21)
Mitochondrial dynamics	*Dnm1l*$^{Py/+}$	Mouse	CM	ATP ↓	(49)
	Mfn1/2 DKO	Mouse	DCM	Mitochondrial fragmentation ↑ Respiration ↓	(39, 42, 43)
	Opa1$^{+/-}$	Mouse	CM	mtDNA stability ↓ Mitochondrial fragmentation ↑	(44)
	Mfn2 KO	Mouse	DCM	O$_2$ consumption ↓ ROS ↑	(40) (41)
	Drp1 cKO	Mouse	DCM	Mitophagy ↑ MPT pore opening ↑	(39)
Induced models	DOX-induced model	Rabbit Mouse Rat	CM	ROS ↑	(79) (78) (80)
	Pacing-induced model	Dog	DCM	Complex I ↓ ROS ↑	(72)
	Fz-induced model	Turkey Rat	DCM	Mitochondrial swelling ↑ Complex II, IV ↓ ΔΨm ↓	(68) (69)
	Autoantibodies (M$_2$-AA)	Rat	DCM-like	Mitochondrial swelling ↑	(26)

CAP^R: chloramphenicol-resistant, mtDNA: mitochondrial DNA, *Tfam*: mitochondrial transcription factor A (mtTFA), KO: knock out, cKO: conditional KO, *Sirt7*: sirtuin 7, GABPβ1: GA binding protein β1, *Ncoa6*: nuclear receptor coactivator 6, DN1: dominant-negative mutant containing an N-terminal LXXLL-1 motif of NCOA6, TG: transgenic, *Scgd*: δ-sarcoglycan, BIO 14.6: Syrian cardiomyopathic hamster, St3: state 3 (ADP-stimulated) respiration, IFM: interfibrillar mitochondria, *Sod2*: superoxide dismutase 2 (mitochondrial, manganese superoxide dismutase), *Ant1*: adenine nucleotide translocator, ROS: reactive oxygen species, DKO: double KO, CryAB: α-B-crystallin, *mdx*: X chromosome-linked muscular dystrophy, LTCC: L-type Ca^{2+} channel, mut: mutant, UNG1: uracil-DNA glycosylase 1, *Dnm1l*: dynamin-1-like, Py: Python (a mouse mutant), *Mfn2*: mitofusin 2, *Opa1*: optic atrophy 1, *Drp1*: dynamin-related protein 1, MPT pore: mitochondrial permeability transition pore, DOX: doxorubicin, Fz: furazolidone, M$_2$-AA: M$_2$ muscarinic receptor, ΔΨm: mitochondrial membrane potential.

dinucleotide (NAD^+)-dependent histone deacetylases (HDACs). Since sirtuins require NAD^+ for their deacetylase activity, they are tightly linked to cellular energy status, such as exercise and caloric restriction. Among the 7 isoforms, mitochondrial Sirt3, and nuclear Sirt6 and Sirt7 are implicated in left ventricular hypertrophy, CM, and lipid metabolism (18). In *Sirt7*-deficient mice, SIRT7-mediated deacetylation of GA binding protein β1 (GABPβ1), a master transcription factor of nDNA-encoded mitochondrial genes, is inhibited. The reduction in deacetylation level of GABPβ1 and the associated transcriptional activity results in multisystemic mitochondrial dysfunction, including CM (19). Recently, we found that either cardiomyocyte-specific ablation of nuclear hormone receptor coactivator (NCOA6) or overexpression of a dominant-negative mutant form of NCOA6 (DN1) leads to development of severe DCM with impaired mitochondrial function (20). *Ncoa6* deficiency reduces activity of peroxisome proliferator-activated receptor δ (PPAR δ), transcription levels of its target genes, the number of mitochondria in cardiomyocytes, and complex II activity (20). However, it is still unknown if morphologic and functional abnormalities of cardiac mitochondria in Ncoa6 deletion mice are causes or results of DCM. Cardiomyocyte-specific expression of a mutant uracil-DNA glycosylase 1 (mutUNG1) leads to development of HCM, demonstrating that mtDNA toxicity-induced mitochondrial dysfunction leads to heart failure. In this transgenic mouse heart, increased mitochondrial mass, reduced mtDNA transcription, and suppressed mitochondrial respiration with impaired mitochondrial fission and fusion dynamics were observed (21).

Morphologic changes of mitochondria

The introduction of the mtDNA 16S ribosomal RNA (rRNA) chloramphenicol (CAP) resistance mutation into the mouse female germ line causes CM in chimeric mice. In CAP-resistant (CAP^R) mouse skeletal muscle and heart, abnormally enlarged mitochondria were observed (22). Autoimmunity has been considered as one of major causes for idiopathic DCM (23), and high titers of autoantibodies (M_2-AA) against the second extracellular loop of the M_2 muscarinic receptor (M_2AChR-el2) has been found in serum of patients with idiopathic DCM (24). Animal models have been established by immunizing rats (25, 26) or mice (27) with synthetic M_2AChR-el2 peptide to induce DCM-like morphological changes in the animals' heart. M_2AChR-el2-immunized rat heart showed decreased systolic and diastolic function with swollen and damaged mitochondria (26).

Two functionally distinct populations exist in cardiac mitochondria, interfibrillar mitochondria (IFM) and subsarcolemmal mitochondria (SSM). IFM is located between the myofibrils, whereas SSM is located beneath the plasma membrane. (28). Although the coupling of respiration is similar in both populations of mitochondria, the rate of substrate oxidation is greater in IFM than in SSM (29, 30). Cardiac muscle cells in cardiomyopathic hamsters showed depressed oxidation rates in IFM, but not in SSM (31).

Dysfunction of mitochondrial dynamics and quality control

Binding of agonist to G protein-coupled receptor (GPCR) family triggers the dissociation of Gq protein into Gαq and Cβγ subunits. GTP-bound Gαq (active form) activates phospholipase C (PLC) and ultimately protein kinase C (PKC). As activation of MAP kinase by Gαq has been implicated in stimulating cardiac growth, transgenically overexpressed Gαq in mouse hearts causes myocardial hypertrophy with systolic dysfunction and DCM with cardiac contractile failure at high-expression levels (32). Combined with a pressure overload model by surgical transverse aortic constriction (TAC) (33), Gαq-mediated myocardial hypertrophy model has been used by the same research group to study roles of pro-apoptotic Bcl-2 family protein Nix (also known as Bnip3L) and Bnip3 in cardiac hypertrophy and apoptotic CM (34-36). Proapoptotic Nix is induced in Gq-dependent and pressure overload hypertrophy, and cardiac-specific expression of Nix triggers apoptotic CM (34). Moreover, BNip3/Nix DKO induces the accumulation of abnormal mitochondria and causes CM due to mitophagic dysfunction (37).

Mitochondrial dynamics, mitochondrial fission and fusion, are involved in the mechanisms of a variety of human diseases including cancer, neurodegenerative diseases, and cardiovascular diseases (38). Dorn and his associates have intensively studied the connection between mitochondrial dynamics and CM (39-44). Using genetic ablations of the pro-fission factor dynamin-related protein 1 (Drp1) and the pro-fusion factors mitofusins (Mfn1, Mfn2, and Mfn1/2) and optic atrophy 1 (Opa1), they demonstrated that mitochondrial fission and fusion dynamism plays an important role in pathophysiology of CM and heart failure (45). Mitophagy, the autophagy of mitochondria, is a selective clearance process of damaged or aged mitochondria (46, 47). Regulation of mitophagy requires two Parkinson's disease factors, the mitochondrial kinase PINK1 (PTEN-induced putative kinase protein 1) and the cytosolic ubiquitin ligase Parkin (48). *Mfn2*-deficient mouse cardiomyocytes exhibited accumulation of morphologically and functionally abnormal mitochondria due to suppressed mitophagy and subsequent reduction of oxygen (O_2) consumption, suggesting that the mitochondrial respiratory impairment contributes to the development of DCM (40). Interrupting mitochondrial fusion by combined *Mfn1/Mfn2* ablation in adult hearts increased mitochondrial fragmentation and induced mitochondrial respiratory dysfunction, causing lethal DCM (39, 42, 43). Since the homozygous mutation is embryonic lethal, heterozygous $Opa^{+/-}$ mice were generated and examined. Abnormal cardiac function with reduced mtDNA stability and fragmented mitochondria was observed in aged $Opa^{+/-}$ mouse heart, which develops late-onset CM (44). In contrast, cardiomyocyte-specific *Drp1* knock out (KO) mice showing increased cardiomyocyte mitophagy and mitochondrial permeability transition (MPT) pore opening, led to cardiomyocyte necrosis and DCM (39). A mutation in another mitochondrial fission gene Dnm1l ($Dnm1l^{Py/+}$) also led to CM. ATP depletion observed in

$Dnm1l^{Py/+}$ mouse heart possibly contributes to CM (49). These studies suggest that the cardiac mitochondrial quality control process is critical in pathophysiology of CM.

Impaired mitochondrial metabolism

Transcription of mtDNA is controlled by nuclear gene-encoded regulatory proteins such as mitochondrial transcription factor A (Tfam, also known as mtTFA) (50, 51). Mutant animals, whose *Tfam* gene is disrupted in heart and muscle, developed DCM similar to Kearns-Sayre syndrome due to cardiac-specific inactivation of mtDNA gene expression (52) and cardiac respiratory chain dysfunction (53). The main function of mitochondria in the cardiac muscle is ATP synthesis via the OXPHOS system. Reducing equivalents, such as the reduced form of nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) generated by the tricarboxylic acid (TCA) cycle following substrate oxidation (fatty acids or glucose), are used by the respiratory chain to produce an electrochemical gradient across the mitochondrial membrane [mitochondrial membrane potential ($\Delta\Psi$m)] that drives ATP synthesis. In the adult myocardium, although both fatty acids and glucose are utilized, fatty acids are preferentially oxidized, supplying approximately 70% of total ATP (54, 97). In the failing heart, oxidation of carbohydrate substrates (glucose and lactate) is impaired and fatty acid oxidation (FAO) is enhanced (55). Heart failure patients may benefit from increasing oxidation of pyruvate which derives glucose and lactate, by pharmacologically activating pyruvate dehydrogenase (PDH) (56) (see section Mitochondria as a Therapeutic Target for Cardiomyopathy). Conditional KO mice of adenine nucleotide translocator (Ant1) revealed a dramatic proliferation of mitochondria and cardiac hypertrophy. As *Ant1* KO also has the metabolic acidosis phenotype and its isolated mitochondria exhibited a severe defect in coupled respiration, it will be a good animal model to study all the biochemical, histological, metabolic and physiological characteristics of mitochondrial myopathy and CM (57). Desmin-related CM is caused by mutations in desmin, α-B-crystallin (CryAB), and other genes (58, 59). In particular an R120G missense mutation in CryAB results in desmin-related CM (60). Although desmin-related CM caused by mutations in desmin is not associated with mitochondria (61), a mouse model of desmin-related CM caused by $CryAB^{R120G}$ showed that reduced complex I activity with alterations in the MPT pore and mitochondrial membrane potential ($\Delta\Psi$m) eventually resulted in cardiac cell death, CM, and heart failure (62). Since deficiencies in the cytoskeletal protein dystrophin have been implicated in the pathogenesis of both genetic and acquired forms of CM (63-65), the dystrophin-deficient *mdx* mouse is considered a good experimental model for CM. In the *mdx* mouse heart, a marked shift in substrate from fatty acids to carbohydrates associated with enhanced oxygen consumption was observed (66). Overexpression of the LTCC β2a subunit enhanced Ca^{2+} influx-induced cellular necrosis and induced CM, supporting the concept that necrosis associated with Ca^{2+} overload can also

contribute to loss of cardiomyocytes in heart failure (67). Furazolidone (Fz), a nitrofuran antibacterial, used to establish a turkey model of DCM, with similarities to DCM in human (68), was also used to establish a rat model of DCM (69). The Fz-induced rat model heart showed significant myocardial degeneration and mitochondrial swelling with decreases in mitochondrial membrane potential ($\Delta\Psi$m) and activities of complex II and IV (69).

Together with small animal models, large animal models are needed to translate discoveries from the basic sciences into clinical applications. The pacing-induced DCM model is obtained by continuous cardiac pacing at a higher frequency than the spontaneous heart rate in dogs, pigs, sheep and monkeys (70, 71). In a pacing-induced canine model of DCM, decreases in the enzymatic activity of complex I were observed (72).

Increased oxidative stress

Superoxide dismutase 2 (SOD2, also known as mitochondrial MnSOD) mutant mice developed a neonatal CM, indicating that mitochondrial ROS can cause accumulation of oxidative DNA damage (73). As described in the section *Dysfunction of Mitochondrial dynamics and quality control*, due to impaired Parkin-mediated mitophagy, *Mfn2* KO hearts exhibit accumulation of damaged mitochondria, which produces ROS. Cardiomyocyte-specific expression of mitochondrial-targeted catalase reversed ROS-mediated dysfunction of mitochondrial quality control mechanisms in *Mfn2* KO hearts (41). Mutation of the *Ant1* gene also increases mitochondrial ROS production (57). The most effective anti-cancer drug, doxorubicin (DOX), has been reported to have cardiotoxicity (74, 75) and thus DOX-induced CM has been considered a good model for studying congestive heart failure induced by anthracycline analogues (76, 77). Various DOX-induced CM animal models have been developed in mouse (78), rabbit (79), and rat (80). The increased superoxide production in pacing-induced canine model of DCM and heart failure suggested that complex I is a potential source of mitochondrial production of ROS in failing hearts (72). All these reports provide important insights into the role of mitochondrial ROS in the pathophysiology of CM.

MITOCHONDRIA AS A THERAPEUTIC TARGET FOR CARDIOMYOPATHY

The development of experimental models of mitochondrial heart diseases also provides the opportunity to investigate the efficacy of new mitochondria-targeting drugs and their underlying mechanisms of action. Many studies have targeted bioenergetic dysfunction of mitochondria by adding cofactors, such as coenzyme Q or L-carnitine to therapies for treatment of heart failure associated with CM (81, 82). Coenzyme Q10 (also known as ubiquinone) functions as an antioxidant and its deficiency is associated with CM (83). The coenzyme Q analog idebenone corrects cardiac diastolic dysfunction and im-

proves exercise performance in the *mdx* mouse model of Duchenne muscular dystrophy (DMD) (84). Although initial trials have shown some benefits in preventing progression of HCM, recent controlled trials reported that idebenone neither decreased left ventricular hypertrophy nor improved cardiac function in patients with Friedreich's ataxia (FRDA) (81, 85). Long-term administration of L-carnitine for the treatment of HF caused by DCM showed improved survival (86). The carnitine palmitoyltransferase 1 (CPT-I) inhibitor, perhexilin, corrected energy deficiency caused by diastolic dysfunction and improved exercise capacity in HCM patients, suggesting its function as a metabolic modulator (shifting FAO to glucose oxidation) (87). Another CPT-I inhibitor, oxfenicine showed similar effects in dog heart, in which CPT-I inhibition prevents ventricular remodeling (88). Dichloroacetate (DCA) is another potential therapy for CM, by stimulating pyruvate dehydrogenase is stimulated to drive pyruvate to mitochondrial OXPHOS, increasing acetyl-CoA and NADH levels. However, treatment of non-ischemic and ischemic DCM patients with DCA showed inconsistent effects on left ventricular mechanical efficiency and myocardial O_2 consumption (89, 90). L-arginine has also been proposed as a treatment for CM. In patients with mitochondrial CM, L-arginine enhanced aerobic metabolism and myocardial efficiency, independent of its vasodilation effect (91). Because the primary target of DOX-induced CM is the mitochondria, in which DOX triggers the generation of ROS and lipid peroxidation (76, 77), numerous studies have tested iron-chelating agents [e.g., dexrazoxane (92)], antioxidants [e.g., MnTBAP and ebselen (93)], and the phosphodiesterase 5 inhibitor sildenafil (94) to prevent the mitochondrial dysfunctions in DOX-induced CM.

CONCLUDING REMARKS

Mitochondrial dysfunction is substantially involved in arrhythmogenic cardiac diseases, including inherited and acquired CM. Alterations in mitochondrial morphology, impaired energy production (i.e. ATP synthesis) and enhanced pathologic function (i.e. ROS generation, Ca^{2+} overload and apoptosis) cause cardiomyocyte injury and/or cell death due to malfunction of cellular mechanisms involved in maintaining intracellular Ca^{2+} homeostasis and normal mechanical and electrical functions of heart.

Although interpretations must be made with appropriate caution, i.e. "a man will never be a mouse", studies of mitochondrial function in cardiac disease using animal models will undoubtedly continue to provide new insights into the mechanisms of cardiac mitochondrial damage and a better understanding of the mitochondrial-mediated injury pathways involved in CM and heart failure. Current pharmacologic strategies have not yet been proven effective, and large randomized controlled trials are needed to establish a link between mitochondria and CM. Cross-disciplinary analysis will hopefully provide unique insights into mitochondrial disorders and

optimum translational models with which to challenge our perspectives on human CM and heart failure while also suggesting new therapeutic strategies.

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