DRUG DISCOVERY

Novel targets for mitochondrial medicine

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Mitochondria—classically viewed as the powerhouses of the cell—have taken center stage in disease pathogenesis and resolution. Mitochondrial dysfunction, which originates from primary defects within the organelle or is induced by environmental stresses, plays a critical role in human disease. Despite their central role in human health and disease, there are no approved drugs that directly target mitochondria. We present possible new druggable targets in mitochondrial biology, including protein modification, calcium ion (Ca^{2+}) transport, and dynamics, as we move into a new era of mitochondrial medicine.

As the powerhouse of the cell, the mitochondrion is essential for life. A large body of research in recent years has established that mitochondria are not simply static, passively producing adenosine 5′-triphosphate (ATP) for fuel, but that they sense and respond to changing cellular environments and stresses. The mitochondria thereby control critical decision points for cells, such as whether to live or die or whether to transform to malignant cancer cells. Acquired mitochondrial dysfunction has been implicated in numerous common diseases and conditions, such as cardiovascular disease; neurodegenerative diseases, including Alzheimer's and Parkinson's; metabolic disorders, such as diabetes and obesity; cancer; and even in normal aging. Despite the central role of mitochondria in human health and disease, there has been no successful therapy for defective mitochondrial function, which is devastating and often fatal. Looking at the pipeline of new drugs, as of the end of 2015, there were more than 200 trials on ClinicalTrials.gov that used "mitochondria" as a key word. However, drugs tested in most of these trials did not target mitochondria (e.g., Table 1); instead, they monitored mitochondrial function as an indicator of indirect effects of the treatments. This begs the question: Why is there a void of mitochondria-targeted therapies when its role in human disease is widely recognized?

To fuel the cell, mitochondria synthesize ATP by oxidative phosphorylation, generating reactive oxygen species (ROS) as a by-product. A long-standing hypothesis is that defective energy supply and/or excessive ROS generation accounts for the pathology caused by mitochondrial dysfunction. Several antioxidants or compounds aimed at boosting energy metabolism, through improving ATP production, have been tested clinically to improve mitochondrial function but have yielded disappointing or inconclusive results (Table 1) (*1*–*4*). Failures of the earlier trials were attributed to the loss of physiological ROS function or the inefficiency of delivering antioxidants to targets inside the mitochondria owing to the organelle's double-membrane structure, which is virtually impermeable to the passive diffusion of most small molecules and hence a barrier for drug delivery.

Newer-generation compounds that bear positive charges [for example, MitoQ (*5*)] and/or have high affinity to the mitochondrial membrane lipids (*6*) are now in clinical trials (Table 1). One promising redox therapeutic from Edison Pharmaceuticals is EPI-743 (*7*, *8*), a designed CoQ10 analog with much more potent antioxidant activity, and is being tested currently in pediatric patients with various mitochondrial diseases, including Rett syndrome, Friedreich's ataxia, and Leigh syndrome (Table 1). Thus, the efficacy of these compounds in human diseases remains to be determined.

There are opportunities for innovation in mitochondrial medicine beyond the conventional focus on bioenergetics and oxidative stress [or, in the oncology field, selective destruction of mitochondria (*9*–*12*)]. Here, we highlight emerging studies in mitochondrial genetics, signaling, and physiology that lead to novel avenues of improving mitochondrial function and/or protect against mitochondria-mediated cell injury in human diseases.

MITOCHONDRIAL REPLACEMENT THERAPY

For mitochondrial disease caused by inborn mutations, mitochondrial genetics research has yielded remarkable progress, particularly advances in mitochondrial replacement therapy (MRT) (*13*–*15*). MRT prevents the passing of mutated mitochondrial DNA (mtDNA) from the mother to the offspring via a three-way in vitro fertilization in which the nuclear genome from a patient's egg is transferred into an enucleated healthy egg with normal mtDNA before fertilization. In this way, mitochondrial diseases caused by mutant mtDNA can be prevented.

However, concerns remain regarding the long-term outcome of this technology. Several major issues, such as the interaction of mitochondrial and nuclear genome and the fate of carryover mutant mtDNA, remain to be clarified. There are several other limitations as well. MRT does not benefit mitochondrial diseases caused by mutations in the nuclear genome that encodes most of the proteins, only inherited diseases resulting from mutations of mtDNA, which encodes transfer RNAs, ribosomal RNAs, and mRNAs that make only 13 of ~1000 proteins in mitochondria. Thus, the target patient population is rather small. Furthermore, MRT only prevents the development of mitochondrial disease in the offspring of mutation carriers but does not treat existing mitochondrial disease or the more prevalent secondary mitochondrial dysfunction caused by maladaptive responses to environmental stresses. Therefore, the technology can potentially complement but not replace the unmet need: a mitochondria-targeted therapeutic.

MITOCHONDRIAL PROTEIN MODIFICATION

Mitochondrial metabolism integrates the energy demand of the cell with the nutrient availability, the redox state, and ion fluxes (Fig. 1) through a tight coupling of the TCA cycle flux with the rate of oxidative phosphorylation. Interruption of the coupling by stresses, such as hypoxia, changes in nutritional status, or energy demand, triggers mitochondrial

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Table 1. Selected clinical trials on drugs affecting mitochondrial function. Trial statuses were confirmed as of 26 January 2016. AMP, adenosine-5′-monophosphate.

Class	Compound name (manufacturer)	Targeting site	Disease	Phase	Outcome	ClinicalTrials.gov identifier
Antioxidant	MitoQ	ROS scavenger	Chronic kidney disease (CKD)	4*	Ongoing	NCT02364648
	Resveratrol	SIRT1 activator	CKD	3	Completed	NCT02433925
	EPI-743 (Edison Pharmaceuticals)	Coenzyme Q10 (CoQ10)–based	Rett syndrome	2	Completed	NCT01822249
	Idebenone (Santhera Pharmaceuticals)	CoQ10-based	Duchenne muscular dystrophy	3	Positive (*74*)	NCT01027884
	Edaravone	Nonspecific ROS scavenger	Acute myocardial infarction (AMI)	4	Positive (*75*)	NCT00265239
	Edaravone	Nonspecific ROS scavenger	Amyotrophic lateral sclerosis	3	Negative (*76*)	NCT00330681
	CoQ10	ROS scavenger	Parkinson's disease	3	Negative (*77*)	NCT00740714
	SS31 (bendavia) (Stealth BioTherapeutics Inc.)	Unknown	AMI	2	Negative†	NCT01572909
Metabolism modulator	Acipimox	Niacin derivative	Obesity	2	Negative (*78*)	NCT01488409
	Metformin		Type 1 diabetes	4	Ongoing	NCT01813929
	Metformin	Metabolism/unknown	Nonalcoholic fatty liver disease	4	Terminated	NCT00736385
	Creatine	Metabolism	Huntington's disease	3	Terminated‡	NCT00712426
	Acadesine (Merck Sharp & Dohme Corp.)	AMP-activated protein kinase, nonspecific	Myocardial infarction	3	Negative (*79*)	NCT00872001
Mitochondrial permeability transition pore (mPTP) inhibitor	Cyclosporine A	Cyclophilin D	Acute kidney injury	2	Ongoing	NCT02397213
	Cyclosporine A	Cyclophilin D	AMI	3	Negative (*46*)	NCT01502774
	Dimebon (Pfizer)	Unknown	Alzheimer's disease	3	Negative (*80*)	NCT00838110
	TRO40303 (Trophos)	Mitochondrial translocator protein	AMI	2	Negative (*81*)	NCT01374321

*The compound entered a phase 4 trial on the basis that it is used as a nutritional supplement. †Results reported at the American College of Cardiology 2015 Scientific Sessions. ‡Terminated as unlikely to be effective.

responses to restore the homeostasis or to initiate cell death if the damage incurred is beyond repair. Mechanisms mediating such responses are poorly understood but are increasingly shown to involve protein modification by mitochondrial metabolites, including acetylation of lysine residue (LysAc), malonylation, and succinylation. LysAc results from the transfer of an acetyl group from acetyl-CoA to the ε-amino group of lysine, which neutralizes the positive charge. A putative mitochondrial acetyltransferase (GCN5L1) has been proposed for LysAc (*16*); however, nonenzymatic LysAc owing to the abundance of acetyl-CoA in mitochondria is likely the primary mechanism in protein acetylation (*17*) (Fig. 1). Enzymatic deacetylation by sirtuins is another major determinant of LysAc level in mitochondria. There are three isoforms of sirtuins in the mitochondria, SIRT3/4/5, among which SIRT3 is the predominant deacetylase. SIRT4 is an ADP (adenosine 5′-diphosphate)–ribose transferase, whereas SIRT5 has been shown to function as desuccinylase, demalonylase, and deglutarylase (*18*). Thus, the level of lysine modifications reflects the availability of the thioester-CoAs and the sirtuin activities in the mitochondria (Fig. 1).

Studies of the SIRT3-deficient mouse have provided the first evidence that mitochondrial LysAc is key to stress response. *Sirt3*$^{-/-}$ mice are normal under unstressed conditions but show higher susceptibility to obesity and cardiovascular disease (*19*–*21*). Sirtuins are NAD^+-dependent enzymes; their activities are sensitive to the NAD^+ level as well as the NAD^+/NADH (reduced form of NAD^+) ratio (*22*). NAD^+ is a coenzyme for a variety of biochemical reactions, and in mitochondria, it also serves as the major electron carrier for oxidative phosphorylation. NAD(H) exists in either oxidized or reduced form; the partition of NAD^+ and NADH in

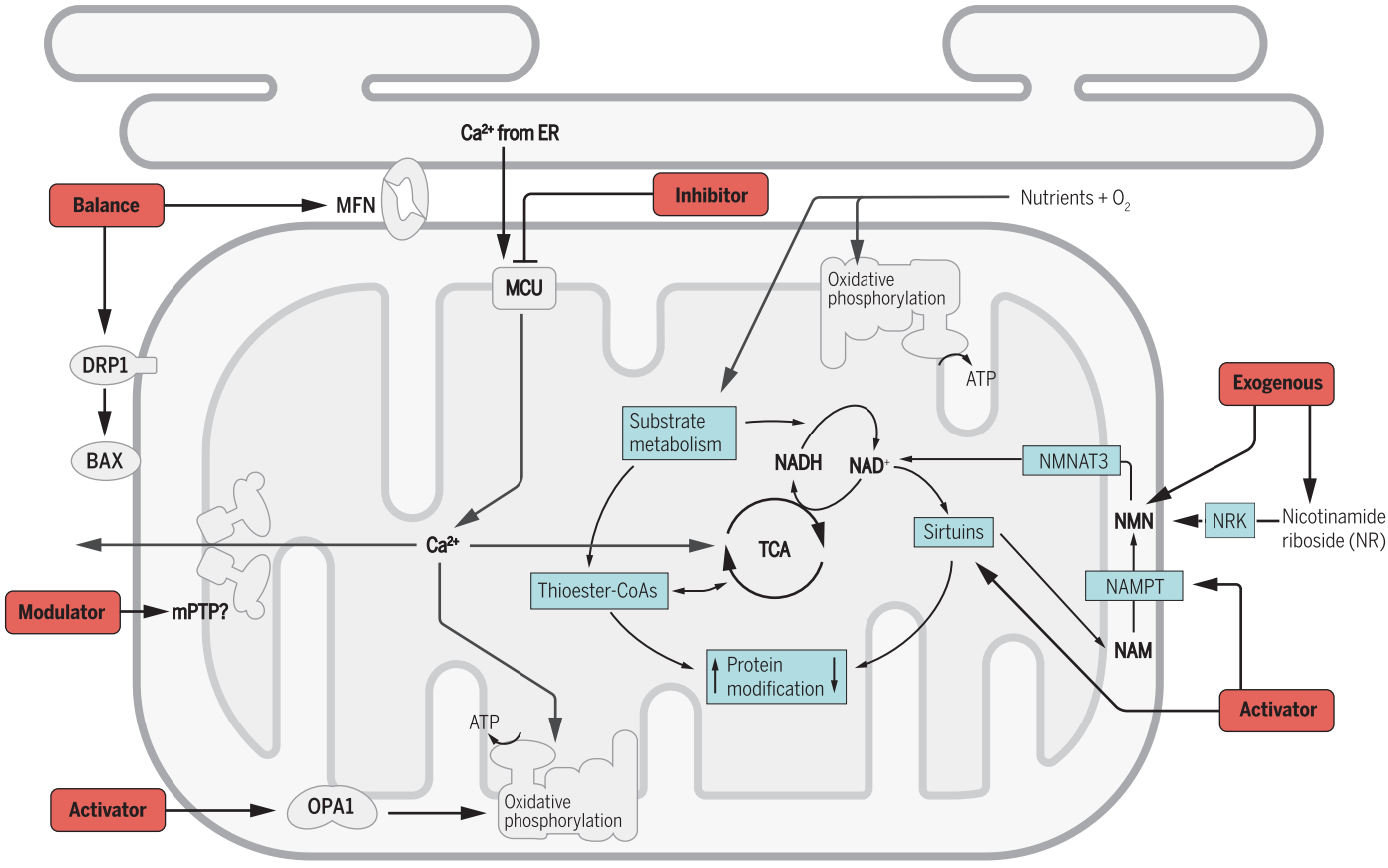

Fig. 1. Key players—and therapeutic targets—in mitochondrial protein modification, Ca^{2+} transport, and dynamics. Mitochondrial protein can be modified by the thioester–coenzyme A (CoA) produced by substrate metabolism, for example, acetyl-CoA, malonyl-CoA, succinyl-CoA. The most commonly studied is the acetylation of lysine residue (LysAc). The LysAc level is determined by the availability of acetyl-CoA and the activity of deacetylases, sirtuins, which catalyze deacetylation at the expenses of nicotinamide adenine dinucleotide (NAD^+). Mitochondrial NAD^+ level is regulated by the activities of tricarboxylic acid (TCA) cycle and oxidative phosphorylation. Nicotinamide (NAM) generated from deacetylation reaction is converted to nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme. Alternatively, NMN is synthesized from nicotinamide riboside (NR) by nicotinamide riboside kinase (NRK). NMN is converted to NAD^+ by nicotinamide mononucleotide adenylyltransferase 3 (NMNAT3) in the mitochondria. Ca^{2+} is a key player in orchestrating metabolism and signaling function of the mitochondria. It is mainly stored in endoplasmic reticulum (ER) and transported into mitochondrial matrix by mitochondrial Ca^{2+} uniporter (MCU) to stimulate enzymes in TCA cycle and oxidative phosphorylation. Mitochondrial Ca^{2+} also triggers the opening of mPTP, which likely plays a physiological role in matrix Ca^{2+} release and a detrimental role in cell death. The mitochondrial dynamic regulatory proteins may bear new roles beyond fusion and fission. The outer membrane fusion protein mitofusin (MFN) tethers the mitochondria and ER membranes and through which facilitate mitochondrial Ca^{2+} uptake. The inner membrane fusion protein optic atrophy 1 (OPA1) controls the cristae structure and through which modulates mitochondrial respiratory chain activity. The fission protein dynamin-related protein 1 (DRP1) also regulates BAX (BCL2-associated X protein) and mPTP. The black arrows indicate potential targets for drug development.

the mitochondria is determined by the balance of substrate metabolism that generates and the oxidative phosphorylation that consumes NADH. Under conditions of increased NADH production, such as in overnutrition/obesity/diabetes, or decreased NADH consumption, such as in mitochondrial disease with impaired oxidative phosphorylation, the balance of NAD^+/NADH could be tilted toward a lower ratio and a concomitant increase in mitochondrial LysAc (Fig. 1 and Table 2) (*22*–*25*). There is increased sensitivity to stress in these and other mouse models of decreased NAD^+/NADH ratio and increased mitochondrial LysAc (*22*, *25*, *26*). Conversely, the NAD^+/NADH ratio increases, and LysAc decreases in animals subjected to caloric restriction, which has been attributed to the health benefits observed in these models (*27*, *28*).

How does increased LysAc increase propensity of disease? This is a challenging question, because the observed phenotype is unlikely attributable to the hyperacetylation of either one single protein or all acetylated proteins. Nevertheless, the concept that protein modification mediates the response of mitochondrial metabolism to stress is an important one. It also proposes a role of NAD^+ redox imbalance and sirtuin-regulated LysAc in the development and progression of diseases. Indeed, several strategies have been shown to improve stress response by targeting the NAD^+-LysAc mechanism. Decreasing protein acetylation via the activation of sirtuins by pharmacological, genetic, or dietary interventions has demonstrated benefits in mouse models (*29*). Stimulating the NAD^+ biosynthetic pathway also effectively increases the NAD^+ levels and promotes protein deacetylation by the sirtuins (Fig. 1) (*30*, *31*). Treating the mouse heart with complex I deficiency with the NAD^+ precursor NMN normalizes the NAD^+/NADH ratio and restores the sensitivity to stress (*22*). NMN treatment in mice also attenuates the

Table 2. Potential new therapeutic targets in mitochondria. These new targets are shown in Fig. 1 and have been tested in preclinical studies.

Compound	Compound type	Target site or mechanism	Disease	Reference
NMN	Small molecule	NAD^+, protein acetylation	Heart failure, metabolic diseases, neurodegeneration, aging	(*22*, *30*, *32*)
NR	Small molecule	NAD^+, protein acetylation	Metabolic diseases, aging	(*24*)
P7C3 chemicals	Small molecule	Activate NAMPT	Neurodegeneration	(*31*)
Honokiol	Small molecule	Activate SIRT3	Cardiac hypertrophy	(*82*)
Ru360	Small molecule	MCU	Myocardial infarction	(*38*, *40*)
Cyclophilin D–specific inhibitors	Small molecule	mPTP	Ischemic disease	(*83*–*86*)
Mdivi-1	Small molecule	DRP1	Ischemic disease, neurodegeneration	(*56*, *87*)
S3	Small molecule	MFN	Neurodegeneration	(*57*)
P110	Small peptide	DRP1	Neurodegeneration	(*55*, *88*)

pathophysiological changes in diabetes (*30*) and aging (*32*) (Table 2). Elevating NAD^+ levels with another NAD^+ precursor, NR, protected mice from high-fat diet–induced obesity (*24*). Recently, a small-molecule activator of NAMPT, the rate-limiting enzyme of the NAD^+ biosynthetic pathway, was neuroprotective in mice (Table 2) (*31*).

Rebalancing the NAD^+/NADH ratio and/or restoring LysAc may therefore be viable strategies in people for protection against downstream consequences of mitochondrial dysfunction (Fig. 1). Supplementation of the NAD^+ precursors or stimulation of the key enzymes in the NAD^+ salvage pathway is a logical approach (Table 2). One NAD^+ precursor, NR, is already available for human consumption as a nutritional supplement. Clinical studies are underway to test its efficacy in raising intracellular NAD^+ levels as well as its safety and tolerability in patients.

MITOCHONDRIAL CA^{2+} TRANSPORT

Calcium is a crucial regulator of mitochondrial function, stimulating dehydrogenases in the TCA cycle and the respiratory chain and triggering mitochondria-mediated cell death (Fig. 1). Insufficient mitochondrial calcium ion (Ca^{2+}) uptake or Ca^{2+} overload can cause various human diseases, such as ischemia/reperfusion injury and neurodegeneration (*33*, *34*). Although Ca^{2+} transport across the inner mitochondrial membrane has been known for decades, the exact protein identities of the channels or transporters involved were unknown until recently. The major Ca^{2+} uptake channel MCU was identified in 2011 (*35*, *36*), and subsequently, the MCU complex that contains several regulatory proteins was described (Fig. 1) (*37*). These landmark discoveries allow further understanding of the regulation of mitochondrial Ca^{2+} and open the door for therapeutic intervention (Table 2).

A major motivation to intervene in mitochondrial Ca^{2+} handling is to protect against ischemia/reperfusion injury, where restoring blood supply to ischemic tissues in diseases such as stroke, myocardial infarction, and peripheral vascular disease causes additional damage. The mitochondrial cell death pathway—which occurs through Ca^{2+}-triggered opening of mPTP, leading to the collapse of mitochondrial membrane potential and release of cytochrome C (Fig. 1)—is proposed to be an important mechanism of reperfusion injury. Preclinical studies have repeatedly shown the pathological roles of mitochondrial Ca^{2+} overload and the effectiveness of MCU blockade by small molecules, such as ruthenium red and Ru360, on ischemia/reperfusion injury (Table 2) (*38*–*40*). It is therefore unexpected that germline knockout of MCU in mice did not lead to protection from cardiac ischemia/reperfusion injury (*41*). However, inducible and cardiac-specific deletion of the MCU in adult mice did protect against acute ischemic injury (*42*, *43*). Because both models reveal that mitochondria lacking MCU cannot take up Ca^{2+} in response to acute metabolic or signaling stress, the difference in these animals in protecting the heart from ischemia/reperfusion injury suggests that germline deletion of MCU might have triggered mechanisms other than mitochondrial Ca^{2+} transport for cell death. Perhaps one of the lessons we can learn from these studies is that genetic inhibition of MCU would not be a clinically preferred approach. Rather, the genetic models can aid the design and testing of small molecules that target MCU and mitochondrial Ca^{2+} uptake. Pharmacological modulators of MCU and its regulatory proteins may have broad utility as mitochondria-targeted therapies.

An end effector of mitochondrial Ca^{2+} overload is prolonged opening of the mPTP and subsequent cell death. The mitochondrial permeability transition phenomenon was first discovered more than 40 years ago, yet the molecular identity of the mPTP has not been fully revealed, making it difficult to develop a therapy. Preclinical studies have provided strong evidence for the causal role of mPTP in cell death, particularly in ischemic diseases (*39*). Pharmacological and genetic inhibitions of cyclophilin D, the confirmed regulator of mPTP, have shown beneficial effects against myocardial infarction in mice (Table 2) (*44*). Multiple clinical trials have tested mPTP inhibitors in various diseases, most notably cyclosporine A, a cyclophilin D inhibitor, for AMI (Table 1) (*1*, *2*, *6*). Cyclosporine A decreased infarct size in a small, pilot phase 2 clinical trial (*45*). In a recent trial with a larger patient population [CIRCUS (Cyclosporine and Prognosis in Acute Myocardial Infarction Patients) trial], cyclosporine A failed to improve clinical outcomes or prevent adverse left ventricular remodeling at 1 year after myocardial infarction (*46*). Other trials with different mPTP inhibitors or in different diseases also failed recently, raising the question whether the mPTP is a meaningful target (Table 1). Alternatively, there could be a fundamental flaw in the current strategy of drug development even though the mPTP is a promising target.

Strategies targeting mPTP components other than cyclophilin D, eliminating the nonspecific effects of cyclosporine A, and balancing the physiological and nonphysiological roles of mPTP are all in consideration for the development of new inhibitors of mPTP. It is increasingly evident that the mPTP has a physiological role, such as transient release of mitochondrial matrix Ca^{2+} (Fig. 1). Mitochondrial respiration and ROS production under resting conditions are coupled with physiological mPTP openings (*47*). Chronic suppression of physiological mPTP openings in cyclophilin D–deficient mice leads to increased mitochondrial matrix Ca^{2+}, metabolic remodeling, and heart failure (*26*). Thus, the mixed results for cyclosporine in the clinical trials may stem from its nonselective inhibition of both physiological and pathological mPTP openings. Therefore, a therapy that specifically inhibits pathological mPTP function (prolonged and massive openings) while preserving physiological mPTP function (transient and controlled openings) would be highly desirable. Alternatively, targeting the sensitivity of mPTP during stress rather than blocking its opening entirely would be more favorable for maintaining the ion homeostasis. To achieve such a goal, again, the physical identity and molecular structure of mPTP are needed. Recent reports on the formation of mPTP by the F_o/F_1 ATP synthase have raised new hope (*48*, *49*). Before any drug is designed to target the ATP synthase, however, it is critical to fully understand how the most abundant and important protein in mitochondria can play dual and seemingly contradictory roles in the life and death of the cell (*48*, *49*).

MITOCHONDRIAL DYNAMICS AND ITS REGULATORS

Research in the past decade has established that mitochondria are not static organelles; their size, shape, and intracellular location undergo constant changes, termed mitochondrial dynamics (*50*). These events are executed by a family of dynamin-related proteins that hydrolyze GTP (guanosine 5′-triphosphate), including the fusion proteins mitofusin (MFN1/2) and OPA1 and the fission protein DRP1 (*51*) (Fig. 1). Mitochondrial dynamics are essential for normal mitochondrial function, transporting and exchanging mitochondrial content and sequestering and removing the damaged mitochondrial subpopulation through mitophagy (*52*). Mutations of fission and fusion proteins have been associated with the inherited human diseases Charcot-Marie-Tooth neuropathy type 2A and dominant optic atrophy (*52*).

Initial studies of mitochondrial morphology during stress showed that fragmented mitochondria are associated with detrimental outcomes such as oxidative stress and cell death, but fusion is protective against stress-induced cell death (*53*). Thus, there is interest in developing fission inhibitors. Inhibition of DRP1 by a small molecule named Mdivi-1 (for mitochondrial division inhibitor) (*54*) or interfering the binding of DRP1 with its outer membrane receptor FIS1 by a small peptide has been shown to prevent excessive fission-induced cell dysfunction and ameliorate ischemia/reperfusion injury in the mouse heart and neurotoxicity in rodent neurons (Table 2) (*55*, *56*). Recently, a compound called S3 that enhances MFN1/2 activity by inhibiting its deubiquitination has been shown to restore the morphology and function of mitochondria in fusion-deficient human and mouse cells in vitro (Table 2) (*57*). Continued success in preclinical studies using these compounds or similar may eventually lead to clinical trials.

More recent work has challenged the dogma that "fusion is always beneficial, whereas fission is detrimental." Deletion of the fission protein DRP1 resulted in elongated but dysfunctional mitochondria, whereas deletion of MFN2 unexpectedly also yielded enlarged (rather than fragmented) mitochondria in the heart (*58*–*61*). In both cases, mitochondrial dysfunction was caused by inadequate mitophagy and poor quality control, suggesting that a healthy balance of fusion and fission is critical. Whether the modulators of DRP1 or MFN1/2 function described above can restore the balance of fusion and fission remains to be determined and is likely critical for their utility in therapeutic application. Furthermore, in some cell types, such as skeletal muscle or cardiac myocytes, the fission and fusion proteins are abundant despite infrequent morphological changes and/or movement of mitochondria. This observation raises the question as to whether the fission and fusion proteins play other roles in the regulation of mitochondrial function (Fig. 1).

Recently, the inner membrane fusion protein OPA1 has been shown to critically maintain the cristae structure of the inner membrane and to facilitate the expression and assembly of the protein complexes in the respiratory chain (Fig. 1) (*62*). Transgenic overexpression of *Opa1* in mice partially rescued mitochondrial disease phenotypes caused by defects in respiration chain complexes (*63*, *64*). Small molecules that enhance OPA1 function are not yet available but are attractive for mitochondrial diseases that exhibit defective respiration and abnormal cristae structures. Other dynamic proteins are also involved in modulating mitochondrial function outside of fission/fusion. For instance, DRP1 regulates BAX oligomerization and mPTP opening (*56*, *65*), and MFN1/2 regulates mitochondrial Ca^{2+} uptake by tethering mitochondria with ER (Fig. 1) (*66*). Identification of these novel functions of the dynamic proteins opens a new window for innovative drug development.

The tethering of mitochondria and ER by MFN1/2 provides a mechanism to manipulate the distance between the two organelles and, as such, alter the local concentration of Ca^{2+} released from the ER. Because the ER is the major intracellular Ca^{2+} storage, a close association between mitochondria and ER would facilitate prompt and appropriate amount of mitochondrial Ca^{2+} uptake for metabolic stimulation (Fig. 1). Indeed, MFN2 has been shown to critically modulate mitochondrial bioenergetics response in *Drosophila* heart tubes during cardiac excitation-contraction coupling through the tethering mechanism (*67*). A recent report showed that such tethering facilitated OPA1 cleavage and cristae remodeling in mice response to nutritional stress (*68*). Excessive nutrition, such as in obesity, enhances mitochondria-ER tethering, which is responsible for mitochondrial Ca^{2+} overload and dysfunction in the liver (*69*). Thus, regulating the distance between mitochondria and ER impacts mitochondrial function and morphology (*70*, *71*). Altering the distance between these two organelles through drug-inducible interorganelle linkers could be a promising novel approach to manipulate the distance between the two organelles and to modify the space available for local Ca^{2+} transfer machinery (*72*). One potential clinical application of this approach is the treatment of heart failure, a chronic condition with drastically deranged T-tubule and sarco(endo)plasmic reticulum systems that lead to compromised intracellular Ca^{2+} handling (*73*). Small-molecule linkers that can reestablish the Ca^{2+} microdomains between sarco(endo)plasmic reticulum and mitochondria would provide double benefits at early stage of heart failure by enhancing the capacity of mitochondrial Ca^{2+} buffering and boosting mitochondrial metabolism to maintain the energy supply.

CONCLUSION AND PERSPECTIVES

In summary, the mitochondrial biology field has experienced fast-paced progress in recent years, yielding numerous opportunities to translate the discoveries to clinical medicine. Small-molecule compounds included in Table 2 represent a starting point for the new generation of therapies, which are expected to grow significantly in the near future. At the same time, we realize that the ultimate success of mitochondrial medicine is dependent on a thorough understanding of mitochondrial biology and function. ATP production by mitochondria and its role in Ca^{2+}, ROS, and redox regulation are intertwined; mitochondrial morphology and function are likely coupled; and the regulators in mitochondrial function are multitasking and interacting with each other. Although modern science has revealed exciting new targets, many challenges are expected on the journey of translation, such as how to target a particular pathway without affecting the other functions of a protein and how to determine the effectiveness of a potential candidate. Thus, this is an exciting time in mitochondrial research and also the beginning of a long expedition.

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