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Mitochondrial Function and Dysfunction: An Update

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ABSTRACT

With the current explosion of knowledge on the role of mitochondrial dysfunction in the genesis of various human disease states, there is an increased interest in targeting mitochondrial processes, pathways, and proteins for drug discovery efforts in cancer and cardiovascular, metabolic, and central nervous system diseases, the latter including autism and neurodegenerative diseases. We provide an update on understanding the central role of the mitochondrion in ATP and reactive oxygen species production and in controlling cell death pathways.

Introduction

For many pharmacologists, the mitochondrion is probably last remembered as a major topic in their undergraduate efforts in biochemistry where the importance of this key intracellular organelle was assessed almost exclusively in the context of its key role in ATP production, some 40 to 50 kg each day, and calcium homeostasis (McBride et al., 2006; Schatz, 2007; Lax et al., 2011). Since then, studies on the role of mitochondria in cell function have evolved considerably with a veritable explosion in knowledge on their role as rheostats or biosensors for oxidative stress and as a focal point for cellular signaling platforms especially those involved in modulating cell death, the latter including necrosis, apoptosis, and autophagy (Edinger and Thompson, 2004; McBride et al., 2006; Kroemer et al., 2009; Huang and Figueiredo-Pereira, 2010; Kitsis and Molkentin, 2010; Martin et al., 2011; Koopman et al., 2012) together with their mitochondrial-specific variations, mitoptosis and mitophagy (Youle and Narendra, 2011).

ABBREVIATIONS: mtDNA, mitochondrial DNA; ΔΨm, mitochondrial membrane potential; Aβ, amyloid β peptide; AD, Alzheimer's disease; AIF, apoptosis-inducing factor; ALS, amyotrophic lateral sclerosis; ANT, adenine nucleotide translocator; Bak, Bcl-2 homologous antagonist killer; Bax, proapoptotic Bcl-2-associated X protein; Bcl-, B-cell lymphoma protein; BH3, proapopotoic Bcl-2 family members; CytC, cytochrome c; Cyp-D, cyclophilin D; DRP1, dynamin-related protein 1; ETC, electron transport chain; FAD, flavin adenine dinucleotide; HD, Huntington's disease; IAP, inhibitor of apoptosis; IMM, inner mitochondrial membrane; MLKL, mixed lineage kinase-domain-like protein; MPT, mitochondrial permeability transition; MPTP, MPT pore; NCE, new chemical entity; Nix/BNip3L, BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like; nDNA, nuclear DNA; NSAID, nonsteroidal anti-inflammatory drug; OMM, outer mitochondrial membrane; Omi/HtrA2, homotrimeric serine protease high-temperature requirement A2; OXPHOS, oxidative phosphorylation; PARP, poly(ADP) ribose polymerase; PD, Parkinson’s disease; PGAM5S, phosphoglycerate mutase/protein phosphatase 5, short form; PGC-1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PKA, protein kinase A; RCT, randomized clinical trial; ROS, reactive oxygen species; RIPK, receptor interacting protein kinase; sAC, soluble adenylyl cyclase; Smac/DIABLO, second mitochondria-derived activator of caspases/direct IAP-associated binding protein with low pI; TNF, tumor necrosis factor; TRAF2, TNF receptor-associated factor 2; VDAC, voltage-dependent anion channel; ABT-737, 6-(3-((2-chlorophenyl)-2-yl)methyl)piperazin-1-yl)-N-[[4-[[3,5-dimethyl-3-(5,5'-bis[1-methylthylidene]-2,2'-binaphthalen-8-yl)dicarboxaldehyde]-3,4-di(1-adamantyl)-4-hydroxyphenyl]-2-naphthiencarboxylic acid; PK 11195, [N-(1-(6-iodo-1,3-benzodioxol-5-yl)-6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine); CGP 37157, 7-chloro-6-(4-propyl-3,5-dimethyl-1,3-thiazol-5-yl)benzo[d]imidazole-2,6-dinitrophenylglycine; PI-H71, 6-amino-8-[[6-iodo-1,3-benzodioxol-5-yl]thio]-N-(1-methylthio)-9H-purine-9-propanamine; KNS-760704, (R)-N6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine; CGP 37157, 7-chloro-5-(2-chlorophenyl)-1,3-dihydro-4,1-benzothiazepin-2(3H)-one; SS-31, arginyl-2',6'-dimethyltyrosyl-lysyl-phenylalaninamide; TRO19622, (NZ)-N-[[BS,SS,10R,13R,14S,17R]-10,13-dimethyl-17-[[2R]-6-methylheptan-2-yl]-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-yldiene]hydroxylamine.

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Deficiencies in energy metabolism, the bioenergetic failure characteristic of both mitochondrial and epigenomic disease states (Wallace and Fan, 2010), have been implicated in a variety of human disease states, especially in those organs in which there is a high level of energy consumption, e.g., the brain, which with only 2% of total body weight represents 20% of the total oxygen consumption in the body. Diseases specifically linked to mitochondrial dysfunction vary from the well known (glaucoma, inflammation, neurodegenerative diseases, type 2 diabetes, cancers, especially those involving prostate and colon, cardiomyopathies, and dysrhythmias) to the less well known (Frederich’s ataxia) to a group of relatively obscure disease states [Kearns-Sayre syndrome (KSS), Leber hereditary optic neuropathy (LHON), mitochondrial encephalopathy lactic acidosis and strokes (MELAS), myoclonic epilepsy with ragged red fibers (MERRF), and mitochondrial neuro-gastrointestinal encephalomyopathy (MNGIE)] (Haas et al., 2008).

These various disease states have been associated in some or all of their manifestations with mutations in both mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) that result in defects in mitochondrial function (Wallace, 1999; Schapira, 2006; Copeland, 2008; Finsterer, 2010) or with an inability to accommodate the consequences of oxidative stress (Poljsak, 2011). While an excess of free radical, e.g., ROS (reactive oxygen species), production leads to both mutations of DNA and the degradation of proteins, lipids, and nucleic acids, the view that ROS is causal to mitochondrially-related diseases has been challenged in the context of “oxidative shielding” (Naviaux, 2012). This concept, albeit controversial, views ROS production as a form of innate immunity to protect the cell with ROS production being the response to tissue trauma or disease, a view similar to that evolving for the role of Aβ in Alzheimer’s disease (Castellani et al., 2009). The spatial proximity of mtDNA to the free radicals produced by the electron transport chain (ETC) (Fig. 1) makes it uniquely susceptible to mutations, especially when the ETC is dysfunctional. This has led to the heuristically engaging, albeit controversial, mitochondrial oxidative stress/free radical/genotoxic stress theory of aging that reflects the negative impact of chronic, accumulating damage to DNA and cellular proteins from free radicals as a function of age (Kujoth et al., 2005; Wallace, 2005; Dagda et al., 2009; Swerdlow and Kahn, 2009; Lapointe and Hekimi, 2010; Durieux et al., 2011; Pamplona, 2011). This involves a progressive loss of functional telomeres that contribute to replicative senescence and apoptosis via decreased mitochondria and mtDNA copy numbers, increased ROS production, and decreased ATP production (Sahin et al., 2011).

With the current evolution in understanding of the contribution of mitochondrial dysfunction to the genesis of human disease states, the majority of them chronic, there is increased interest in targeting mitochondrial processes and proteins for drug discovery efforts in cancer (Fulda et al.,

![Fig. 1. Schematic of the mitochondrion showing the mitochondrial permeability transition pore and the respiratory chain. See text for details. G6P, glucose 6-phosphate; HK, hexokinase; TCA, tricarboxylic acid cycle.](image-url)
2010; Maldonado and LeMasters, 2012), cardiovascular disease (Ballinger, 2005; Akar and O’Rourke, 2011; Ong and Hausenloy, 2010), metabolic diseases (Gilliam and Neufert, 2012; Szendroedi et al., 2012), central nervous system diseases including autism (Rossignol and Frye, 2012), and neurodegenerative diseases including Alzheimer’s disease (AD), Parkinson’s disease (PD), Huntington’s disease (HD), multiple sclerosis, amyotrophic lateral sclerosis (ALS), and pain (Moreira et al., 2010; Reyes et al., 2010; Witte et al., 2010; Ferrari et al., 2011; Lax et al., 2011; Johri and Beal, 2012; Cooper et al., 2012). Progress in these efforts from a traditional small-molecule perspective has, however, been challenging (Finsterer, 2010; Kerr, 2010; Stacpoole, 2011; Davis, 2012).

The present review highlights the current state of knowledge on the role of mitochondrial dysfunction in various disease states and identifies potential drug targets.

The Role of Mitochondria in Cell Function

Mitochondrial Genetics. Mitochondria are unique in that they have their own DNA pool (mtDNA) distinct from that of nDNA. mtDNA is almost exclusively maternally inherited and has independent evolutionary origins from nDNA that date back to the time when mitochondria were separate organelles before forming a symbiotic relationship with eukaryotes (Schapira, 2006; Wallace and Fan, 2010). Human mtDNA is approximately 16.6 base pairs long, forming a closed, double-stranded structure (Legros et al., 2004). Each mitochondrion contains between 2 and 10 mtDNA copies that consist of 37 genes coding for 22 transfer and 2 ribosomal RNAs and 13 proteins, the latter including the enzymes involved in the oxidative phosphorylation (OXPHOS) pathway involved in ATP production. OXPHOS units are coded by both nDNA and mtDNA, with the former contributing somewhere in excess of 1000 proteins that are essential for mitochondrial function (Wallace and Fan, 2010; Eichner and Giguère, 2011). Of these, 705 are under the transcriptional control of estrogen-related receptors, α, β, and γ, that are responsible for the integrated control of mitochondrial metabolism (Eichner and Giguère, 2011). Although the mtDNA sequence in most cells is identical and is consequently termed homoplasmic, the coexistence of wild-type and mutant mtDNA in the same mitochondrion and/or cell is known as heteroplasmy.

The OXPHOS pathway consists of five different ETC complexes located on the inner mitochondrial membrane that together contribute to the generation of the mitochondrial electrochemical gradient (Fig. 1). These complexes are composed of proteins that originate from both nDNA and mtDNA (Schon et al., 2010; Wallace and Fan, 2010). Complex I consists of 45 peptide subunits, 7 originating from mtDNA with the remainder from nDNA. Complex II has four subunits, all of which are derived from nDNA, and Complex III has 11 subunits, only one of which originates from mtDNA. Complex IV has 12 subunits, 3 of which are derived from mtDNA, and complex IV has approximately 16 subunits, 2 of which are from mtDNA. The fifth ETC complex, Complex V, is ATP synthase.

Mutation rates in mtDNA are generally 2- to 3-fold higher than those occurring in nDNA (although some experts estimate a 10-fold or more difference), a consequence, as already noted, of the proximity of mtDNA to the ROS produced by electron leakage from complexes I and III of the ETC (Fig. 1), coupled with inefficient DNA repair mechanisms and a lack of protective histones on mtDNA. To date, some 270 disease-related mtDNA point mutations have been identified (http://www.mitomap.org/bin/view.pl/MITOMAP/MutationsCodingControl) that are thought to affect mitochondrial protein synthesis, protein-encoding genes and mRNA, and ultimately mitochondrial function. These are complemented by rearrangements, deletions, and insertions in mtDNA and their altered interactions with nDNA, the latter reflecting defects in mitochondrial transport processes (Schon et al., 2010).

In heteroplasmic situations, the percentage of mutant mtDNA dictates the degree of mitochondrial dysfunction and disease occurrence. Thus the age-related accumulation of somatic mtDNA mutations that can lead in time to decreased mitochondrial function has been associated with an increased rate of aging and cancer incidence (Wallace, 2005; Schapira, 2006; Wallace and Fan, 2010). A variety of conditions (hypoxia, stress, trauma, blood glucose levels, aberrant circadian rhythms, etc.) and agents/mechanisms (phosphorylation, DNA methylation/acetylation, Akt/protein kinase B signaling, calcium homeostasis, estrogen-related receptor signaling, heat shock proteins, soluble adenylly cyclase (sAC), receptor-interacting protein 3 kinase, Target of Rapamycin kinases, peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), Signal Transducer and Activator of Transcription 3, AMP-activated protein kinase, PGAMSS (phosphoglycerate mutase/protein phosphatase 5, short form), β-amyloid, sirtuin-1, etc.) are involved in both modulating transcription of the mitochondrial genome and the function of the transcribed proteins. Mutated proteins such as huntingtin in HD, amyloid (Aβ) in AD, superoxide dismutase 1 in ALS, and parkin, DJ1, and α-synuclein in PD have been localized to mitochondrial membranes (Reddy, 2009) where they can alter ETC function to increase ROS production.

The increased interest in mtDNA as a risk factor and/or causative to human disease states parallels the renewed focus on noncoding or “junk” nuclear DNA that was originally dismissed as lacking importance when the map of the human genome was finally annotated. Far from being unimportant, junk DNA has been found to contain key regulatory sequences that modify gene expression and activity (Biémont, 2010), adding an additional level of complexity to understanding gene function and disease risk. This has the potential to negate the validity of many of the genomewide association studies (GWAS) conducted to date that sought to establish the relationship between specific genes and specific disease states (Mullane and Williams, 2012). The superimposition of mtDNA as yet another overlooked/underestimated component of the human genome coupled with its potential interactions with nDNA adds yet another level of complexity to deciphering gene-driven risk factors and causality. It is noteworthy that, more than a decade ago, Wallace (1999) noted that a specific mtDNA mutation could produce very different human disease phenotypes, whereas different mutations could result in the same phenotype. This insight is not limited to the mitochondrial genome and seems equally applicable to the total cellular genome, a conclusion that is supported by the identification of multiple, and often concep-
At the Qi site where the quinone moiety in ubiquinone is the electron carrier located in the intermembrane space and four temporarily transferred to two molecules of CytC, a water-soluble gradient [mitochondrial membrane potential ($\Delta \Psi_m$)] across the mitochondrial inner membrane (Bertram et al., 2006) via the ETC (Fig. 1). Approximately 90% of ATP arises from mitochondria. In complex I (NADH dehydrogenase) two electrons are removed from NADH and transferred to the lipid-soluble carrier, ubiquinone (Q) forming the reduced product, ubiquinol ($QH_2$) that can freely diffuse in the membrane. Complex I thus leads to the translocation of four protons ($H^+$) across the membrane to produce a proton gradient (Fig. 1). In complex II (succinate dehydrogenase) additional electrons are delivered from succinate via flavin adenine dinucleotide (FAD) to the quinone pool (Q) and transferred via FAD to Q. In complex III [ubiquinol-cytochrome $c$ (CytC) reductase] six electrons are removed from $QH_2$, two of which are sequentially transferred to two molecules of CytC, a water-soluble electron carrier located in the intermembrane space and four to the $Q$ site where the quinone moiety in ubiquinone is reduced to quinol contributing to the proton gradient. In complex IV (cytochrome $c$ oxidase), four electrons contributed by four CytC molecules are transferred to molecular oxygen ($O_2$), resulting in two molecules of water. Concomitantly, four protons translocate across the membrane, adding further to the proton gradient. The latter is then used in complex V, the $F_0F_1$ ATP synthase complex to produce ATP via OXPHOS. The $\Delta \Psi_m$ for ATP production is 100 to 120 mV with $\Delta \Psi_m$ values more than 140 mV leading to increased ROS production at the expense of ATP generation (Hüttemann et al., 2011).

The function of CytC, other key OXPHOS proteins, and necrosis signaling pathways (Wang et al., 2012) can be dynamically modulated by phosphorylation. One example is the negative feedback effects of ATP to control ETC function involve phosphorylation-dependent changes that alter the ability of CytC to bind to cytochrome $c$ oxidase, which is determined by the ATP/ADP ratio. ATP is also a key substrate in generically determining kinase activity (Dagda et al., 2009).

**Mitochondrial Dynamics and Cell Death Signaling**

Mitochondria are dynamic organelles that form networks throughout the cell via the opposing processes of fission and fusion (Sheridan and Martin, 2010). The latter is critical to the maintenance of mitochondrial function because it affects the repair of dysfunctional and damaged mitochondria in addition to intermixing DNA and proteins between mitochondria (Chan, 2006). Fusion involves the merging of the inner and outer membranes from two mitochondria to facilitate the GTPase-dependent exchange of materials to aid in mitochondrial repair. Fission occurs when a mitochondrion splits in two. When this process occurs in the presence of decreased fusion, it can lead to a fragmented mitochondrial phenotype that is widespread in both necrosis and apoptosis. Deficient fission and fusion mechanisms are thus key events in mitochondrial disease causality. In HD, fission is facilitated via the action of dynamin-related protein 1 (DRP1), leading to fragmented mitochondria that are fewer in number (Song et al., 2011). The mutant form of huntingtin, a protein associated with HD, enhances DRP-1 activity. Although fission seems to be involved in mitoptosis, there is considerable debate as to whether this is a primary or secondary event, in the former instance being causative to mitochondrial permeability transition (MPT) pore (MPTP) formation with a secondary, passive role in promoting mitochondrial network disassembly (Sheridan and Martin, 2010).

Mitochondria can promote both necrotic and apoptotic cell death via an abrupt increase in the permeability of the inner mitochondrial membrane (IMM) that allows the passage of molecules with molecular masses below 1.5 kDa (Zamzami et al., 2005; Baines, 2010). The MPT event results in the uncoupling of OXPHOS, resulting in the dissipation of the proton electrochemical gradient with decreased ATP production, increased ROS production, calcium overload, and mitochondrial swelling (Rodriguez-Enriquez et al., 2004). The degree to which the level of mitochondrial ATP is depleted is thought to be the major determinant as to whether cell death proceeds by necrosis or apoptosis, with very low ATP levels leading to necrosis. The relationship between apoptosis and necrosis is complex with data suggesting that: 1) necrosis is more important in cell death than apoptosis; 2) necrosis is an alternative death pathway to apoptosis when caspases are inhibited; and 3) necrosis is engaged as a cell death pathway when mitochondria form a complex with the endoplasmatic reticulum (Baines, 2010). Until recently, necrosis was thought to be a random, uncontrolled process (Kitis and Molkentin, 2010) that like apoptosis produced its effects via MPTP formation and mitochondrial membrane permeabilization. However, necrosis has now been recognized as a programmed process, the effects of which are mediated through pathways that, although distinct from those mediating apoptosis, may involve common pathway members (Sun et al., 2012; Wang et al., 2012) (Fig. 2) with canonical apoptotic molecules being involved in programmed necrosis (Baines, 2010). The effects of these common proteins may be antagonistic. For instance, caspase 8, which is involved in chromatin degradation and apoptosis formation, can inhibit necroptosis function (Fig. 2).

**Mitochondrial Membrane Permeability.** The increase in mitochondrial membrane permeability in the IMM is mediated via the MPTP, the composition of which remains a subject of active debate (Halestrap, 2009; Javadov et al., 2011). Although early studies had indicated that the MPTP was comprised of three subunits, a voltage-dependent anion channel (VDAC) (Shoshan-Barmatz and Ben-Hail, 2012), the adenine nucleotide translocator (ANT) (Kunji and Crichton, 2010), and mitochondrial cyclophilin D (Cyp-D) (Schinzel et al., 2005), the latter a matrix peptidyl-prolyl cis-trans isomerase, gene knockout studies have questioned the involvement of VDAC while relegating ANT to a modulatory role because MPT can still occur in mitochondria lacking VDAC or ANT (Baines, 2010). ANT also exists in several forms that have different and opposing functions. ANT-1 and ANT-3 are proapoptotic, whereas ANT-2 is antiapoptotic (Fulda et al., 2010).
A variety of other proteins have been associated with the MPTP, including the antiapoptotic and proapoptotic proteins, Bcl-2 and Bax, hexokinase, the mitochondrial phosphate carrier (Varanyuwatana and Halestrap, 2012), the peripheral benzodiazepine receptor also known as the 18-kDa translocator protein (Papadopoulos et al., 2006), and complex I of the ETC (Roestenberg et al., 2012). The mitochondrial phosphate carrier can form complexes with ANT (Halestrap, 2009) as part of an “ATP synthasome” providing a phosphate-sensing entity that can bind to Cyp-D.

Determining the functional structure of the MPTP, in addition to being key to understanding its contributions to disease pathogenesis and aging, is critical in providing a rationale basis for targeting the pore for drug discovery efforts, because a compound that would specifically and potently inhibit MPTP formation and function would have potential utility in ameliorating cardiac (Halestrap, 2009), metabolic (Szendoredi et al., 2012), and neurodegenerative (Martin et al., 2011) disease states, whereas an agent that would facilitate or enhance MPTP formation and function would increase apoptosis and be useful in the treatment of cancer. Halestrap (2009) has suggested, however, that the MPTP may be intrinsically heterogeneous, its molecular composition varying as a function of the local availability of subunits that can contribute to a functional MPTP. If correct, this will inevitably complicate targeting molecular targeting approaches. An additional complicating factor is whether MPTP formation in the IMM occurs as the key event in both necrotic and apoptotic signaling pathways via the “BH3-only-like” protein, Nix/BNip3L, BCL2/adenovirus E1B 19-kDa protein-interacting protein 3-like; PARP, poly(ADP) ribose polymerase; RIPK, receptor interacting protein kinase; TNFR, TNF receptor; TRAF2, TNF receptor-associated factor 2.

Fig. 2. Mitochondrial cell death pathways: necrosis and apoptosis. See text for discussion of extrinsic and intrinsic necrotic and apoptotic pathway activities. Apaf, apoptosis protease-activating factor; Bax, proapoptotic Bcl-2-associated X protein; Bak, Bcl-2 homologous antagonist killer; Bcl, B-cell lymphoma protein; BH3, proapoptotic Bcl-2 family members; Endo G, endonuclease G; FADD, FAD domain protein; Fasl, Fas ligand type II transmembrane protein; FLIP, FADD-like interleukin-1-β-converting enzyme FLICE-like inhibitory protein; McI, induced myeloid leukemia cell differentiation protein; MLKL, mixed-lineage kinase-domain-like protein; Nix/BNip3L, BCL2/adenovirus E1B 19-kDa protein-interacting protein 3-like; PARP, poly(ADP) ribose polymerase; RIPK, receptor interacting protein kinase; TNFR, TNF receptor; TRAF2, TNF receptor-associated factor 2.
ways, the composition of which has increased in complexity as new members, and their interactions, continue to be identified.

**Apoptotic Cell Death Pathway.** Proteins released via a combination of MPTP formation and OMM collapse include CytC, Bcl-2, Smac/DIABLO [second mitochondria-derived activator of caspasess/direct inhibitor of apoptosis (IAP)-associated binding protein with low pi], Omi/HtrA2 (homotrimeric serine protease high-temperature requirement A2), apoptosis-inducing factor (AIF), and endonuclease G. CytC is the key protein in the initiation of apoptosis. Together with the protein APAF-1 (apoptosis protease-activating factor) and pro-caspase-9, CytC forms an “apoptosome” that facilitates activation of the cysteine protease, caspase-9, which then activates effector caspases to enable apoptosis. AIF and endonuclease G are key mediators in the DNA fragmentation and chromosomal condensation that occurs in apoptosis.

The function of CytC, like many of the other proteins in the cell death pathways, depends on its state of phosphorylation, a point that was not fully appreciated in early studies when it was isolated and studied in its dephosphorylated state (Hüttemann et al., 2011). Phosphorylation of tyrosines in CytC inhibits interactions with cytochrome c oxidase, supporting the concept that under normal conditions when there is adequate ATP OXPHOS runs at a reduced activity, a “controlled” state, to maintain ΔΨm, below those leading to free-radical formation (Hüttemann et al., 2011). Smac/DIABLO and Omi/HtrA2 are antagonists of the protein IAPs that promote caspase activation. Mitochondrial membrane permeabilization can also occur independently of pore formation and involves Bcl-2 family members that include both proapoptotic (Bax, Bak, and Bok) and anti-apoptotic (Bcl-2 and Bcl-xL) members.

Apoptosis in mitochondria comprises the intrinsic apoptotic or Type I pathway as contrasted to the extrinsic pathway (Type II) that involves activation of the cell surface death receptor family, a subclass of the tumor necrosis factor (TNF) superfamily. The intrinsic and extrinsic apoptotic pathways are linked by the Bcl2 family protein, Bid (Bcl-2 interacting domain), a BH-3 proapoptotic regulator. sAC is a proapoptotic mediator that translocates to mitochondria under conditions of acidic stress. The effects of sAC are mediated via activation of Protein Kinase A (PKA), which facilitates translocation of Bax from the cytosol to the mitochondrion where Bax is involved in OMM permeabilization. PKA is also thought to block the effects of Akt on inactivating Bax, thus attenuating apoptosis (Kumar et al., 2009).

**Necrotic Cell Death Pathway.** Like apoptosis, necrosis has both extrinsic and intrinsic components, the former involving death receptor activation and the latter involving ROS production and PARP-1/calpain activation (Fig. 2). The receptor interacting protein kinases (RIPKs) together with TRAP2 and MLKL (mixed lineage kinase-domain-like protein) initiate necrosome formation that is then activated by sequential phosphorylation events (Sun et al., 2012). It then forms a complex with the mitochondrial protein phosphatase, PGAM5S, which in turn recruits the mitochondrial fission factor, DRP1. The resultant necrosome complex can then activate DRP-1 GTPase to induce mitochondrial fragmentation, initiating necrosis execution (Wang et al., 2012). Mitochondrial PGAM5S is also involved in ROS-induced necrosis and may thus represent a major convergence point for necrotic pathways.

**Autophagy.** Autophagy, an organelle-specific autophagic elimination, is responsible for both the elimination of damaged mitochondria and the regulation of their number and involves ubiquitination that recruits the ubiquitin-binding autophagic components histone deacetylase (HDAC) 6 and p62 to facilitate mitochondrial clearance (Lee et al., 2010). Mitophagy can be regulated by parkin and PTEN-induced putative kinase protein 1 (PINK1) (Youle and Narendra, 2011).

**Mitochondrial Disease States**

As noted, the tissues that are most susceptible to mitochondrial-driven disease states are those with a high metabolic demand. These include brain, eye, liver, heart, and skeletal muscle. Mitochondrial disease states include the mitochondrial myopathies, a group of neuromuscular diseases that includes KSS, MELAS, MERRF, and MNGIE that have genetic origins (Schapira, 2006; Wallace and Fan, 2010), disorders of mitochondrial ETC that affect ETC assembly and/or stability and function and involve both genetic factors and cofactor deficiencies (coenzyme Q10) that can lead to decreased ATP production and increased free-radical production, the latter potentially leading to neurodegenerative diseases (AD, PD, HD, and ALS) (Johri and Beal, 2012). LHON, which is associated with visual failure caused by the degeneration of retinal ganglion cells, is the most common disease associated with mtDNA mutations with a prevalence of approximately 12 cases per 100,000 in the population (Schapira, 2006).

A unifying enabling theme in neurodegenerative disease states involves the misfolding of key cellular proteins that lead to the amyloidopathies (AD), tauopathies (AD, PD, Pick’s disease, progressive supranuclear palsy, corticobasal degeneration, and argyrophilic grain disease), α-synucleinopathies (PD, dementia with Lewy bodies, multiple system atrophy, and some instances of AD), and the TAR DNA-binding protein 43 proteinopathies/ubiquinopathies (ALS, frontotemporal dementias, and argyrophilic grain disease) (Geser et al., 2009). In PD, defects in complex I activity involve mtDNA mutations, alterations in mitochondrial kinase signaling (e.g., PTEN-induced kinase I, Akt/PKB, JNK, and ERK; Dagda et al., 2009), and can be caused by the effects of environmental toxins (rotenone) that lead to increased free-radical production and reduced activity in complex IV. In PD, dysregulation of the ubiquitin-proteasomal system, which is energy sensitive, leads to destruction of dopamine cells in the substantia nigra. In HD, the mutant form of huntingtin protein (mHtt) alters mitochondrial function, leading to a loss of membrane potential, decreased expression of OXPHOS enzymes (Mochel and Haller, 2011), and increased fission events that lead to decreases in the number, size, and distribution of mitochondria (Song et al., 2011). Alterations in ETC function also occur in AD where the major culprit thought to be responsible for disease causation, Aβ, can inhibit OXPHOS and specifically inhibit the mitochondrial enzyme, ABAD (Aβ-binding alcohol dehydrogenase) also known as ERAB (ER amyloid-β-peptide binding protein) that exacerbates Aβ-induced cell stress, leading to mitochondrial and neuronal dysfunction (Lustbader et al., 2004; Tillement et
and the SKQs (Skulachev ions), the SS-peptide arginyl-2, and E) and supplements that include carnitine, coenzyme the ETC chain or adding membrane penetrating antioxidants diseases have focused on replacing deficient components of current interest involve modulation of MPTP formation pathways associated with mitochondrial function, there is no

given the exquisite complexity of the structural proteins and function (Eichner and Gigue `re, 2011).

mitochondrial dysfunction has also been associated with multiple sclerosis (Witte et al., 2010) and autism spectrum disorders (Rossignol and Frye, 2012).

Type II diabetes (T2DM) is associated with reductions in OXPHOS capacity and decreased mitochondrial plasticity and numbers in skeletal muscle and liver, resulting in insulin resistance (Szendroedi et al., 2012). Mitochondrial dys

function appears to be a key link between AD and diabetes (Moreira et al., 2007) having been described as “type 3 diabetes” (de la Monte et al., 2006). Changes in cardiac mito

ochondrial morphology that are linked to changes in mitochondrial metabolism have been associated with heart failure, coronary artery disease, and responses to ischemic episodes (Ong and Hausenloy, 2010).

Mitochondria as a Target for Drug Discovery

The explosion of knowledge regarding the key role of mitochondria in human disease states has led to efforts to develop drugs based on the considerable knowledge base. Given the exquisite complexity of the structural proteins and pathways associated with mitochondrial function, there is no shortage of potential targets, although the majority of those of current interest involve modulation of MPTP formation and function (Eichner and Giguère, 2011).

Seminal efforts in addressing inherited and acquired ETC diseases have focused on replacing deficient components of the ETC chain or adding membrane penetrating antioxidants and free-radical scavengers. The former include vitamins (D and E) and supplements that include carnitine, coenzyme Q10 and its analogs, thiamine pyrophosphate, mitoquione and the SKQs (Skulachev ions), the SS-peptide arginyl-2, 6'-dimethyltyrosyl-lysyl-phenyalaninamide (SS-31), 7-chloro

5-(2-chlorophenyl)-1,5-dihydro-4,1-benzothiazepin-2(3H)-one (CGP 37157), riboflavin, trolox, thiamine, creatine, pyruvate, the pyruvate analog dichloracetate, succinate, folate, omega-3 fatty acids [docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)], and methylene blue that can improve the efficiency of the ETC, increase ATP production, and reduce ROS production (Reddy, 2009; Kerr, 2010; Schon et al., 2010; Roestenberg et al., 2012). Removal of noxious metabolites, such as lactate, by using bicarbonate and/or dichloracetic acid (Finsterer, 2010) and free-radical scavenging entities, both dietary and synthetic, are other approaches to improving ETC function, although the latter, while effective in cell lines and animal models, have questionable efficacy in the clinical setting (Halliwell, 2011; Poljsak, 2011).

Such agents have shown varying levels of success in treating mitochondrial disorders, and work continues to improve their access to, and selectivity for, their mitochondrial sites of action. Much of the current effort is focused on finding new chemical entities (NCEs) that facilitate or block mitochondrial cell death pathways. This represents the Yin and Yang of cell death-related disease treatment where accelerating/facilitating apoptosis to develop more effective anticancer drugs is counterindicated in cardiovascular, metabolic, and neurodegenerative disease states where abrogating cell death processes is the target for therapeutics to address and improve mitochondrial energetics in these disease states (Javadov et al., 2011). These drug discovery efforts are focused primarily on small molecules, including peptides, that can modulate MPTP formation and function (Finsterer, 2010; Kerr, 2010; Stacpoole, 2011; Davis, 2012) and calcium homeostasis (Giorgi et al., 2012). A number of compounds have been found to interact with the putative MPTP-constituent protein VDAC and include the antisense 18mer G3139 (oblimersen, TCTCCAGCGTGCCCAT), the avicin class of plant stress metabolites, the antidepressant fluoxetine, cisplatin, and endostatin (Shosnan-Barmatz and Ben-Hail, 2012).

A major issue in mitochondrial-targeted drug discovery is the challenge of delivering NCEs at sufficient levels to be therapeutically useful to targets located inside an intracellular organelle, requiring effective passage through cell membrane, cytosol, and the mitochondrial membrane. Analogs of CoQ10, like MitoQ and SKQ1 (Fink et al., 2012), are preferentially absorbed in the IMM, whereas the peptide SS-31 shows a 5000-fold accumulation in mitochondrial fractions (Roestenberg et al., 2012). Functionalized polymeric and metallic nanoparticles are also being explored as potential mitochondrial delivery systems (Durazo and Komplella, 2012) as novel approach proteomic polyanionic or amphipathic cell-penetrating peptides. The latter contain epitopes that act as vectors for the highly efficient delivery of bioactive cargoes into the intracellular milieu (Jones et al., 2010).

Cell-penetrating peptides from human CytC, specifically CytC77–101 and CytC86–101, can mimic the apoptogenic effects of CytC to induce tumor cell apoptosis. Nup153-CytC, a chimeric N-terminal extension of CytC77–101 with a target mimetic of FG nucleoporin, enhanced the apoptotic potency of the parent compound (LD50 CytC77–101 = 80.6 μM; LD50 Nup153-CytC = 730 nM) by facilitating redistribution of nuclear pore complex proteins and targeting inositol trisphosphate receptors on the endoplasmic reticulum involved in calcium homeostasis to amplify apoptotic signaling events (Jones et al., 2010). Other mitochondrialy targeted anticancer NCEs that are focused on enhancing apoptosis include modulators of BCL-2 family function [4-[4-[[4'-chlorobiphe

nyl-2-yl]methyl] piperazin-1-yl]-N-[4-[[1R]-3(dimethylamino)-1-[phenylsulfonyl] methyl] propylamino]-3-nitrophenylsulfonyl] benzamide (ABT-737), (R)-(−)-3′,5′-bis(1-methyl) [2′,2′,6′,6′-tetrachloro-3,3′-dimethyl-5,5′-dimethyl-2-naphthalenecarboxylic acid (CD437), N-butan-2-yl-1-(2-chlorophenyl)-N-(methylsulfinyl)-3-carboxamide (PK 11195), arsenite trioxide, clodronate], ROS regulators [choline tetrathiomolybdate (ATN-224), N1, N3, dimethyl-N1, N3- bis(phenylcarbothiol) propanedihydrazide
(STA-4783)], Hsp-90 inhibitors [6-amino-8-[(6-iodo-1,3-benzodioxol-5-yl)thio]-N-(1-methylethyl)-9H-purine-9-propanamine (PI-H71), phenethyl isothiocyanates], and F1-ATPase inhibition (resveratrol) (Fulda et al., 2010). The sirtuin resveratrol, an NAD+ -dependent deacetylase with many diverse and controversial biological effects (Couzin-Frankel, 2011), can improve mitochondrial function by inducing the genes for OXPHOS and mitochondrial biogenesis. In addition to acting as sensors for the redox/nutritional state of mitochondria, the sirtuins have the potential to modulate the acetylation state of mitochondrial proteins and, consequently, their functions (Pereira et al., 2012).

The effects of resveratrol are mediated by an increase in PGC-1α activity (Lagouge et al., 2006; Roestenberg et al., 2012). PGC-1α is a master regulator of mitochondrial biogenesis and function, ensuring tight coupling between mitochondrial respiration and ROS production (Austin et al., 2011). Nonsteroidal anti-inflammatory drugs (NSAIDs), e.g., aspirin and indomethacin, in addition to their ability to inhibit the cyclooxygenase enzymes responsible for prostaglandin production affect mitochondrial function by uncoupling OXPHOS, decreasing ATP production, and inducing MPTP formation and apoptosis. Although these effects may be responsible for many of the side effects of NSAIDs, they are also thought to mediate the beneficial prophylactic effects of NSAIDs in preventing colorectal cancer (Suzuki et al., 2010).

Blocking the formation and/or function of the MPTP is a conceptually promising approach to treating metabolic, cardiac, and neurodegenerative diseases. The immunosuppressants cyclosporin A and sanglierin A block MPTP function by binding to Cyp-D, an effect independent of their immunosuppressant actions. Cyclosporin A has beneficial effects in reducing cardiac hypertrophy and countering the adverse effects of ischemia (Szewczyk and Wojtczak, 2002). There are also various reports of beneficial actions in preclinical models of AD, PD, HD, and ALS. Antamanide, a cyclic decapeptide from the fungus Amanita phalloides, also blocks the MPTP by targeting Cyp-D and inhibiting its cis-trans isomerase activity (Azzolin et al., 2011). Oleosxime ([(NZ)-N-((8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2-yl]-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-ylidene]hydroxylamine (TRO19622)), an orally active cholesterol-oxime that crosses the blood-brain barrier, targets proteins in the OMM to prevent MPTP formation in response to oxidative stress, resulting in neuroprotection (Bordet et al., 2010). Blockade of apoptosis is beneficial in animal models of ALS (Reyes et al., 2010), and oleosxime is currently in clinical trials for this indication and being developed for use in the treatment of spinal muscular atrophy. Dexpramipexole [(R)-N-[(S)-propyl-4,5,6,7-tetrahydrobenzol[d]thiazole-2,6-diamine (KNS-760704)], the “inactive” isomer of the dopamine agonist, pramipexole, which has neuroprotectant activity via blockade of ROS production and the activation of apoptotic pathways, has shown positive outcomes in phase II trials in ALS (Cudkowicz et al., 2011). Dimebon (latrepirdine), another modulator of MPTP pore function/formation that can enhance mitochondrial function (Zhang et al., 2010), had major therapeutic benefits in a phase II AD trial (Doody et al., 2008) but showed no beneficial effects in a subsequent pivotal phase III trial, leading to concerns regarding the use of this generic antihi-

The cholesterol-lowering statins can also activate cardiac mitochondrial biogenesis and increase antioxidant capacity via effects that involve the ROS/PGC-1 signaling pathway (Bouitbir et al., 2011).

Other approaches to restoring mitochondrial function include blood transfusions, diet, somatic stem cell, germ line, and gene therapy, the latter involving the introduction of engineered mitochondrial genes, the manipulation of heteroplasmy levels, and rescue of mtDNA mutations (Finsterer, 2010; Poljsak, 2011).

**Translational Aspects of Mitochondrial Disease Therapy**

Drug discovery effects targeting mitochondria have evolved through two distinct research eras: the first, treating inherited and acquired ETC disorders and the second, now underway, focused on modulating MPTP function and understanding the role of mtDNA-based genetics in disease etiology and the “drugability” of key protein products.

Identification of therapeutics for mitochondrial diseases has, however, proved to be a challenge with clinical trials in the area being characterized as “generally ineffective . . . inadequately designed, often anecdotal and underpowered” (Schon et al., 2010). In a 2011 PubMed analysis of clinical trials related to mitochondrial diseases, Stacpoole (2011) identified 75 trials of which 43 (57%) were double-blind, placebo-controlled, randomized clinical trials (RCTs). Of these, only 10 were conducted in patients with identified mitochondrial cytopathies. The entities evaluated were desoxycorticosterone acetate, several natural products, and a mixture of nutraceuticals that together led to concerns regarding both the limited number of RCTs tested and the fact that they did not represent the diversity of potential therapeutics. In addition, Stacpoole (2011) expressed concerns regarding the approach to the funding, design, and endpoint designation of RCTs for mitochondrial diseases that were thought to reflect “a persistence of clinical anecdotes as substitutes for scientifically and ethically rigorous clinical trials,” echoing similar concerns that had been raised previously by Kerr (2010). Clearly these concerns do not apply to trials for NCES targeted at the MPTP being tested as anticancer agents or for the treatment of cardiovascular, metabolic, or neurodegenerative disorders where the format of RCTs is well established, although these are not without their concerns. As in other areas of drug discovery, the development of reliable biomarkers for mitochondrially-associated disease states will be key in facilitating translational efforts.
Conclusions

Advances in understanding mitochondrial function and the role of these intracellular organelles presents a novel paradigm for drug discovery, “a dawn for evolutionary medicine” (Wallace, 2005) that although in its infancy has considerable potential for identifying drugs for a diversity of chronic human disease states. An increased appreciation of the complexity of putative drug targets in the mitochondrion and their associated signaling pathways together with drug discovery efforts that are specifically focused on mitochondrial targets and improved translational paradigms will facilitate the discovery of novel compounds, which, on their own or in combination with drugs acting at other complementary targets, have the potential to treat a myriad of human disease states for which there currently are no effective treatments.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Davis and Williams.

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Akar FG and O'Rourke B (2011) Mitochondria are sources of metabolic sink and their associated signaling pathways together with drug discovery efforts that are specifically focused on mitochondrial targets and improved translational paradigms will facilitate the discovery of novel compounds, which, on their own or in combination with drugs acting at other complementary targets, have the potential to treat a myriad of human disease states for which there currently are no effective treatments.

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