Perspectives in Pharmacology

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; $\Delta\Psi_m$, 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, proapoptoic 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, 4-{4-[(4′-chlorobiphenyl-2-yl)methyl]piperazin-1-yl}-N-{[4-({(1R)-3-(dimethylamino)-1-[(phenylsulfanyl)methyl]propyl}amino)-3-nitrophenyl]sulfonyl}benzamide; AT-101, (−)-1,1′,6,6′,7,7′-hexahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-[2,2′-binaphthalene]-8,8′-dicarboxaldehyde; CD437, 6-(3-(1-adamantyl)-4-hydroxyphenyl)-2-naphthalenecarboxylic acid; PK 11195, N-butan-2-yl-1-(2-chlorophenyl)-N-methylisoquinoline-3-carboxamide; ATN-224, choline tetrathiomolybdate; STA-4783, $N′^1$, $N′^3$-dimethyl-$N′^1$, $N′^3$- bis(phenylcarbonothioyl)propanedihydrazide; PI-H71, 6-amino-8-[(6-iodo-1,3-benzodioxol-5-yl)thio]-N-(1-methylethyl)-9H-purine-9-propanamine; KNS-760704, (R)-N^6-propyl-4,5,6,7-tetrahydrobenzo[d]thiazole-2,6-diamine; CGP 37157, 7-chloro-5-(2-chlorophenyl)-1,5-dihydro-4,1-benzothiazepin-2(3H)-one; SS-31, arginyl-2,′6′-dimethyltyrosyl-lysyl-phenylalaninamide; TRO19622, (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.

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 (Freiderich'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.,
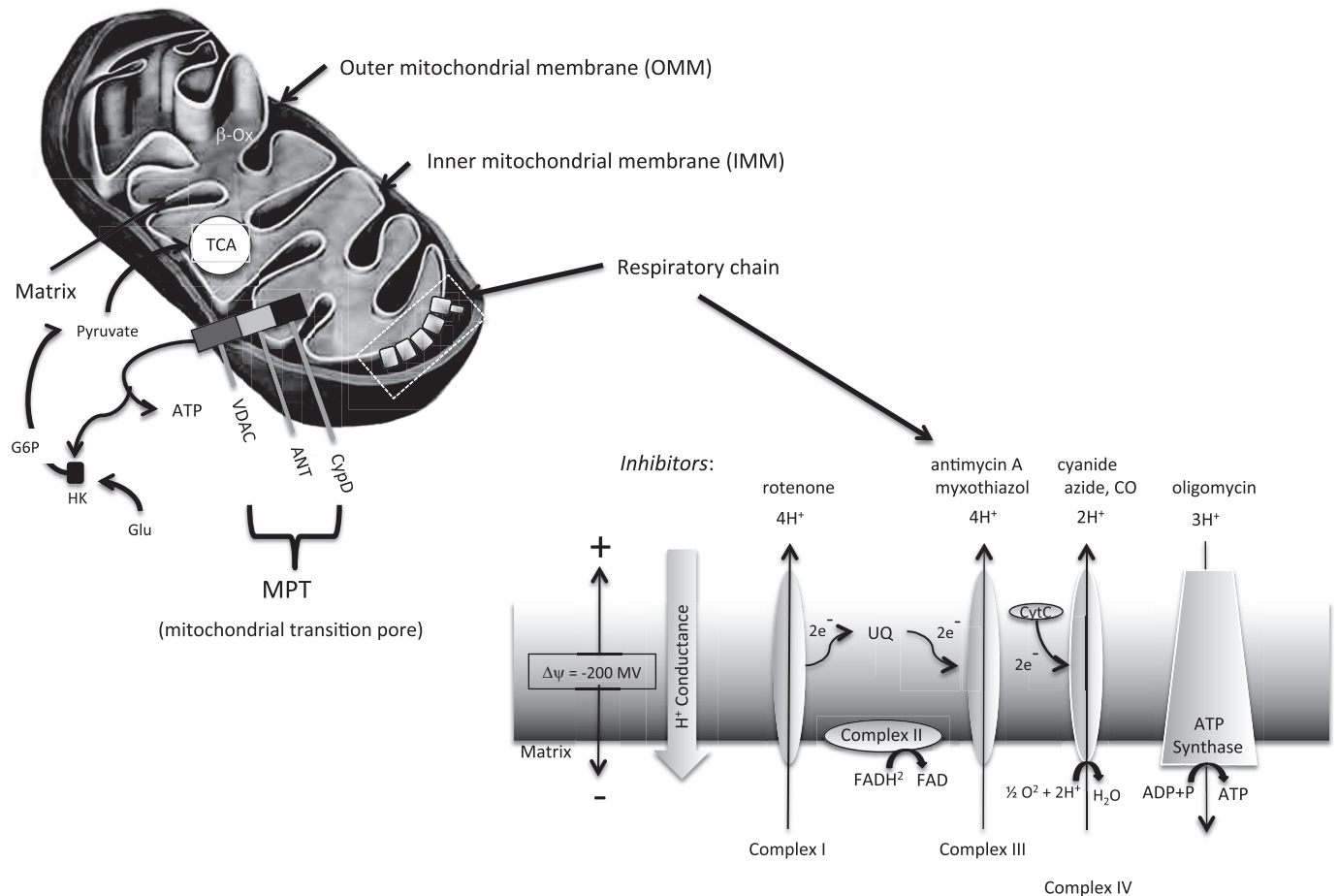

Fig. 1. Schematic of the mitochondrion showing the mitochondrial permeability transition pore and the respiratory chain. See text for details. G6P, glucose 6-phosphate; HK, hexokinase; TCA, tricarboxylic acid cycle.

2010; Maldonado and LeMasters, 2012), cardiovascular disease (Ballinger, 2005; Akar and O'Rourke, 2011; Ong and Hausenloy, 2010), metabolic diseases (Gilliam and Neufer, 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 organisms 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 DNAs 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 adenylyl 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, PGAM5S (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-

tually puzzling, gene candidates/associations for disease states such as asthma, schizophrenia, and AD with the latter currently numbering in excess of 130 and still growing (Mullane and Williams, 2012).

ATP Production. ATP is produced in mitochondria via OXPHOS, a complex process involving mitochondrial respiration and the generation of a proton (or electrochemical) 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_i 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_0/F_1 ATP synthase complex to produce ATP via OXPHOS. The $\Delta\Psi_m$ is normally in the range of 80 to 140 mV. The optimal $\Delta\Psi_m$ 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 huntintin, 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) (see below) 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 decoupling 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 endoplasmic reticulum (Baines, 2010). Until recently, necrosis was though to be a random, uncontrolled process (Kitsis and Molkentin, 2010) that like apopotosis 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 apoptoic 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 apoptosome formation, can inhibit necrosome 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).
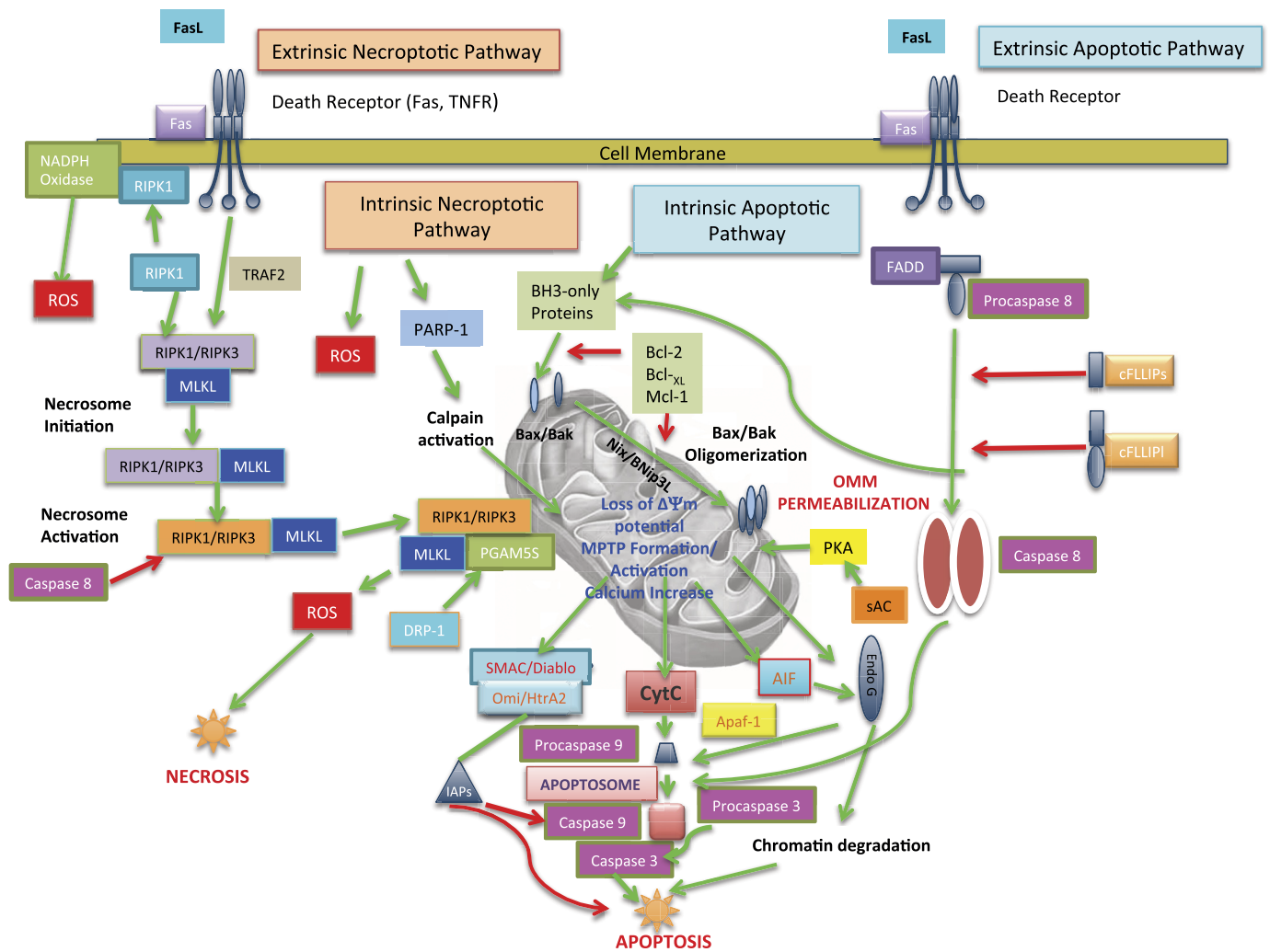
Fig. 2. Mitochondrial cell death pathways: necrosis and apoptosis. See text for discussion of extrinsic and intrinsic necrotic and apoptotic pathway activities. Apaf, apoptosis protease-activating factor; Bax, proapoptotic Bcl-2-associated X protein; Bak, Bcl-2 homologous antagonist killer; BcL, B-cell lymphoma protein; BH3, proapoptoic Bcl-2 family members; Endo G, endonuclease G; FADD, FAD domain protein; FasL, Fas ligand type II transmembrane protein; FLLIP, FADD-like interleukin-1β-converting enzyme FLICE-like inhibitory protein; McL, 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.

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 (Szendroedi 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), or is unique to necrosis with apoptosis being mediated via rupture of the outer mitochondrial membrane (OMM) (Kitsis and Molkentin, 2010).

The various triggers that activate the various mitochondrial death pathways (e.g., viral infection, ischemia, ATP depletion, oxidative stress, p53 activation, DNA damage, nitric oxide, toxins, etc.) increase MPTP formation and function and result in the leakage of multiple soluble apoptogenic/proapoptotic proteins (Fig. 2). The release of these proteins can then engage a diversity of downstream signaling path-

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 caspases/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 $\Delta\Psi_m$ 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 antiapoptotic (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 apootopic 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 TRAF2 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. Mitophagy, 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, MELSAS, 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 Q_{10}) 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-synucleopathies (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

al., 2011). ABAD inhibition in a mouse transgenic APP (amyloid precursor protein) model of AD reduces Aβ accumulation and improves mitochondrial function (Yao et al., 2011). Aβ also binds to ANT in the MPTP and to complexes IV and V of the ETC, resulting in changes in calcium homeostasis, OXPHOS efficiency, decreases in DRP-1, enhancement of nitric oxide production, ROS-induced oxidative stress, tau toxicity, cytokine production, and inflammation (Moreira et al., 2010; Tillement et al., 2011). Alterations in XIAP (X-linked inhibitor of apoptosis), caspase-3, and lipofuscin accumulation are also observed in AD, the latter decreasing autophagy and reducing mitochondrial recycling. Nonetheless, mitochondrial autophagocyotosis is increased in AD and may reflect differential roles for autophagy depending on the stage of the disease (Moreira et al., 2010). 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 dysfunction 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 mitochondrial 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, mitoquinone and the SKQs (Skulachev ions), the SS-peptide arginyl-2,′6′-dimethyltyrosyl-lysyl-phenylalaninamide (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 contraindicated 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, TCTCCCAGCGTGCGCCAT), 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 are novel approach proteomimetic 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 CytC[77−101] and CytC[86−101], can mimic the apopotogenic effects of CytC to induce tumor cell apoptosis. Nup153-CytC, a chimeric N-terminal extension of CytC[77−101] with a target mimetic of FG nucleoporin, enhanced the apopotogenic potency of the parent compound (LD_{50} CytC[77−101] = 80.6 μM; LD_{50} 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 mitochondrially targeted anticancer NCEs that are focused on enhancing apoptosis include modulators of BCL-2 family function [4-{4-[(4′-chlorobiphenyl-2-yl)methyl] piperazin-1-yl}-N-{[4-{((1R)-3-(dimethylamino)-1-[(phenylsulfanyl) methyl] propyl}amino)-3-nitrophenyl]sulfonyl} benzamide (ABT-737), (−)-1,1′,6,6′,7,7′-hexahydroxy-3,3′-dimethyl-5,5′-bis(1-methylethyl)-[2,2′-binaphthalene]-8,8′-dicarboxaldehyde(AT-101)], metabolic inhibitors (dichloracetate, orlistat), ANT/VDAC ligands [lonidamine, 6-(3-(1-adamantyl)-4-hydroxyphenyl)-2-naphthalenecarboxylic acid (CD437), N-butan-2-yl-1-(2-chlorophenyl)-N-methylisoquinoline-3-carboxamide (PK 11195), arsenite trioxide, clodronate], ROS regulators [choline tetrathiomolybdate (ATN-224), N'^1, N'^3-dimethyl-N'^1, N'^3- bis(phenylcarbonothioyl) propanedihydrazide

(STA-4783)], Hsp-90 inhibitors [6-amino-8-[(6-iodo-1,3-benzodioxol-5-yl)thio]-*N*-(1-methylethyl)-9*H*-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 sangliferin 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 counteracting 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). Olesoxime [(*NZ*)-*N*-[(8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-10,13-dimethyl-17-[(2*R*)-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 olesoxime is currently in clinical trials for this indication and being developed for use in the treatment of spinal muscular atrophy. Dexpramipexole [(*R*)-*N*^{6}-propyl-4,5,6,7-tetrahydrobenzo[*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 formation/function 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 antihis-

tamine as a selective MPTP blocker, the depth and quality of its preclinical pharmacological and pharmaceutical characterization especially given its known polypharmic actions, and the execution of the phase II trial in Eastern Europe (Sabbagh and Berk, 2010; Williams and Coyle, 2012). Whether these misgivings can be extrapolated to questioning a key role for the MPTP in AD etiology remains to be determined.

Noninvasive approaches to improving mitochondrial function in AD are also being evaluated and include transcranial laser therapy, which normalized Aβ neuropathology in an AD transgenic mouse model while improving mitochondrial function and brain ATP levels (De Taboada et al., 2011).

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 are currently no effective treatments.

Authorship Contributions

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

References

Akar FG and O'Rourke B (2011) Mitochondria are sources of metabolic sink and arrhythmias. *Pharmacol Ther* **131**:287–294.

Austin S, Klimcakova E, and St-Pierre J (2011) Impact of PGC-1α on the topology and rate of superoxide production by the mitochondrial electron transport chain. *Free Radic Biol Med* **51**:2243–2248.

Azzolin L, Antolini N, Calderan A, Ruzza P, Sciacovelli M, Marin O, Mammi S, Bernardi P, and Rasola A (2011) Antamanide, a derivative of *Amanita phalloides*, is a novel inhibitor of the mitochondrial permeability transition pore. *PLoS One* **6**:e16280.

Baines CP (2010) Role of the mitochondrion in programmed necrosis. *Front Physiol* **1**:156.

Ballinger SW (2005) Mitochondrial dysfunction in cardiovascular disease. *Free Radic Biol Med* **38**:1278–12795.

Bertram R, Gram Pedersen M, Luciani DS, and Sherman A (2006) A simplified model for mitochondrial ATP production. *J Theor Biol* **243**:575–586.

Biémont C (2010) A brief history of the status of transposable elements: from junk DNA to major players in evolution. *Genetics* **186**:1085–1093.

Bordet T, Berna P, Abitbol J-L, and Pruss RM (2010) Olesoxime (TRO19622): a novel mitochondrial-targeted neuroprotective compound. *Pharmaceuticals* **3**:345–368.

Bouitbir J, Charles A-L, Echaniz-Laguna A, Kindo M, Daussin F, Auwerx J, Piquard F, Geny B, and Zoll J (2011) Opposite effects of statins on mitochondria of cardiac and skeletal muscles: a 'mitohormesis' mechanism involving reactive oxygen species and PGC-1. *Eur Heart J* **33**:1397–1407.

Castellani RJ, Lee HG, Siedlak SL, Nunomura A, Hayashi T, Nakamura M, Zhu X, Perry G, and Smith MA (2009) Reexamining Alzheimer's disease: evidence for a protective role for amyloid-beta protein precursor and amyloid-beta. *J Alzheimers Dis* **18**:447–452.

Chan DC (2006) Mitochondrial fusion and fission in mammals. *Annu Rev Cell Dev Biol* **22**:79–99.

Copeland WC (2008) Inherited mitochondrial diseases of DNA replication. *Annu Rev Med* **59**:131–146.

Cooper O, Seo H, Andrabi S, Guardia-Laguarta C, Graziotto J, Sundberg M, McLean JR, Carrillo-Reid L, Xie Z, Osborn T. et al., (2012) Pharmacological rescue of mitochondrial deficits in iPSC-derived neural cells from patients with familial Parkinson's disease. *Sci Transl Med* **4**:141ra90.

Couzin-Frankel J (2011) Aging genes: the sirtuin story unravels. *Science* **334**:1194–1198.

Cudkowicz M, Bozik ME, Ingersoll EW, Miller R, Mitsumoto H, Shefner J, Moore DH, Schoenfeld D, Mather JL, Archibald D, et al. (2011) The effects of dexpramipexole (KNS-760704) in individuals with amyotrophic lateral sclerosis. *Nat Med* **17**:1652–1656.

Dagda RK, Zhu J, and Chu CT (2009) Mitochondrial kinases in Parkinson's disease: converging insights from neurotoxin and genetic models. *Mitochondrion* **9**:289–298.

de la Monte SM, Tong M, Lester-Coll N, Plater M Jr, and Wands JR (2006) Therapeutic rescue of neurodegeneration in experimental type 3 diabetes: relevance to Alzheimer's disease. *J Alzheimers Dis* **10**:89–109.

De Taboada L, Yu J, El-Amouri S, Gattoni-Celli S, Richieri S, McCarthy T, Streeter J, and Kindy MS (2011) Transcranial laser therapy attenuates amyloid-β peptide neuropathology in amyloid-β protein precursor transgenic mice. *J Alzheimers Dis* **23**:521–535.

Doody RS, Gavrilova SI, Sano M, Thomas RG, Aisen PS, Bachurin SO, Seely L, Hung D, and dimebon investigators (2008) Effect of dimebon on cognition, activities of daily living, behaviour, and global function in patients with mild-to-moderate Alzheimer's disease: a randomised, double-blind, placebo-controlled study. *Lancet* **372**:207–215.

Durazo SA and Kompella UB (2012) Functionalized nanosystems for targeted mitochondrial delivery. *Mitochondrion* **12**:190–201.

Durieux J, Wolff S, and Dillin A (2011) The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell* **144**:79–91.

Edinger AL and Thompson CB (2004) Death by design: apoptosis, necrosis and autophagy. *Curr Opin Cell Biol* **16**:663–669.

Eichner LJ and Giguère V (2011) Estrogen related receptors (ERRs): a new dawn in transcriptional control of mitochondrial gene networks. *Mitochondrion* **11**:544–552.

Ferrari LF, Chum A, Bogen O, Reichling DB, and Levine JD (2011) Role of Drp1, a key mitochondrial fission protein, in neuropathic pain. *J Neurosci* **31**:11404–11410.

Fink BD, Herlein JA, Yorek MA, Fenner AM, Kerns RJ, and Sivitz WI (2012) Bioenergetic effects of mitochondrial-targeted coenzyme Q analogs in endothelial cells. *J Pharmacol Ex Ther* **342**:709–719.

Finsterer J (2010) Treatment of mitochondrial disorders. *Eur J Paediatr Neurol* **14**:29–44.

Fulda S, Galluzzi L, and Kroemer G (2010) Targeting mitochondria for cancer therapy. *Nat Rev Drug Discov* **9**:447–464.

Geser F, Martinez-Lage M, Kwong LK, Lee VM, and Trojanowski JQ (2009) Amyotrophic lateral sclerosis, frontotemporal dementia and beyond: the TDP-43 diseases. *J Neurol* **256**:1205–1214.

Gilliam LAA and Neufer PD (2012) Transgenic mouse models resistant to diet-induced metabolic disease: is energy balance the key? *J Pharmacol Exp Ther* **342**:631–636.

Giorgi C, Agnoletto C, Bononi A, Bonora M, De Marchi E, Marchi S, Missiroli S, Patergnani S, Poletti F, Rimessi A, et al., (2012) Mitochondrial calcium homeostasis as potential target for mitochondrial medicine. *Mitochondrion* **12**:77–85.

Haas RH, Parikh S, Falk, MJ, Saneto RP, Wolff NI, Darin N, Wong LJ, Cohen BH, and Naviaux RK (2008) The in-depth evaluation of suspected mitochondrial disease. *Mol Genet Metab* **94**:16–37.

Halestrap AP (2009) What is the mitochondrial permeability transition pore? *J Mol Cell Cardiol* **46**:821–831.

Halliwell B (2011) Free radicals and antioxidants–*quo vadis*? *Trends Pharmacol Sci* **32**:125–130.

Huang Q and Figueiredo-Pereira ME (2010) Ubiquitin/proteasome pathway impairment in neurodegeneration: therapeutic implications. *Apoptosis* **15**:1292–1311.

Hüttemann M, Pecina P, Rainbolt M, Sanderson TH, Kagan VE, Samavati L, Doan JW, and Lee I (2011) The multiple functions of cytochrome c and their regulation in life and death decisions of the mammalian cell: From respiration to apoptosis. *Mitochondrion* **11**:369–381.

Javadov S, Hunter JC, Barreto-Torres G, and Parodi-Rullan R (2011) Targeting the mitochondrial permeability transition: cardiac ischemia-reperfusion versus carcinogenesis. *Cell Physiol Biochem* **27**:179–190.

Johri A and Beal MF (2012) Mitochondrial dysfunction in neurodegenerative diseases. *J Pharmacol Exp Ther* **342**:619–630.

Jones S, Holm T, Mäger I, Langel U, and Howl J (2010) Characterization of bioactive cell penetrating peptides from human cytochrome c: protein mimicry and the development of a novel apoptogenic agent. *Chem Biol* **17**:735–744.

Kerr DS (2010) Treatment of mitochondrial electron transport chain disorders: a review of clinical trials over the past decade. *Mol Genet Metab* **99**:246–255.

Kitsis RN and Molkentin JD (2010) Apoptotic cell death "Nixed" by an ER mitochondrial necrotic pathway. *Proc Natl Acad Sci U S A* **107**:9031–9032.

Koopman WJ, Willems PH, and Smeitink JA (2012) Monogenic mitochondrial disorders. *N Engl J Med* **366**:1132–1141.

Kroemer G, Galluzzi L, Vandenabeele P, Abrams J, Alnemri ES, Baehrecke EH, Blagosklonny MV, El-Deiry WS, Golstein P, Green DR, et al. (2009) Classification of cell death: recommendations of the Nomenclature Committee on Cell Death 2009. *Cell Death Differ* **16**:3–11.

Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, et al. (2005) Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* **309**:481–484.

Kumar S, Kostin S, Flacke JP, Reusch HP, and Ladilov Y (2009) Soluble adenylyl cyclase controls mitochondria-dependent apoptosis in coronary endothelial cells. *J Biol Chem* **284**:14760–14768.

Kunji ER and Crichton PG (2010) Mitochondrial carriers function as monomers. *Biochim Biophys Acta* **1797**:817–831.

Lagouge M, Argmann C, Gerhart-Hines Z, Meziane H, Lerin C, Daussin F, Messadeq N, Milne J, Lambert P, Elliott P, et al. (2006) Resveratrol improves mitochondrial function and protects against metabolic disease by activating SIRT1 and PGC-1α. *Cell* **127**:1109–1122.

Lapointe J and Hekimi S (2010) When a theory of aging ages badly. *Cell Mol Life Sci* **67**:1–8.

Lax NZ, Turnbull DM, and Reeve AK (2011) Mitochondrial mutations: newly discovered players in neuronal degeneration. *Neuroscientist* **17**:645–658.

Lee JY, Nagano Y, Taylor JP, Lim KL, and Yao TP (2010) Disease-causing mutations in parkin impair mitochondrial ubiquitination, aggregation, and HDAC6-dependent mitophagy. *J Cell Biol* **189**:671–679.

Legros F, Malka F, Frachon P, Lombès A, and Rojo M (2004) Organization and dynamics of human mitochondrial DNA. *J Cell Sci* **117**:2653–2662.

Lustbader JW, Cirilli M, Lin C, Xu HW, Takuma K, Wang N, Caspersen C, Chen X, Pollak S, Chaney M, et al. (2004) ABAD directly links Abeta to mitochondrial toxicity in Alzheimer's disease. *Science* **304**:448–452.

Maldonado EN and LeMasters JL (2012) Warburg revisited: regulation of mitochondrial metabolism by voltage-dependent anion channels in cancer cells. *J Pharmacol Exp Ther* **342**:637–641.

Martin LJ, Adams NA, Pan Y, Price A, and Wong M (2011) The mitochondrial permeability transition pore regulates nitric oxide-mediated apoptosis of neurons induced by target deprivation. *J Neurosci* **31**:359–370.

McBride HM, Neuspiel M, and Wasiak S (2006) Mitochondria: more than just a powerhouse. *Curr Biol* **16**:R551–R560.

Mochel F and Haller RG (2011) Energy deficit in Huntington's disease: why it matters. *J Clin Invest* **121**:493–499.

Moreira PI, Carvalho C, Zhu X, Smith MA, and Perry G (2010) Mitochondrial dysfunction is a trigger of Alzheimer's disease pathophysiology. *Biochim Biophys Acta* **1802**:2–10.

Moreira PI, Santos MS, Seiça R, and Oliveira CR (2007) Brain mitochondrial dysfunction as a link between Alzheimer's disease and diabetes. *J Neurol Sci* **257**:206–214.

Mullane K and Williams M (2012) Translational semantics and infrastructure: another search for the emperor's new clothes? *Drug Discov Today* **17**:459–468.

Naviaux RK (2012) Oxidative shielding or oxidative stress? *J Pharmacol Exp Ther* **342**:608–618.

Ong SB and Hausenloy DJ (2010) Mitochondrial morphology and cardiovascular disease. *Cardiovasc Res* **88**:16–29.

Pamplona R (2011) Mitochondrial DNA damage and animal longevity: insights from comparative studies. *J Aging Res* **2011**:807108.

Papadopoulos V, Baraldi M, Guilarte TR, Knudsen TB, Lacapère JJ, Lindemann P, Norenberg MD, Nutt D, Weizman A, Zhang MR, et al. (2006) Translocator protein (18kDa): new nomenclature for the peripheral-type benzodiazepine receptor based on its structure and molecular function. *Trends Pharmacol Sci* **27**:402–409.

Pereira CV, Lebiedzinska M, Wieckowski MR, and Oliveira PJ (2012) Regulation and protection of mitochondrial physiology by sirtuins. *Mitochondrion* **12**:66–76.

Poljsak B (2011) Strategies for reducing or preventing the generation of oxidative stress. *Oxid Med Cell Longev* **2011**:194586.

Reddy PH (2009) The role of mitochondria in neurodegenerative diseases: mitochondria as a therapeutic target in Alzheimer's disease. *CNS Spectr* **14**:8–13; discussion 16–18.

Reyes NA, Fisher JK, Austgen K, VandenBerg S, Huang EJ, and Oakes SA (2010) Blocking the mitochondrial apoptotic pathway preserves motor neuron viability and function in a mouse model of amyotrophic lateral sclerosis. *J Clin Invest* **120**:3673–3679.

Rodriguez-Enriquez S, He L, and Lemasters JJ (2004) Role of mitochondrial permeability transition pores in mitochondrial autophagy. *Int J Biochem Cell Biol* **36**:2463–2472.

Roestenberg P, Manjeri GR, Valsecchi F, Smeitink JA, Willems PH, and Koopman WJ (2012) Pharmacological targeting of mitochondrial complex I deficiency: the cellular level and beyond. *Mitochondrion* **12**:57–65.

Rossignol DA and Frye RE (2012) A review of research trends in physiological abnormalities in autism spectrum disorders: immune dysregulation, inflammation, oxidative stress, mitochondrial dysfunction and environmental toxicant exposures. *Mol Psychiatry* **17**:389–401.

Sabbagh MN and Berk C (2010) Latrepirdine for Alzheimer's disease: trials and tribulations. *Future Neurol* **5**:645–665.

Sahin E, Colla S, Liesa M, Moslehi J, Müller FL, Guo M, Cooper M, Kotton D, Fabian AJ, Walkey C, et al. (2011) Telomere dysfunction induces metabolic and mitochondrial compromise. *Nature* **470**:359–365.

Schapira AH (2006) Mitochondrial disease. *Lancet* **368**:70–82.

Schatz G (2007) The magic garden. *Annu Rev Biochem* **76**:673–678.

Schinzel AC, Takeuchi O, Huang Z, Fisher JK, Zhou Z, Rubens J, Hetz C, Danial NN, Moskowitz MA, and Korsmeyer SJ (2005) Cyclophilin D is a component of mitochondrial permeability transition and mediates neuronal cell death after focal cerebral ischemia. *Proc Natl Acad Sci U S A* **102**:12005–12010.

Schon EA, DiMauro S, Hirano M, and Gilkerson RW (2010) Therapeutic prospects for mitochondrial disease. *Trends Mol Med* **16**:268–276.

Sheridan C and Martin SJ (2010) Mitochondrial fission/fusion dynamics and apoptosis. *Mitochondrion* **10**:640–648.

Shoshan-Barmatz V and Ben-Hail D (2012) VDAC, a multi-functional mitochondrial protein as a pharmacological target. *Mitochondrion* **12**:24–34.

Song W, Chen J, Petrilli A, Liot G, Klinglmayr E, Zhou Y, Poquiz P, Tjong J, Pouladi MA, Hayden MR, et al. (2011) Mutant huntingtin binds the mitochondrial fission GTPase dynamin-related protein-1 and increases its enzymatic activity. *Nat Med* **17**:377–382.

Stacpoole PW (2011) Why are there no proven therapies for genetic mitochondrial diseases? *Mitochondrion* **11**:679–685.

Sun L, Wang H, Wang Z, He S, Chen S, Liao D, Wang L, Yan J, Liu W, Lei X, et al. (2012) Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. *Cell* **148**:213–227.

Suzuki Y, Inoue T, and Ra C (2010) NSAIDs, mitochondria and calcium signaling: special focus on aspirin/salicylates. *Pharmaceuticals* **3**:1594–1613.

Swerdlow RH and Khan SM (2009) The Alzheimer's disease mitochondrial cascade hypothesis: an update. *Exp Neurol* **218**:308–315.

Szendroedi J, Phielix E, and Roden M (2012) The role of mitochondria in insulin resistance and type 2 diabetes mellitus. *Nat Rev Endocrinol* **8**:92–103.

Szewczyk A and Wojtczak L (2002) Mitochondria as a pharmacological target. *Pharmacol Rev* **54**:101–127.

Tillement L, Lecanu L, and Papadopoulos V (2011) Alzheimer's disease: effects of β-amyloid on mitochondria. *Mitochondrion* **11**:13–21.

Varanyuwatana P and Halestrap AP (2012) The roles of phosphate and the phosphate carrier in the mitochondrial permeability transition pore. *Mitochondrion* **12**:120–125.

Wallace DC (1999) Mitochondrial diseases in man and mouse. *Science* **283**:1482–1488.

Wallace DC (2005) A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* **39**:359–407.

Wallace DC and Fan W (2010) Energetics, epigenetics, mitochondrial genetics. *Mitochondrion* **10**:12–31.

Wang C and Youle RJ (2009) The role of mitochondria in apoptosis*. *Annu Rev Genet* **43**:95–118.

Wang Z, Jiang H, Chen S, Du F, and Wang X (2012) The mitochondrial phosphatase PGAM5 functions at the convergence point of multiple necrotic death pathways. *Cell* **148**:228–243.

Williams M and Coyle JT (2012) Historical perspectives on the discovery and development of drugs to treat neurological disorders, in *Translational Neuroscience Applications in Psychiatry, Neurology and Neurodevelopmental Disorders* (Barrett JE, Coyle JT and Williams M eds) pp 129–148, Cambridge University Press, Cambridge, UK.

Witte ME, Geurts JJ, de Vries HE, van der Valk P, and van Horssen J (2010) Mitochondrial dysfunction: a potential link between neuroinflammation and neurodegeneration? *Mitochondrion* **10**:411–418.

Yao J, Du H, Yan S, Fang F, Wang C, Lue LF, Guo L, Chen D, Stern DM, Gunn Moore FJ, et al.. (2011) Inhibition of amyloid-β (Aβ) peptide-binding alcohol dehydrogenase-Aβ interaction reduces Aβ accumulation and improves mitochondrial function in a mouse model of Alzheimer's disease. *J Neurosci* **31**:2313–2320.

Youle RJ and Narendra DP (2011) Mechanisms of mitophagy. *Nat Rev Mol Cell Biol* **12**:9–14.

Zamzami N, Larochette N, and Kroemer G (2005) Mitochondrial permeability transition in apoptosis and necrosis. *Cell Death Differ* **12**:1478–1480.

Zhang S, Hedskog L, Petersen CA, Winblad B, and Ankarcrona M (2010) Dimebon (latrepirdine) enhances mitochondrial function and protects neuronal cells from death. *J Alzheimers Dis* **21**:389–402.

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