Mitochondrial Function and Dysfunction – An Update

Robert E. Davis and Michael Williams

3-D Pharmaceutical Consultants, La Jolla, CA (RED)

and

Department of Pharmacology and Physiology, Drexel University College of Medicine,

Philadelphia, PA (MW)
Running Title Page

*Correspondence

Michael Williams

Phone: 847 234 1079

email: rivoli1635@comcast.net

Running title – Mitochondrial Function and Dysfunction

Keywords – Mitochondria, cell death pathways, apoptosis, necrosis, drug discovery

Document statistics

Text Pages - 16

Tables – None

Figures – 2

References - 93

Abstract – 78 words

Introduction – 669 words

Discussion – 119 words

The authors declare no conflicts of interest.
Non-standard Abbreviations

\( \Delta \Psi \text{m} \) - mitochondrial membrane potential

AIF - apoptosis inducing factor

ANT - adenine nucleotide translocator

ApaF - apoptosis protease activating factor

Akt/PKB – Akt/Protein Kinase B

Bax - pro-apoptotic Bcl-2-associated X protein

Bak - Bcl-2 homologous antagonist killer

BcL - B-cell lymphoma protein

BH3 – pro-apoptotic Bcl-2 family members

CypD - cyclophilin-D

DRP1 - dynamin-related protein 1

Endo G - endonuclease G

ERR - estrogen-related receptor

ETC - electron transport chain

FA - Freiderich’s ataxia

FADD - Fas-associated death domain protein

FasL - Fas ligand type-II transmembrane protein

FLLIP - FLICE-like inhibitory protein

G6P – glucose-6-phosphate
HK – hexokinase

IAP - inhibitor of apoptosis

IMM_ Inner mitochondrial membrane

KSS - Kearns-Sayre syndrome

LHON - Leber hereditary optic neuropathy

McL - Induced myeloid leukemia cell differentiation protein

MELSAS - mitochondrial encephalopathy lactic acidosis and strokes

MLKL - mixed lineage kinase-domain-like protein

MNGIE - mitochondrial neuro-gastrointestinal encephalomyopathy

MPTP - mitochondrial permeability transition pore

mtDNA - mitochondrial DNA

Nix/BNip3L - BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like

nDNA - nuclear DNA

OMM – outer mitochondrial membrane

Omi/HtrA2 - homotrimeric serine protease high temperature requirement A2

OXPHOS – oxidative phosphorylation

PARP - poly ADP ribose polymerase

PBZR – peripheral benzodiazepine receptor

PiC – mitochondrial phosphate carrier

PINK1 – PTEN-induced putative kinase protein 1
PGAM5S - Phosphoglycerate mutase/protein phosphatase 5, short form

PGC-1α - peroxisome proliferator-activated receptor-γ coactivator 1α

PKA – protein kinase A

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 pl

SOD - superoxide dismutase

STAT-3 - Signal transducer and activator of transcription 3

TCA - tricarboxylic acid cycle

TNFR - tumor necrosis factor receptor

TOR - Target of Rapomycin

TRAF2 - TNF receptor-associated factor 2

TSPO – 18 kDa Translocator Protein

VDAC - voltage-dependent anion channel

XIAP - X-linked inhibitor of apoptosis
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, cardiovascular, metabolic and CNS diseases, the latter including autism and neurodegenerative diseases. The present Perspective provides an update in understanding the central role of the mitochondrion in ATP and reactive oxygen species (ROS) 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 – 50 kg each day, and in 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; Huang and Figueiredo-Pereira, 2009; Kroemer et al., 2009; 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).

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 while 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, e.g., glaucoma, inflammation, neurodegenerative diseases, type 2 diabetes, cancers especially those involving prostate and colon, cardiomyopathies and dysrhythmias, to the less well known, e.g. Freiderich’s ataxia (FA), to a group of relatively obscure disease states that are typically described by acronyms, e.g., KSS (Kearns-Sayre syndrome), LHON (Leber hereditary optic neuropathy), MELSAS (mitochondrial encephalopathy lactic acidosis and strokes), MERRF (myoclonic epilepsy with ragged red fibers) and MNGIE (mitochondrial neuro-gastrointestinal encephalomyopathy) (Haas et al., 2008).

These various disease states have been associated in some or all of their manifestations with mutations in in both mitochondrial (mtDNA) and nuclear (nDNA) DNA that result in defects in mitochondrial function (Wallace, 1999; Schapira, 2006; Copeland, 2008;
Finisterer, 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 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 b-amyloid 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 an increased interest in targeting mitochondrial processes and proteins for drug discovery efforts in cancer (Fulda et al., 2010; Maldonado and LeMasters, 2012), cardiovascular (Ballinger, 2005; Akar and O’Rourke, 2011; Ong and Hausenloy, 2011), metabolic (Szendroedi et al., 2011; Gilliam and Neufer, 2012) and CNS disease states, the latter including autism (Rossignol and Frye, 2012) and neurodegenerative diseases including Alzheimer’s (AD), Parkinson’s (PD) and Huntington’s (HD), multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS) and pain (Moreira et al., 2010; Reyes et al., 2010; Witte et al., 2010; Lax et al., 2011; Ferrari et al., 2011; Johri and Beal, 2012). Progress in these efforts from a traditional small
molecule perspective has however, been challenging (Kerr, 2010; Finsterer, 2010; Stacpoole, 2011).

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 prior to 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 2-10 mtDNA copies that consists 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). While 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 (Wallace and Fan, 2010; Schon et al., 2010). Complex I comprises 45 peptide subunits, 7 originating from mtDNA with the remainder from nDNA. Complex II has 4 subunits, all of which are derived from nDNA, Complex III has 11 subunits,
only one of which originates from mtDNA. Complex IV has 12 subunits, 3 of which are derived from mtDNA while Complex IV has approximately 16 subunits, 2 of which are from mtDNA.

Mutation rates in mtDNA are generally 2-3 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 (Mitomap, 2011) that are thought to affect mitochondrial protein synthesis, protein-encoding genes and mRNA and ultimately mitochondrial function. These are complimented 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 have 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/PKB signaling, calcium homeostasis, estrogen-related receptor (ERR) signaling, heat shock proteins, soluble adenylyl cyclase, RIP3 kinase, TOR kinases, PGC-1α, STAT-3, AMPK (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 like huntingtin in HD, amyloid (Aβ) in AD, superoxide dismutase 1 (SOD1) 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 non-coding 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 genome-wide 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. Interestingly, more than a decade ago, Wallace (1999) had noted that a specific mtDNA mutation could produce very different human disease phenotypes while different mutations could result in the same phenotype. This insight is not limited to the mitochondrial genome and appears equally applicable to the total cellular genome, a conclusion that is supported by the identification of multiple, and often conceptually puzzling, gene candidates/associations for disease states like 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 ($\Delta \Psi_m$) 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 FAD to the quinone pool (Q) and transferred via
flavin adenine dinucleotide (FAD) to Q. In Complex III (Ubiquinol-cytochrome-c reductase), six electrons are removed from QH₂ two of which are sequentially transferred to two molecules of cytochrome c (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 (O₂) 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 Fₒ/F₁ ATP synthase complex to produce ATP via OXPHOS. The mitochondrial membrane potential ΔΨₘ, is normally in the range of 80-140 mV. The optimal ΔΨₘ potential for ATP production is 100-120 mV with ΔΨₘ values greater than 140 mV leading to increased ROS production at the expense of ATP generation (Hutteman 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 as it effects the repair of dysfunctional and damaged mitochondria in addition to intermixing DNA and proteins between mitochondria (Chen, 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 into two and 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 DRP1 (dynamin-related protein 1) leading to fragmented mitochondria that are fewer in number (Song et al., 2011). The mutant form of huntin (mHtt), a protein associated with HD enhances DRP-1 activity. While fission appears 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 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 weights below 1.5 kDa (Baines, 2010; Zamzami et al., 2005). The mitochondrial permeability transition (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: a) necrosis is more important in cell death than apoptosis; b) that necrosis is an alternative death pathway to to apoptosis when caspases are inhibited and c) that necrosis is engaged as a cell death pathway when mitochondria form a complex with the endoplasmic reticulum (Baines, 2010). Until recently, necrosis was thought to be a random, uncontrolled process (Kitsis 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 while 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 apoptosome formation can inhibit necosome
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). While 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 relegateing ANT to a
modulatory role as 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 pro-apoptotic while ANT-2 is anti-apoptotic (Fulda et al., 2010).

A variety of other proteins have been associated with the MPTP including the anti-
and pro-apoptotic proteins, Bcl-2 and Bax, hexokinase, the mitochondrial phosphate carrier
(PiC; Varanyuwatana and Halestrop, 2012), the peripheral benzodiazepine receptor (PBZR)
also known as the 18 kDa Translocator Protein (TSPO; Papadopoulos et al., 2006) and
Complex I of the ETC (Roestenberg et al., 2012). PiC can form complexes with ANT
(Halestrup, 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 as 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., 2011) and
neurodegenerative disease states (Martin et al., 2011) while 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 inner mitochondrial membrane (IMM) occurs as the key event in both necrotic and apoptotic signaling pathways via the “BH3-only-like” protein, Nix/BNip3L 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, NO, toxins, etc.) increase MPTP formation and function and result in the leakage of multiple soluble apoptogenic/pro-apoptotic proteins (Fig 2). The release of these proteins can then engage a diversity of downstream signaling pathways 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 Cyt C, bcl-2, Smac/DIABLO (second mitochondria-derived activator of caspases/direct 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 that 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 Cyt C, like many of the other proteins in the cell death pathways is dependent 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 (Hutteman 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, in order to maintain ∆Ψm at potentials below those leading to free radical formation (Hutteman et al., 2011). Smac/DIABLO and Omi/HtrA2 are antagonists of the protein inhibitors of apoptosis (IAPs) that promote caspase activation. Mitochondrial membrane permeabilization can also occur independently of pore formation and involves Bcl-2 family members that include both pro- (Bax, Bak, Bok) and anti-apoptotic (Bcl-2, 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 (DR) 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 pro-apoptotic regulator. Soluble adenylyl cyclase (sAC), is a pro-apoptotic 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 and 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, ROS production and PARP-1/calpain activation (Fig. 2). The receptor interacting protein kinases (RIPKs) together with TRAF2 (TNF receptor-associated factor 2) 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 that 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, HDAC6 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 include KSS, MELSAS and MERR 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 co-factor deficiencies, e.g., CoQ10, that can lead to decreased ATP production and increased free radical production, the latter potentially leading to neurodegenerative diseases (AD, PD, HD, ALS; Johri and Beal, 2012). LHON, which is associated with visual failure due to 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 (Scjapira, 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, argyrophilic grain disease), α-synucleopathies (PD, dementia with Lewy bodies, multiple system atrophy and some instances of AD), and the TDP-43 proteinopathies/ubiquinopathies (ALS, frontotemporal dementias, argyrophilic grain disease (Geser et al.,2010). In PD, defects in Complex I activity involve mtDNA mutations, alterations in mitochondrial kinase signaling (e.g., PTEN-induced kinase I, Akt/PKB, JNK, ERK; Dagda et al., 2009) and can be caused by the effects of environmental toxins (e.g. rotenone) that lead to increased free radical production and reduced activity in Complex IV. In PD, dysregulation of the ubiquitin-proteasomal system (UPS) 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 oxidative phosphorylation (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 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 NO production, ROS-induced oxidative stress and tau toxicity and cytokine production and inflammation (Moriera 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 autophagocytosis is increased in AD and may reflect differential roles for autophagy depending on the stage of the disease (Moriera 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, decreased mitochondrial plasticity and numbers in skeletal muscle and liver resulting in insulin resistance (Szendroedi et al., 2011). Mitochondrial dysfunction appears to be a key link between AD and diabetes (Moriera 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, CoQ10 and its analogs, the TPP, MitoQ and the SKQs, the SS-peptide, SS-31, CGP 37157, riboflavin, trolox, thiamine, creatine, pyruvate, the pyruvate analog, dichloracetate, succinate, folate, omega-3 fatty acids, e.g., 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 like lactate 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 (Poljsak, 2011; Halliwell, 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 primarily focused on small molecules, including peptides, that can modulate MPTP formation and function (Kerr, 2010; Finsterer,
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, the avicin class of plant stress metabolites, the antidepressant fluoxetine, cisplatin and endostatin (Shosan-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 while 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 (CPPs). The latter contain epitopes that act as “vectors for the highly efficient delivery of bioactive cargoes into the intracellular milieu” (Jones et al., 2010). CPPs in human CytC, specifically CytC\textsuperscript{77-101} and CytC\textsuperscript{86-101}, can mimic the apoptogenic effects of CytC to induce tumor cell apoptosis. Nup153-CytC, a chimeric N-terminal extension of CytC\textsuperscript{77-101} with a target mimetic of FG nucleoporin, enhanced the apoptogenic potency of the parent compound \( \text{LD}_{50} \text{CytC}^{77-101} = 80.6 \mu M; \text{LD}_{50} \text{Nup153-CytC} = 730 \text{nM} \) by facilitating redistribution of nuclear pore complex proteins and targeting IP\textsubscript{3} receptors on the endoplasmic reticulum involved in calcium homeostasis to amplify apoptotic signaling events (Jones et al., 2010). Other mitochondrially-targeted anti-cancer NCEs that are focused on enhancing apoptosis include modulators of BCL-2 family function (ABT-737, AT-101), metabolic inhibitors (dichloracetate, orlistat), ANT/VDAC ligands (lonidamine, CD437, PK 11195, arsenite trioxide, clodronate), ROS regulators (ATN-224, STA-4783), Hsp-90 inhibitors (PI-H71, PEITCs) and \( F_{1}-ATPase \) inhibition (resveratrol; Fulda et al., 2010). The sirtuin resveratrol, an NAD\textsuperscript{+}-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 (peroxisome proliferator-activated receptor-γ coactivator 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).

Non-steroidal anti-inflammatory drugs (NSAIDs), e.g., aspirin and indomethacin, in addition to their ability to inhibit the cyclo-oxygenase (COX) enzymes responsible for prostaglandin production affect mitochondrial function by uncoupling OXPHOS, decreasing ATP production and inducing MPTP formation and apoptosis. While 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 (CsA) and sangliferin A (SfA) block MPTP function by binding to CyP-D, an effect independent of their immunosuppressant actions. CsA 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 (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 is also being developed for use in the treatment of spinal muscular atrophy (SMA). Dexpramipexole (KNS-760704), the “inactive” isomer of the dopamine agonist, pramipexole, that 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 antihistamine 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 key role for the MPTP in AD etiology remains to be determined.

Non-invasive 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 can 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, the treatment of 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, proven 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 DCA, 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 these did not represent the diversity of potential therapeutics. Additionally, 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 while 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 complimentary targets, have the potential to treat a myriad of human disease states for which there are currently no effective treatments.

Wrote the manuscript: Davis, Williams
References


http://www.mitomap.org/bin/view.pl/MITOMAP/MutationsCodingControl


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,


Figure Legends

Figure 1: Schematic of the mitochondrion showing the mitochondrial permeability transition pore and the respiratory chain.

See text for details.

Abbreviations: ANT - adenine nucleotide translocator; CypD - cyclophilin-D; G6P – glucose-6-phosphate; HK – hexokinase; MPTP - mitochondrial permeability transition pore; TCA - tricarboxylic acid cycle; VDAC - voltage-dependent anion channel

Figure 2: Mitochondrial cell death pathways – necrosis and apoptosis.

See text for discussion of extrinsic and intrinsic necrotic and apoptotic pathway activities.

Abbreviations:

ΔΨm- mitochondrial membrane potential; AIF - apoptosis inducing factor; Apaf - apoptosis protease activating factor; Bax - pro-apoptotic Bcl-2–associated X protein; Bak - Bcl-2 homologous antagonist killer; BclL- B-cell lymphoma protein; BH3 – pro-apoptotic Bcl-2 family members; DRP1 - dynamin-related protein 1; Endo G - endonuclease G; FADD - Fas-associated death domain protein; FasL - Fas ligand type-II transmembrane protein; FLLIP - FLICE-like inhibitory protein; IAP - inhibitor of apoptosis; McL - Induced myeloid leukemia cell differentiation protein; MLKL - mixed lineage kinase-domain-like protein; MPTP - mitochondrial permeability transition pore; Nix/BNip3L - BCL2/adenovirus E1B 19 kDa protein-interacting protein 3-like; OMM – outer mitochondrial membrane; Omi/HtrA2 - homotrimERIC serINE protease high temperature requirement A2; PARP - poly ADP ribose polymerase; PGAM5S - Phosphoglycerate mutase/protein phosphatase 5, short form; PKA – protein kinase A; 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; TNFR - tumor necrosis factor receptor; TRAF2 - TNF receptor-associated factor 2)
Figure 1: Schematic of the mitochondrion showing the mitochondrial permeability transition pore and the respiratory chain.
Figure 2: Mitochondrial cell death pathways – necrosis and apoptosis.