Published in final edited form as:
Drug Discov Today Ther Strateg. 2013 ; 10(2): e91–e98. doi:10.1016/j.ddstr.2014.04.002.

From a Cell's Viewpoint: Targeting Mitochondria in Alzheimer's disease

Emily Ann Carlson, **Valasani Koteswara Rao**, and **Shirley ShiDu Yan***
Department of Pharmacology & Toxicology and Higuchi Biosciences Center, School of Pharmacy, University of Kansas, Lawrence, Kansas 66047, USA

Abstract

Mitochondria are well-known cellular organelles widely studied in relation to a variety of disease states, including Alzheimer's disease. With roles in several metabolic processes, numerous signal transduction pathways, and overall cell maintenance and survival, mitochondria are essential to understanding the inner workings of cells. As mitochondria are able to be utilized by diverse illnesses to increase the likelihood of disease progression, targeting specific processes in these organelles could provide beneficial therapeutic options.

Introduction

Mitochondria are double-membrane-bound organelles composed of an outer membrane (OM) and an inner membrane (IM), which is further constructed into compartments: the intermembrane space, the cristae interior, and the matrix. These organelles are central players in cellular function with roles in sustaining cell survival, maintaining energy metabolism, balancing reactive oxygen species (ROS), and mediating cell death pathways. Given their extensive role in cells, mitochondrial function and dysfunction has been examined in many diseases, including neurodegeneration. Studying the functions of mitochondria in diseased cells, in comparison with healthy cells, may elucidate the modified mechanisms and molecular components involved in specific disease states. Within this review, mitochondrial processes necessary for cell survival under normal conditions are discussed. Cell death pathways mediated by mitochondria are examined, with particular attention given to various components in view of their relevance in neurodegeneration, specifically Alzheimer's disease (AD). Finally, current and prospective treatment strategies will be reviewed.

*Correspondence should be addressed to Dr. Shirley ShiDu Yan, 2099 Constant Avenue, University of Kansas, Lawrence, KS 66047; Telephone: 785-864-3637; shidu@ku.edu.

Mitochondrial Roles in the Cell

Calcium Homeostasis

Mitochondria within the vicinity of cytosolic Ca^{2+} or organelle Ca^{2+} release sites rapidly take up ions through voltage dependent anion channels (VDACs) on the OM. Along the IM, Ca^{2+}-sensitive uniporter channels pass ions into the matrix. Once inside a mitochondrion, Ca^{2+} levels accelerate adenosine triphosphate (ATP) production by stimulating dehydrogenases in the tricarboxylic acid (TCA) cycle (Figure 1.1). Ca^{2+} extrusion from intramitochondrial stores occurs primarily through cation exchangers. Also, a nonselective mitochondrial Ca^{2+}-activated pore, termed the mitochondrial permeability transition pore (MPTP), takes in and extrudes Ca^{2+} ions during membrane permeability fluctuations. Although the MPTP is often connected with cell death elicited by Ca^{2+} overload and stress, Barsukova et al. demonstrated that it acts as a Ca^{2+} release channel under normal conditions [1] (Figure 1.7). Utilizing these influx and efflux mechanisms, mitochondria sustain Ca^{2+} levels within a narrow range for normal functioning of cellular processes.

Energy Metabolism

ATP manufacturing is dependent upon oxygen consumption, the TCA cycle, and the electron transport chain (ETC). Glucose enters glycolysis for conversion into pyruvate (Figure 1.2), which is subsequently fed into the TCA cycle (Figure 1.3). Enzymes of the TCA cycle are stimulated by mitochondrial Ca^{2+} to reduce nicotinamide adenine dinucleotide (NADH). Reduced NADH molecules are then targeted to the ETC to act as electron donors (Figure 1.4). Complex I accepts electrons from NADH, while complex II receives electrons from succinate. Electrons from complexes I and II transfer to complex III, and are incorporated into cytochrome c for delivery to complex IV. This last complex reduces oxygen to water and aids in ATP synthesis. Throughout the ETC process, protons are generated and used by ATP synthase (complex V) in the oxidative phosphorylation (OXPHOS) pathway to synthesize ATP from ADP and inorganic phosphate.

Oxidative Stress

While Ca^{2+} enhances ATP production via increasing ETC activity, more oxygen is reduced to water. This contributes to greater leakage of free electrons from the respiratory complexes, resulting in the formation of ROS. As a compensatory mechanism, Ca^{2+} also activates ROS scavenging enzymes, such as superoxide dismutase, to eradicate harmful oxidants. Healthy cells tightly regulate the balance between oxidants and antioxidants to prevent destructive consequences (Figure 1.5). However, deficiencies in ETC complex activities are associated with depleted energy stores and enhanced ROS production [2]. With low levels of ROS scavengers, harmful oxidants cause extensive damage. Healthy cells can prevent ROS-inflicted harm using uncoupling proteins (UCPs) (Figure 1.6), which block ROS formation and sequester Ca^{2+} in mitochondria.

Mitochondrial Permeability Transition Pore

The mitochondrial permeability transition is defined as an increase in IM permeability to molecules smaller than 1500 Daltons. In cells undergoing stress, this phenomenon is

initiated by opening of the MPTP, typically due to high levels of Ca^{2+} or ROS. MPTP induction can lead to loss of the transmembrane potential ($\Delta\Psi$), release of proapoptotic mediators, and cell death.

Cyclophilin D (CypD) has been identified as a modulatory component of the MPTP. Upon Ca^{2+} overload or oxidative stress in cells, CypD translocates from the matrix to the IM to initiate opening of the MPTP [3] (Figure 2.1 and 2.2). Along with CypD, two other channels have been strongly suggested as constituents of the MPTP. As a channel along the OM responsible for taking up ions into mitochondria, VDAC was a promising candidate. However, recent data suggests that VDAC is not a vital component of the MPTP structure [4]. Adenine nucleotide translocase (ANT) was another favorable contender, as it catalyzes the ATP/ADP exchange through the IM. Nevertheless, studies have shown that it is not essential for MPTP formation [5]. Thus, VDAC and ANT may be regulators of the MPTP, not physical units.

Mitochondrial-mediated Cell Death Pathways

Apoptosis can be initiated via two distinct pathways. The extrinsic route involves death receptors on the cell surface, whereas the intrinsic path originates in mitochondria (Figure 2.1). Induction of apoptosis signaling stimulates initiator caspases, which activate executioner caspases for cleavage of death substrates. In mitochondria, antiapoptotic Bcl-2 and Bcl-xL release tBid upon its activation by caspases. Proapoptotic Bax and Bak form a multidomain after stimulation from tBid, propelling the release of cytochrome c and other factors from the MPTP. The proapoptotic factors perpetuate the process, causing the organized collapse and shrinkage of the cell. Finally, the cell body is engulfed by nearby cells.

Necrosis can be launched in the event of cellular injury (Figure 2.2). Activation of death receptor adaptors leads to their translocation to the IM. This disrupts ANT-mediated ATP/ADP exchange, resulting in ATP depletion and ROS accumulation. Excessive levels of ROS and Ca^{2+} promote mitochondrial uncoupling and disruption of the $\Delta\Psi$ via MPTP opening. Imbalances activate degradative enzymes that propagate the damage until the plasma membrane ruptures, leaking the cellular contents.

Alzheimer’s disease and Mitochondria

As the sixth leading cause of death in the United States, Alzheimer’s disease (AD) is expected to become more prevalent as the world population ages [6]. Since there are few available treatments for AD, it is essential to continue investigating this devastating disease. The amyloid hypothesis has propelled much AD research over the last decade concerning mitochondrial dysfunction in neurons.

AD is characterized by the loss of neuronal synaptic density and synapse number, and collection of abnormally folded amyloid tau proteins and amyloid-beta (Aβ)-peptides in the brain. These atypical proteins give rise to two hallmarks of AD pathology: neurofibrillary tangles and amyloid plaques. Prior to plaque formation, Aβ-peptides selectively accumulated within mitochondria promotes metabolic dysfunction [7], implicating mitochondria in AD

pathogenesis. As the disease progresses, Aβ impairs patients' learning and memory [8]. Eventually, the accrual of Aβ is toxic to neuronal cells, ultimately leading to cell death. While the mechanism of Aβ toxicity remains to be clarified, it is thought to involve the overproduction of ROS, loss of Ca^{2+} homeostasis, and reduction in energy metabolism [9].

Disrupted Calcium Homeostasis in AD

Much evidence has indicated that exposure of Aβ causes an abnormal increase in intracellular Ca^{2+} levels in AD-affected neurons (Figure 3.1). Accumulation of large amounts of Ca^{2+} ions is well known to promote the induction of cell death. However, the mechanisms underlying Aβ-mediated disruption of calcium homeostasis are still largely unknown. It was reported that Aβ influences a variety of receptors that contain Ca^{2+} channels, resulting in Aβ-regulated intracellular Ca^{2+} concentrations during membrane depolarization and organelle release [10]. Moreover, Kawahara et al. recently proposed that Aβ-induced intracellular Ca^{2+} changes occur due to the formation of amyloid channels on neuronal cell membranes [11]. As Aβ-peptides have been shown to bind the membranes of wild type hippocampal neurons [12], and aggregation of Aβ leads to ion channel formation, the resulting unregulated channel would allow a continuous flow of Ca^{2+} ions into the cell, thus disrupting the cell's Ca^{2+} balance. While this concept shows promise, additional studies need to be performed in AD models to verify that amyloid channels occur within diseased neurons.

Reduced Energy Metabolism in AD

Alterations in intracellular Ca^{2+} concentrations can profoundly impact cellular function. If not properly managed, Ca^{2+} levels inside mitochondria become toxic and can activate a series of events, which lead to cell death. A common occurrence in AD is decreased activity of many TCA cycle enzymes, correlating with diminished ATP production (Figure 3.2). Interestingly, Aβ has been observed to bind to the α-subunit of ATP synthase in amyloid precursor protein/presenilin-1 (APP/PS1) transgenic mice, thereby negatively regulating ATP production [13]. Furthermore, decreases in glucose metabolism have been reported in AD patients, suggesting that both major metabolic processes are compromised in the disease. While several studies confirm that energy metabolism is reduced in AD, the reasoning for this is unclear. One explanation for this energy deficiency is as a result of the side effect of ROS damage, as AD mitochondria display impairments in the ETC complexes leading to elevated ROS generation. In contrast, Sun et al. propose that the reduction in energy metabolism seen in AD is a protective response against lower levels of nutrients and oxygen [14]. Hence, AD-affected neurons may modify energy metabolism in a final effort to regain a healthy condition.

Elevated Oxidative Stress in AD

Besides changes in TCA cycle enzymes, AD mitochondria also display impairments in the ETC complexes leading to elevated ROS generation (Figure 3.3). Expression and functional variations have been observed in all complexes of multiple AD mouse models, though the most severe defects caused by Aβ are typically seen in complex IV [15]. Additionally, increased oxidative stress promotes the overexpression of β-secretase in brain tissue of both AD patients and triple-transgenic AD mice [16]. β-secretase is an enzyme responsible for Aβ

production, and thus elevation in its expression resulted in excessive amounts of Aβ-peptide. This becomes a feed-forward loop as Aβ aggregate formation enhances the generation of mitochondrial ROS by damaging the ETC, leading to even higher levels of oxidative stress. As seen from the vast amounts of data, the oxidative stress hypothesis is another well-recognized theoretical mechanism for AD development. While the mechanisms underlying this concept are still under investigation, a conceivable method is through Aβ-induced mitochondrial dysfunction.

Modified MPTP Initiation in AD

Neuronal cell death in AD involves MPTP opening, chiefly due to Aβ toxicity. Modifications to MPTP constituents may have an impact on AD cells undergoing apoptosis. Indeed, interactions between CypD and Aβ-peptide have been visualized in diseased mitochondria, in which CypD-Aβ complexes promote organelle swelling and MPTP opening [17]. Our group postulates that in AD-affected mitochondria, Aβ-binding alcohol dehydrogenase (ABAD) disrupts CypD-Aβ interactions [18]. Once ABAD-Aβ complexes form, CypD is left uninhibited in the matrix. Due to Aβ-induced mitochondrial stress, CypD translocates to the IM to initiate MPTP-mediated cell death (Figure 3.4). This complex exchange of CypD for ABAD mediated by Aβ may hold promise as a therapeutic target, since ABAD is seen as a direct link between Aβ and mitochondrial toxicity in AD progression [19].

Likewise, ANT is thought to have a role in AD. Singh and colleagues propose that Aβ-peptides localize to the IM near ANT channels, as strong ANT-Aβ interactions were observed using the STITCH database [20]. This interaction could possibly drive mitochondrial dysfunction in AD through the dysregulation of ATP/ADP transport. Similarly, VDAC is thought to be involved in AD as it is overexpressed in the hippocampus of AD transgenic mouse models [21] and postmortem brain tissue from AD patients [22]. Overall, further studies are needed to explore the roles of CypD, VDAC, and ANT in AD as the reasons behind these changes in expression and function remain elusive.

Adjusted Cell Death in AD

Much evidence has been generated in support of apoptosis-mediated cell death in AD pathogenesis (Figure 3.5). Aβ deposits have been found concurrently with increased caspase activity, amplified DNA damage, and altered apoptotic gene expression. Additionally, tissue from AD patients exhibit TUNEL-positive cells with apoptotic-like morphology, implicating apoptosis in the disease. However, other studies saw few or none of the traditional morphological characteristics of apoptosis, suggesting another pathway is also involved.

Although necrosis is only just beginning to be examined in AD, data already advocates that it plays a role in the disease. For instance, brain sections from patients with familial AD display the standard morphological features of necrotic cell death. This is further supported by studies in mice containing mutated presenilin-1, a gene linked to early-onset of familial AD; the animals exhibited increased susceptibility to necrosis induction. Although information on necrosis in AD is scarce, the current data indicate that necrotic cell death is important in familial AD, and perhaps sporadic AD as well.

Treatment Strategies Targeting Mitochondria in AD

Since AD is largely dependent upon metabolic dysfunction, research targeting mitochondrial-driven events is being explored to slow disease advancement. A few drugs have been approved for AD treatment, including donepezil [23], rivastigmine [24], galantamine [25], and memantine [26]. While the current medications address the symptoms of AD, there is no evidence that they modify disease progression.

A group of insulin sensitizers, thiazolidinediones (TZDs), have been shown to increase glucose uptake, thereby adjusting cellular metabolism. Since glucose metabolism is generally reduced in AD, manipulating the process could be useful. Several TZDs are being investigated in relation to AD. Rosiglitazone prevents memory impairment in an AD mouse model [27] and exhibited promising results in Phase 2 studies. However, a recent Phase 3 clinical trial saw no beneficial effect of rosiglitazone treatment in AD patients [28]. Pioglitazone also reduces cognitive defects and decreases Aβ deposits in AD transgenic mice [29]. Some small clinical studies have been performed in AD patients to evaluate the safety of pioglitazone [30]. At present, pioglitazone shows great promise as a drug candidate as it is able to penetrate the blood-brain barrier, unlike rosiglitazone.

Targeting of organelles and specific cellular processes is a relatively new approach to AD therapy. As mitochondrial dysfunction occurs early in the development of AD, therapeutic targeting of this organelle and its functions could counteract the molecular components driving AD progression. Another TZD drug candidate that specifically targets mitochondria, mitoglitazone (MSDC-0160), has completed Phase 2 clinical trials in AD patients [31]. Additionally, dimebon (latrepirdine), a non-selective antihistamine, enhances mitochondrial function in human neuroblastoma cells, in particular mitochondrial membrane potential and ATP production [32]. As dimebon alone failed to meet primary and secondary endpoints in Phase 3 studies [33], it was recently assessed in combination with donepezil. However, co-treatment of dimebon with donepezil in Phase 3 AD clinical trials did not achieve substantial results for primary efficacy endpoints [34]. Taking the Aβ channel concept into consideration, two amphipathic pyridinium salts (MRS2481 and MRS2485) were found to provide protection in neuronal cells against Aβ neurotoxicity, essentially by blocking Aβ channel formation to regain Ca^{2+} homeostasis [35]. If the Aβ channel theory shows merit, animal studies using these salts will need to be performed to assess the applicability of the drugs in AD clinical trials.

MPTP induction in AD is also being examined, focusing particularly on the interactions of Aβ with CypD, ABAD, and perhaps ANT. Once CypD is released from Aβ complexes, it initiates MPTP opening. Ablation of CypD in AD transgenic mice results in enhanced mitochondrial function, and improved learning and memory [36, 37], suggesting that blocking CypD has potential for AD treatment. The addition of the pharmacological CypD inhibitor, cyclosporine A (CsA), to cultured neurons and isolated cortical mitochondria also attenuates Aβ-induced impairments in MPTP opening, and reduces mitochondrial and synaptic dysfunction [38]. Unfortunately, CsA lacks clinical significance in AD treatment because of its immunosuppressive effect of inhibiting calcineurin (a Ca^{2+}-dependent protein phosphatase), severe renal toxicity, and inability to pass the blood-brain barrier. Also,

inhibition of ABAD-Aβ interactions in transgenic APP/ABAD mice reduces Aβ buildup and improves mitochondrial function [39]. Given the promising data, our group designed several small molecule inhibitors of ABAD for further study in AD [40]. Lastly, since the relationship between ANT and Aβ is fairly new, further inquiry may elucidate its involvement in MPTP-mediated cell death in AD.

Conclusion

Neuronal cells affected by AD undergo changes due largely to Aβ toxicity, many of which impact mitochondrial-driven processes for preparation toward cell death. With effective treatment options limited for AD, research has shifted to target-specific and/or concurrent therapeutic regimens. Since mitochondrial dysfunction is highly implicated in AD progression, targeting specific mitochondrial processes could enhance the susceptibility of diseased cells to available drugs at all stages of AD. This fresh approach toward disease treatment will likely increase the quantity and quality of therapeutic options for AD, and potentially open new avenues for use in other disease states as well.

Acknowledgments

This work is supported by grants from the National Institute of Aging (R37AG037319), the National Institute of General Medical Science (R01GM095355), and the National Institute of Neurological Disorders and Stroke (R01NS65482).

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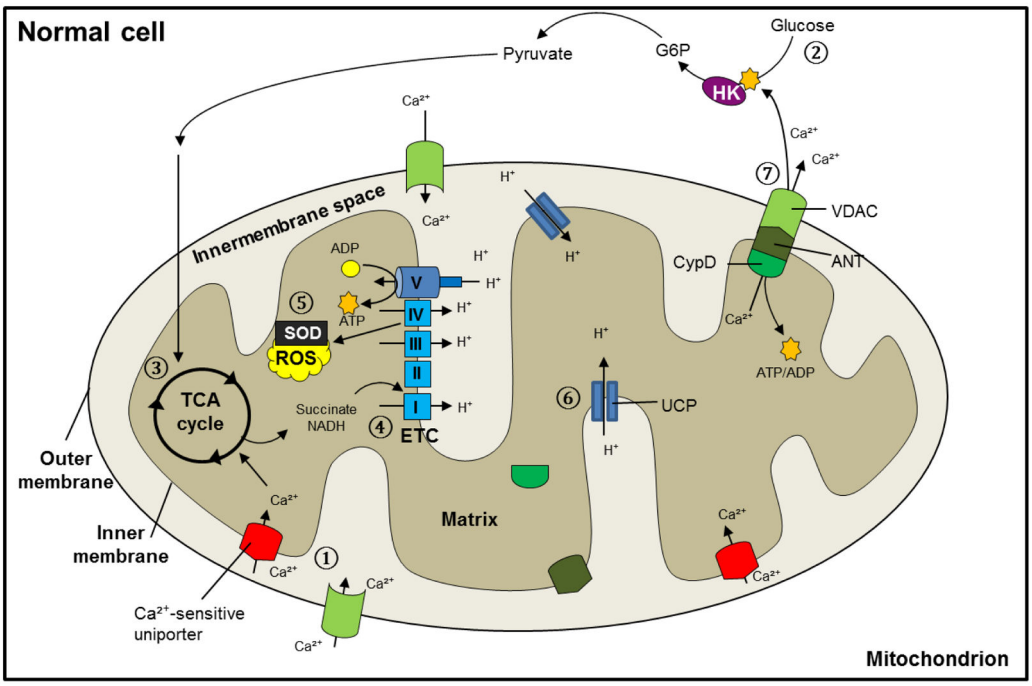

Figure 1. Mitochondrial function in normal cells

Under regular oxygen conditions, healthy cells primarily rely on mitochondrial OXPHOS for ATP production. **1)** Ca^{2+} ions are taken up into a mitochondrion through VDACs and Ca^{2+}-sensitive uniporter channels, and stimulate ATP generation. **2)** Glucose is converted into pyruvate during glycolysis which is **3)** imported into the mitochondrion for entry into the TCA cycle. **4)** Reduced succinate and NADH molecules are used by the ETC complexes to power ATP generation. **5)** ROS created along the ETC are balanced by antioxidants, such as superoxide dismutase (SOD), and **6)** UCP channels. **7)** Excess Ca^{2+} ions are expelled from the mitochondrion through the MPTP comprised of VDAC, ANT, and CypD.

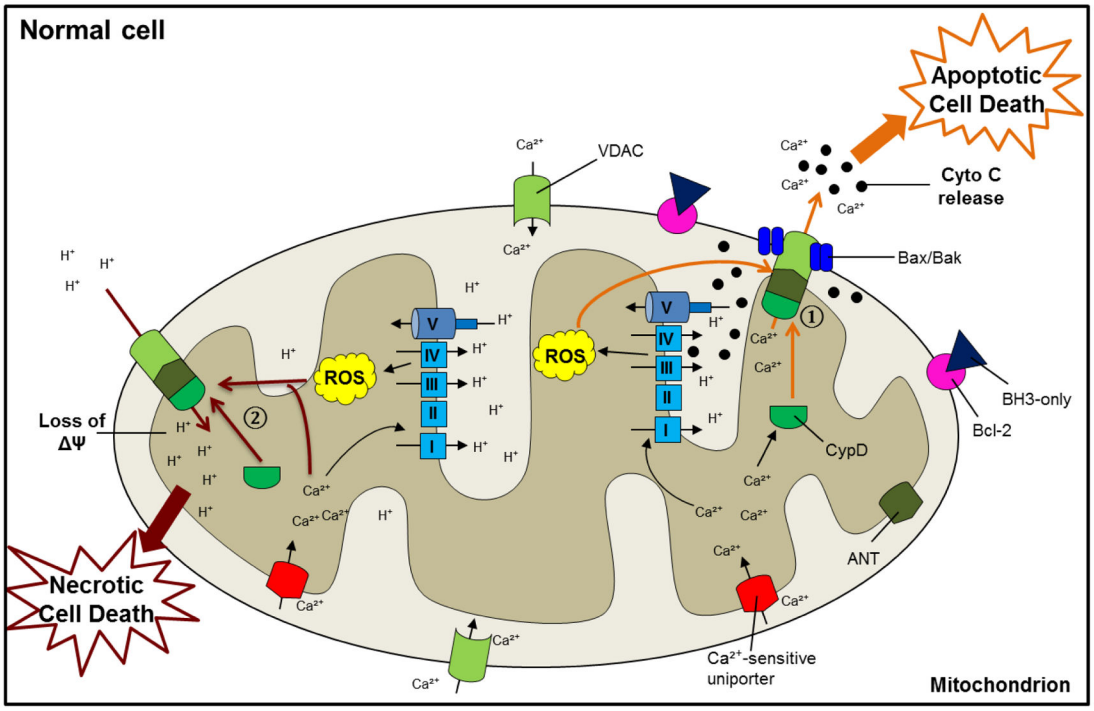

Figure 2. Mitochondrial-mediated cell death pathways in normal cells

In the event of cellular injury, a mitochondrion can signal cell death pathways for destruction of the whole cell. **1) Apoptosis** (orange pathway) is initiated due to excessive Ca^{2+} and ROS levels. Bcl-2 is inhibited by BH3-only proteins, allowing Bax and Bak to interact, which induces MPTP opening for Ca^{2+} and cyto c release into the cytosol. These proapoptotic factors further propel the process until the cell collapses in an organized manner. **2) Necrosis** (dark red pathway) results from ATP depletion and enhanced Ca^{2+} and ROS accrual. This leads to the loss of ΔΨ and eventual unplanned rupture of the cell.

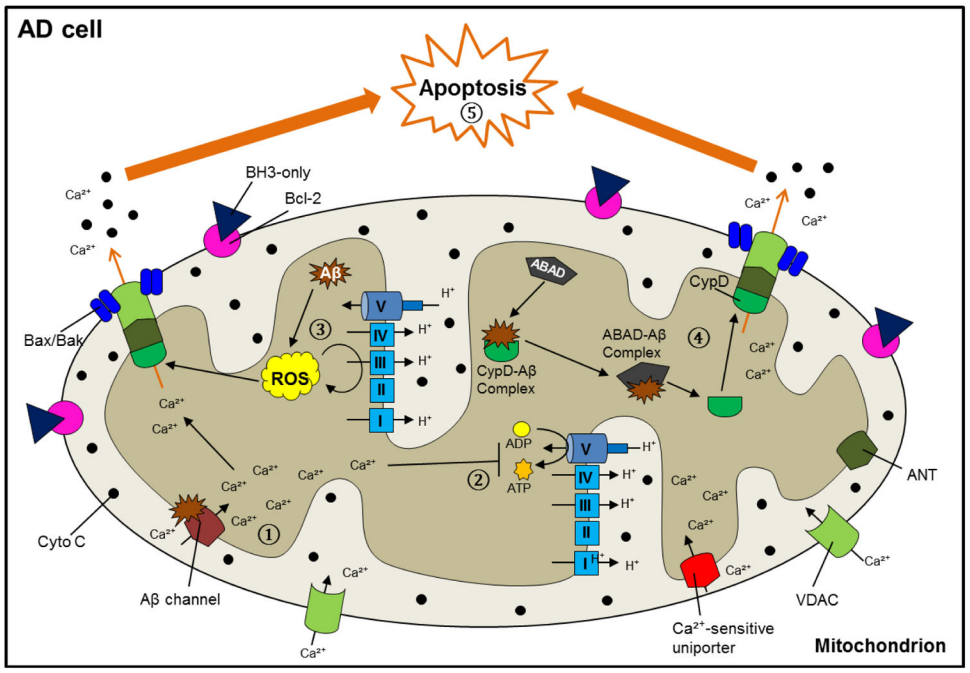

Figure 3. Altered mitochondrial processes during AD

In comparison with normal cells, unhealthy cells affected by AD contain dysfunctional mitochondria with altered processes. **1)** Aβ enters the mitochondrion, causing disruption of Ca^{2+} homeostasis possibly via Aβ channel formation. **2)** Ca^{2+} imbalance and Aβ accumulation cause diminished energy metabolism, leading to **3)** increased ROS production. **4)** Ca^{2+} toxicity and enhanced ROS levels promote MPTP formation. ABAD binds to Aβ, displacing CypD from bound complexes. Once alone, CypD translocates to the IM and initiates MPTP opening. **5)** Together, Aβ toxicity, high Ca^{2+} levels, and ROS accrual stimulate apoptotic cell death.

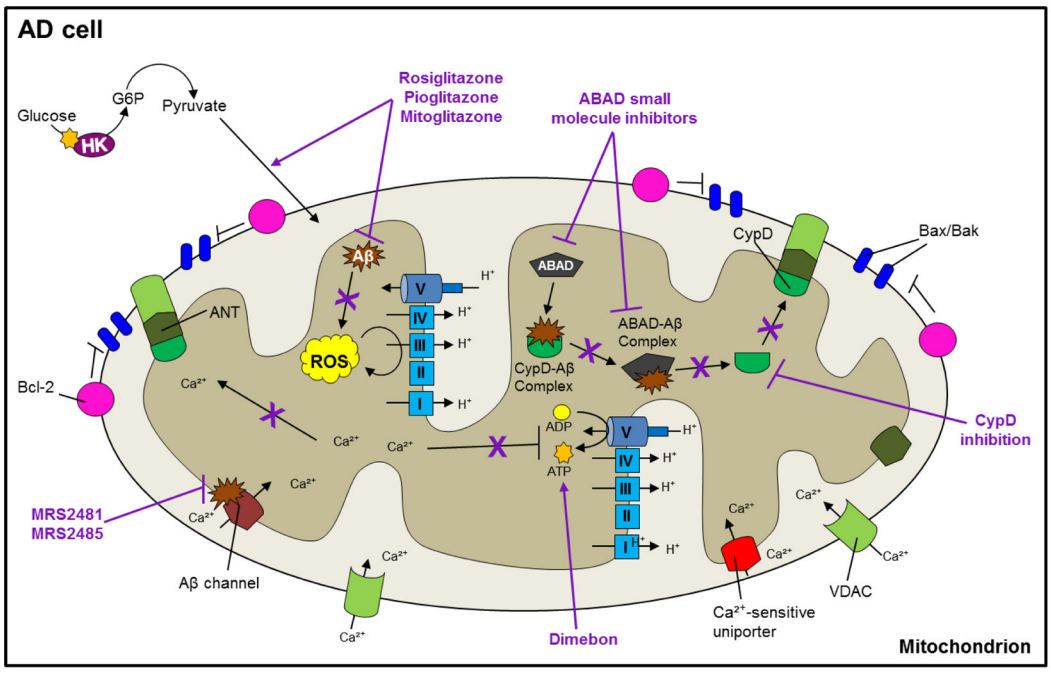

Figure 4. Mitochondrial targets for treatment of AD

As mitochondrial processes are altered in AD, targeting dysfunctional pathways could lead to novel treatment regimens (labeled in purple). In AD cells, TZDs can be used to modulate glucose metabolism. **Rosiglitazone** and **pioglitazone** have also been shown to decrease Aβ accumulation within mitochondria. **Mitoglitazone** is thought to have similar properties, though further study is needed. **ABAD inhibition** blocks the formation of ABAD-Aβ complexes; this causes CypD to remain bound to Aβ, resulting in blockade of MPTP opening. **Inhibition of CypD** targets bound and unbound forms of CypD to prevent its translocation to the IM and MPTP induction. **Dimebon** increases ATP generation and stabilizes the mitochondrial membrane potential, thereby helping to prevent membrane depolarization and cell death. **MRS2481** and **MRS2485** have been observed to block Aβ channel formation, potentially resulting in re-attainment of Ca^{2+} homeostasis. Basically, preventing Aβ-mediated events in AD-affected mitochondria with the indicated therapeutics/target areas could allow cells to regain a healthy condition.