ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

Chemistry & Biology Interface

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Review Paper

Small molecules targeting mitochondria: applications for cancer and neurodegenerative disease therapeutics

Ekaterina A. Korobkova*, Leonid Sukala

**John Jay College of Criminal Justice, City University of New York*

Received 31January 2012; Accepted 23 March 2012

Keywords: mitochondria, apoptosis, necrosis, neurodegenerative disease, cancer

Abstract: Mitochondria are known to play an important role in cellular physiology and homeostasis. Over the last ten years, we have witnessed a growing appreciation by scientific community of mitochondrial involvement in cellular processes. A mitochondrion appears to be a very vulnerable organelle, which is subject to far more aggressive environment than the other cellular components. The accumulation of various types of damage in mitochondria including mtDNA lesions and modifications on the membrane leads to the development of a number of disorders and aging. These findings prompt many researchers around the world to investigate the processes in mitochondria that may lead to cellular malfunction. One of the major processes known to be regulated by mitochondria is cellular apoptosis. Certain conformational changes of the proteins located on the inner membrane trigger the swelling of the matrix and the outer membrane opening, which in turn leads to the release of the apoptotic proteins from the mitochondrial matrix into the cellular cytoplasm. The overregulation of membrane opening leads to the accelerated apoptosis and consequently to the development of neurodegenerative diseases. On the other hand, slow down in apoptosis causes the proliferation of cancer cells. One of the most popular ways of medicinal intervention into the apoptotic pathways is chemical targeting of the proteins involved in these pathways. In this review, we discuss the role of mitochondria in the development of diseases and present molecules found to target key proteins associated with mitochondrial membranes and involved in the regulation of apoptosis and necrosis. We also examine the methods of molecular design and discovery as well as the mechanisms underlying the drugs' interference with the selected biochemical pathways linked to mitochondrial membranes.

Introduction

It has been known for decades that mitochondria act as power engines in cells supplying living organisms with energy carriers ATP molecules. The synthesis of ATP is performed by mitochondria following the electron transfer through the protein complexes embedded in mitochondrial inner membrane. However, it

Corresponding Author* Address: 445 W 59th St., New York, NY 10019, USA; Tel: 212-237-8064; Fax: 212-237-8318; E-mail: ekorobkova@jjay.cuny.edu

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

was not until about twenty years ago that scientists started to appreciate the active participation of mitochondria in the development of a number of disorders including various cancers (1-3), ischemic heart disease (4), neurodegenerative diseases such as Alzheimer's (5), Huntington's (6) and Parkinson's diseases (7), diabetes (8,9), and liver diseases (10). Organism malfunctioning, which manifests itself in the onset and the progression of disease, is associated directly with cell death processes, necrosis and apoptosis.

Apoptosis is an extremely complex cellular process that is triggered by external stimuli and is regulated by a network of biochemical signals. The regulation occurs both externally by the receptors located on cellular membrane (11) and internally by mitochondria. The control of apoptosis by mitochondria is currently an area of intensive research. There have been identified a number of agents that help trigger apoptosis. These include active oxidants (12), viruses (13), calcium cations (14), and some natural compounds found in vegetables, fruits (15,16), and herbs (17). The complex of signals that brings about the destruction of mitochondria followed by a set of biochemical events leading to the cellular death is not comprehensively understood. However, many aspects concerning mitochondrial apoptosis have been described in detail. For instance, it is known that during apoptosis the spatial distribution of mitochondria in cells changes from almost homogenous to localized, as the organelles tend to gather near the nucleus (18). In response to the external agents the permeability of the inner membrane may increase such that molecules having a size under 1.5 kDa can freely escape the mitochondrial matrix. This is followed by the swelling of the matrix, the loss of the electric potential on the inner mitochondrial membrane, the breakage of the outer mitochondrial membrane and the release of the cytochrome c into the cytoplasm (19). Cytochrome c is a protein located in the intermembrane mitochondrial space and attached to the inner membrane. It is an indispensable component of the electron transport respiratory chain located on the inner mitochondrial membrane and coupled to the synthesis of ATP (20,21). When in the cytoplasm, cytochrome c associates with Apaf-1 to produce the "apoptosome". The structure of this complex was solved with electron cryo-microscopy and revealed a wheel-shaped fragment with a seven-fold symmetry (22,23). The formation of this complex primed with the binding of dATP or ATP (24) is the irreversible process that inevitably leads to apoptosis through the activation of procaspase-9 (23) and executive caspases procaspase-3 and procaspase-7 (25).

While apoptosis as a genetically programmed cell death is considered advantageous to a living organism, necrosis occurs when cells are exposed to a severe stress such as highly elevated temperature, drastic decrease in oxygen supply, trauma, or infection. For a long time necrosis had been considered as an accidental cell death process. Recently, a number of works has appeared that support the hypothesis of the programmed nature of necrosis (26). A mitochondrion has also been shown to play a significant role in the initiation of necrotic cell death (27-29). For instance, nitrogen oxide was shown to mediate necrosis through the interactions with complex I of mitochondrial respiratory chain (29).

The release of the cytochrome c into the cytoplasm was found to be associated with a number of neurodegenerative diseases. This finding motivated many researchers to search for effective chemical inhibitors that

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

would prevent the detachment of cytochrome c from the inner membrane and its transportation to the cytoplasm followed by the cellular death.

The detailed analysis of the mitochondrial biochemical events preceding mitochondrial rupture and cytochrome c release is also required in order to develop strategies for the manipulation of apoptosis and consequently the control of cancer cell growth.

This review discusses the particular role of mitochondrial membranes in the development of neurodegenerative diseases and cancer. Below, we summarize recent work done towards the discovery of chemical compounds that regulate intrinsic apoptotic and necrotic pathways through the interactions with a number of mitochondrial or mitochondria-associated proteins. We also describe the specific approaches utilized in search for bioactive molecules and discuss their mechanism of action.

Neurodegenerative diseases

Small molecules for the treatment of Huntington disease

Huntington disease (HD) is an inherited syndrome in which nerve cells progressively die in the brain. It causes mental regression, psychiatric morbidity, weakened muscle coordination and is associated with involuntary writhing movements (30). The disease symptomes usually manifest approximately between 30 and 45 years of age. In the later stage, a person with HD becomes completely dependent on caregivers. In 1993, it was discovered that HD is caused by a mutation consisting of an expanded $(CAG)_n$ repeat in a huntingtin gene (31). While in a normal gene, CAG fragment is repeated 10 to 35 times, people with HD have from 36 to 120 repeated segments if CAG. A normal huntingtin protein plays an important role in neurons. The extended protein divides into smaller parts that accumulate in nerve cells in the brain and lead to the disruption of normal cellular activity and finally to death (32,33). The mechanisms of neurons dysfunction brought about by the mutated huntingtin protein are not precisely understood.

No effective pharmacological treatment is known for HD. However, there have been a number of studies that identified molecules exhibiting exceptional neuroprotective properties and featuring cell death. One of the best known examples is minocycline (Figure 1A). The mechanism of its action is not comprehensively understood. There is evidence of minocycline being associated with the prevention of cytochrome c release from mitochondria to the cytoplasm, where cytochrome c triggers apoptotic pathways. Specifically, it was shown that minocycline inhibits the upregulation of mRNA for caspase-1 and caspase-3 and decreases the activity of inducible nitric oxide synthetase activity thereby inhibiting the production of nitric oxide, which plays a significant role in HD (34). The later work from the same group showed that minocycline also inhibits caspase-independent mitochondrial pathways of cell dysfunction and death (35).

To test the hypothesis that it is the release of the cytochrome c from mitochondria to cytoplasm that initiates HD and to identify a potential inhibitor, Wang et al. (2008) developed a cell free screening assay (36). In this assay, mouse-liver mitochondria were isolated and incubated with test drugs. Calcium cations were added to the mitochondria to stimulate the release of cytochrome c. The concentration of cytochrome c in the samples with different drugs was determined with ELISA. 1040

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

FDA-approved compounds from a National Institute of Neurological Disorders and Stroke library were tested using the cell-free assay. Many of these compounds cross blood-brain barrier. The cell-free assay narrowed down the set of 1040 molecules down to 21 compounds that exhibited outstanding cytochrome c release inhibition capacity. The compounds were then tested on neuronal cells stably expressing a mutant huntingtin fragment. 16 out of 21 compounds prevented cell death. It is noteworthy that monocycline previously found to be neuroprotective was identified as the second best hit in the list of the successful compounds. The study thus confirms that the mechanism of the neuroprotective action shown by monocycline is based on the inhibition of the release of cytochrome c from the intermembrane mitochondrial space. The authors performed further evaluations of the drugs' efficacy using the animal model. Methazolamide (Figure 1A) was chosen from the list of the best hits to investigate the ability of the drug to slow chronic neurodegeneration in mice. The results revealed the neuroprotective properties of the drug. Moreover, the drug was shown to inhibit the release of the cytochrome c *in vivo* in mice model.

Even though the cell free high throughput approach proved to be extremely effective in identifying molecules inhibiting the release of cytochrome c, the chemical basis of the activity of compounds identified as best hits in the screen remains obscure. It is unclear whether the molecules interact directly with cytochrome c or with proteins responsible for the opening of the outer membrane. Further investigations in this area would facilitate the discovery of novel methods in curing Huntington's disease.

In a later study, Hoffstrom et al. (2010) performed a cell based screening assay using a PC12 model of HD and tens of thousands of molecules including natural and synthetic compounds (37). The authors identified five novel compounds that suppressed apoptosis in cells transfected with the expanded huntingtin gene. Using the cycloaddition chemistry, the authors then identified the molecular mechanism underlying the inhibition of apoptosis. The authors found that the compounds found from high throughput screen target protein disulfide isomerase (PDI), an enzyme that assists in the formation and cleaving disulfide bonds in other proteins thereby regulating their folding. In cells transfected with the mutated Huntington gene, PDI accumulates in the mitochondrial-associated-endoplasmic reticulum-membranes. This accumulation triggers the opening of the outer mitochondrial membrane and the release of the apoptotic factors into the cytoplasm.

Small molecules for the treatment of Altzheimer disease

Altzheimer disease (AD) is an age-related neurodegenerative disorder and is diagnosed in patients over 65 years of age. The symptoms of the disease include the loss of long-term memory, difficulty with language, irritability, fluctuations of mood and aggression. AD is associated with the presence of extracellular amyloid plaques, intracellular neurofibrillary tangles, amyloid β-peptide (Aβ) deposits, and the loss of synapse (38,39). There is a substantial amount of evidence attesting a crucial role of mitochondrial dysfunction in the development of AD (40-44). As the cerebral blood flow reduces with age, the brain struggles to produce more mitochondria to satisfy the neuronal demand in energy. As a result, a lot of immature mitochondria are produced. In normal mitochondria, the

ISSN: 2249 –4820
Chemistry & Biology Interface, 2012, 2, 2, 59-75

energy generation is coupled to electron transport through protein complexes embedded in the inner membrane. The electron transport is linked to oxidative phosphorylation and ATP production. In immature mitochondria, the electron transport process appears not effective and is associated with the increased production of free radicals, particularly superoxide anion. It is noteworthy that mitochondrial disorder is observed in all types of brain cells including various classes of neurons, glia, endothelial cells, and pericytes. To combat the oxidative stress, the Aβ precursor is overexpressed in brain, which leads to the Aβ accumulation and subsequently to the development of AD. The free radicals generated by unsuccessful electron transport chains in turn damage other mitochondria and brain enzymes. Additionally, the brain is known to be abundant with neuropeptides and polyunsaturated fatty acids, biomolecules susceptible to easy oxidation, which makes the brain especially vulnerable to various types of oxidizing agents. The oxidative modifications of brain proteins lead to variations in their activity, which in turn alters neuronal signal transduction pathways (44), ultimately promoting the onset and the progression of the disease.

The therapeutic strategies of AD treatment include anti-amyloid, anti-inflammatory, and antioxidant approaches, the later one being the most commonly mentioned approach. Aliev and coworkers (2009) analyzed the effect of selective mitochondrial antioxidants on aged rats using electron microscopy techniques (45). The antioxidants included acetyl-L-carnitine (ALCAR) and R-lipoic acid (LA) (Figure 1B). The authors found that feeding the animals with the antioxidants reduced the number of damaged mitochondria. In a later study, the same group of researchers investigated the effect of the antioxidants on the cognitive behavior of ApoE4 transgenic mice (46). The spatial and temporal memory was tested in mice fed with acetyl-L-carnitine and R-lipoic acid, and the results of the study demonstrated the improved exploratory behavior, attention, and spatial orientation in mice.

The chemical basis of the antioxidant action of ALCAR and LA has been a subject of intensive research. An acetylated product ALCAR is synthesized from a natural cellular product L-carnitine and acetyl-CoA with the aid of carnitine O-acetyltransferase. The scavenging mechanism of L-carnitine is proposed by Gulcin (2006) who studied its reaction with 1,1-Diphenyl-2-picryl-hydrazil (DPPH) using UV/vis spectroscopy (47). Gulcin proposed that the radical formed on L-carnitine after the abstraction of a hydrogen atom from an alpha-carbon atom is stabilized through the conjugation between the unpaired electron and the π-orbital of the carbonyl group. The stabilization of the resulting radical product lowers the reaction enthalpy change and increases the likelihood of radical scavenging. We hypothesize that a similar radical quenching mechanism pertains to ALCAR.

LA is present in living cells naturally and plays an important role in the synthesis of ATP as it is a cofactor for mitochondrial alpha-keto-dehydrogenase (48). In cells, LA is reduced to dihydrolipoc acid (DHLA), and there is evidence that the reduced form also exhibits antioxidant activity (49). The electron transfer reactions within this redox pair may affect the conformations of proteins by varying the amount of disulfides, which in turn adjusts the network of signal transduction pathways. A number of studies have demonstrated that both LA and DHLA directly scavenge reactive oxygen species such as hydroxyl radicals, hypochlorous

ISSN: 2249 –4820
Chemistry & Biology Interface, 2012, 2, 2, 59-75

acid, and singlet oxygen. However, in addition to the direct scavenging affect, indirect mechanisms of antioxidant activity of LA have been discovered. Thus, LA acts as a transcription inducer of genes responsible for the synthesis of glutathione, which is the most widely known natural cellular antioxidant (50). There is also evidence that LA increases the level of ascorbic acid, an essential cellular nutrient and antioxidant (51). Both LA and DHLA act as chelating agents of transitional metals, thereby hampering their redox activity (51).

At present, mechanisms underlying the antioxidant activity of ALCAR and LA acid are not fully understood. However, it is clear that these molecules are very promising as therapeutic agents and the mechanisms of their ameliorating action need to be exploited, specifically in regard to AD.

Small molecules potentially targeting Parkinson disease

Parkinson disease (PD) is a neurodegenerative disorder characterized by the dysfunction or death of neurons responsible for the production of dopamine. Since dopamine is responsible for the coordination of movements, the symptoms of PD are related to movement impairment such as hands and legs trembling and stiffness, poor coordination, and sensory problems. Several therapeutic agents increasing the level of dopamine in the brain have been employed in medicine. These include levodopa that is converted to dopamine in neurons, tolcapone that inhibits an enzyme degrading dopamine, and others. Even though these medications may be effective in controlling symptoms, there is no cure for the disease.

The biomolecular basis of the death of dopamine-generating cells is not understood. However, a significant number of recent studies indicate the essential role of mitochondria and oxidative stress in the initiation and progression of PD (52-55). A set of mitochondrial and mitochondria-associated proteins were found to be directly linked to the etiology and pathogenesis of the disease. Thus, it is established that PTEN-induced putative kinase 1 (PINK1) and PARK2 (Parkin) proteins trigger the degradation of damaged mitochondria in healthy cells. PINK1 is a serine/threonine protein kinase localized to mitochondria. Parkin is a part of a ligase complex which in turn represents a part of a proteasome system responsible for the degradation of deteriorated and unnecessary proteins. Mutations in PINK1 and Parkin cause mitochondrial dysfunction and early-onset Parkinson disease.

Until recently, the biochemical mechanism underlying mitochondrial autophagy had not been described and a network of signals connecting Parkin and PINK1 in one complex pathway leading to the disposal of damaged mitochondria had not been understood. A novel study performed by Narendra and coworkers (2010) shed light on the biophysics of this key pathway (55). The authors discovered that in healthy mitochondria with a stable inner-membrane potential PINK1 is promptly turned over by proteolysis. However, in the case of damaged depolarized mitochondria, PINK1 spans through the outer membrane with its kinase domain facing the cytosol. Once PINK1 is stabilized on a depolarized mitochondrion, Parkin is recruited to the spot of PINK1-mitochondria assembly, where it triggers the degradation of the mitochondrion (Figure 2A). The precise mechanism of signals conveying between these proteins is not established. Geisler and coworkers (2010) suggested that PINK1 controls a mitochondrial voltage-dependent

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

anion channel-1, which serves as a target for Parkin (56).

Even though the precise mechanism of PINK1-Parkin signaling is not yet completely understood, the pharmacological treatment has been proposed for Parkinson that suppresses pathologic phenotypes in these mutant-proteins. Tain and coworkers (2009) identified 4E-BP1 gene as a factor that inhibits pathology of PINK1 and Parkin mutants and thus mediates the cell survival response (57). The authors also demonstrated that treating *Drosophila* with rapamycin (Figure 2B), which activates 4E-BP1, led to similar curative effects including slowing down in the relapse of dopamine-generating neurons and the amendment of damaged mitochondria in *Drosophila* (57). The activation mechanism involves the inhibition of TOR, which is known to negatively regulate 4E-BP1. mTOR is a protein kinase and performs multiple tasks such as regulation of transcription and translation in response to nutrients, protein degradation, and membrane trafficking. Interestingly, Harrison and coworkers (2009) also reported that rapamycin can extend lifespan in mammalian species and thus be implicated in the treatment of age-related diseases (58). The biochemical mechanism of TOR-rapamycin interaction is discussed by Faivre and coworkers, and the TOR binding site is shown in Figure 2A (59).

A significant number of studies indicates the deficiency of complex I (NADH:ubiquinone oxidoreductase), an essential component of the electron transport chain, in mitochondria of patients with PD. The reduced activity of complex I was revealed in brain tissue (60), skeletal muscle (61), and blood cells (62). The discovery of the electron transport chain imparity in mitochondria of PD patients suggested the usage of co-enzyme Q10 and creatine as therapeutic agents against the disease (Figure 3A). Co-enzyme Q10 or ubiquinone possesses unique chemical properties that allow it to switch between three oxidation states: ubiquinone (fully oxidized), semiquinone (half oxidized), and ubiquinol (fully reduced). A wide range of oxidation states allows this molecule to perform an exclusive task of carrying electrons from complex I and complex II to complex III, all embedded in the inner mitochondrial membrane (Figure 3B). Creatine is synthesized naturally from arginine and glycine or is taken up from meat and fish. It is transported to brain and skeleton muscles through blood with the aid of an effective transport system. The fundamental role of creatine in cells is founded on its ability to be converted to phosphocreatine with creatine kinase. The phosphocreatine in turn donates its phosphate group to ADP leading to the production of ATP, molecular energy source of cells (63). Yang and coworkers (2009) investigated the collective effect of co-enzyme Q10 and creatine in mouse model of PD (64). The authors found that the combination of the two drugs produced additive neuroprotective effects, which included the inhibition of dopamine depletion and the decrease of lipid peroxidation (64).

Cancer

A tremendous amount of research efforts is directed at mitochondria in regards to the treatment of cancer. A mitochondrion is a key cellular component regulating apoptosis through the membrane permeability transition (MPT) and the release of proapoptotic factors into the cytosol. While a healthy organism benefits from apoptosis, cancer cells develop a complex of self-protective mechanisms stimulating their proliferation and inhibiting apoptosis (65).

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

The resistance of cancer cells to apoptosis challenges therapeutic endeavors to combat tumors. The comprehensive understanding of the function of mitochondrial and mitochondria-associated proteins participating in the regulation of apoptosis would allow the development of therapeutic strategies towards the stimulation of apoptosis and the inhibition of the growth of cancer cells. The following sections summarize recent findings concerning central mitochondrial pathways controlling apoptosis and methods of chemical intervention with these pathways.

Inhibition of Bcl-2 antiapoptotic proteins

Bcl-2 family members play a crucial role in the regulation of apoptosis (66). Bcl-2 is an anti-apoptotic protein known to inhibit the MPT. The mechanism behind Bcl-2-mediated apoptosis inhibition involves protein interaction with the voltage-dependent anion channel (VDAC) on the outer mitochondrial membrane (67). Bcl-2 and structurally related Bcl-X_L bind to and thus inactive several proapoptotic proteins including Bax/Bak (68,69) and BH3 proteins - Bad, Bid, and Bim (70). Bcl-2 was found to be overexpressed in various types of cancer cells, while the expression of proapoptotic proteins in the same cells were found below normal (71,72).

The design of small molecules interfering with the binding of Bcl-2 and proapoptotic proteins has been viewed as the potential strategy to arrest the growth of cancer cells. Oltersdorf and coworkers (2005) used NMR-based screening, parallel synthesis, and structure-based design to develop a small molecule named ABT-737 (Figure 4). The molecule blocked the binding groove of Bcl-2 or Bcl-X_L thus preventing their binding to BH3 and augmenting the effects of death signals (73). ABT-737 exhibited the binding affinity two to three orders of magnitude stronger than that of previously reported antagonists. The authors demonstrated that ABT-737 caused the regression of solid tumors. In a later work, investigators from Abbott Laboratories developed another small molecule ABT-263 (Figure 4), which disrupted the interactions between Bcl-2/Bcl-X_L and a proapoptotic protein Bim. ABT-263 induced cytochrome c release and the subsequent apoptosis (74). Lessene et al. discuss other peptide mimetics designed for antiapoptotic proteins from Bcl-2 family and list the affinity constants and the values of IC_{50}/EC_{50} (75).

Recently, Dalafave and Prisco employed a computer-based strategy to design five novel molecules that blocked antiapoptotic proteins, Bcl-2, Bcl-X_L, and Mcl-1. Each molecule was predicted to exhibit significant affinity to each of the three proteins (76). The authors discuss the advantage of using computational methods to predict potential capability of molecules to induce apoptosis before performing laboratory and preclinical trials.

Inhibition of TRAP1 and HSP90 proteins

In addition to proapoptotic proteins that stimulate the outer membrane opening in mitochondria from the cytosol side, the MPT is also regulated internally, at the inner membrane. A protein which is believed to be a key regulator of MPT is called cyclophilin D (CypD). It is located at the inner membrane on the side of the mitochondrial matrix. The relationship of this protein with MPT and subsequently apoptosis is currently a subject of intensive research. Several chaperones were found to associate with CypD directly thereby changing its conformations and thus preventing MPT. These include HSP90, TRAP1 (77), and HSP60 (78). One exciting finding is that the

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

CypD-chaperones interactions, antagonizing the opening of the mitochondrial outer membrane pore and thus the release of cytochrome c into the cytoplasm, only occur in mitochondria of cancer cells. A chaperone-CypD complex thus presents an attractive target for cancer therapy, and a significant amount of research efforts are directed towards the design of chemical inhibitors targeting these proteins (79,80).

HSP90 plays an essential role in cellular survival in general and the survival of cancer cells in particular (81,82). Markedly, HSP90 was not only found overexpressed in cancer cells compared with normal tissues, but its ATPase activity in cancer cells appeared to be 100 times stronger than in healthy cells. The discovery of the augmented ATPase activity prompted investigators to design molecules inhibiting ATP-binding sites in HSP90. Novobiocin, epilgallocatechin-3-gallate and taxol (Figure 5A) are antitumor agents that bind a C-terminal ATPase pocket in HSP90 (83). The molecules, inhibiting an ATPase pocket located on the N-terminal domain of the protein, include radicicol and derivatives of the natural compound geldanamycin (GA), 17-allylamino-17-desmethoxygeldanamycin (17-AAG) and 17-dimethylaminoethylamino-17-desmethoxygeldanamycin (17-DMAG) (Figure 5B). The compounds were shown to reduce the viability of cancer cells and to cause substantial apoptosis (84,85). However, the outcomes of the clinical trials of 17-AAG were considerably below expectations. The low efficacy of the drug was probably associated with its inability to accumulate in mitochondria.

Plescia and coworkers (2005) designed a peptidomimetic called shepherdin. Shepherdin was modeled on the binding surface between HSP90 and survivin, a protein inhibiting apoptosis. Since the function of survivin is regulated by HSP90, the disruption of their interaction with shepherdin led to cancer cells' death (86). A later work from the same group showed that shepherdin induced the disruption of TRAP1/HSP90 complex leading to the conformational switch of CypD (Figure 5C), which in turn stimulated the opening of the mitochondrial permeability transition pore followed by apoptosis (77). Unlike 7-AAG, shepherdin is directed to mitochondria, where it actively antagonizes the function of TRAP1 and HSP90. Costantino and coworkers (2009) also showed that the inhibition of TRAP1 with shepherdin increased the sensitivity of colorectal carcinoma cells to standard chemotherapeutic agents oxaliplatin and irinotecan (87).

Later, Kang and coworkers (2009) used a combinatorial modular design approach to develop a new class of compartmentalized and pathway-oriented molecules called gamitrinibs (GA mitochondrial matrix inhibitors). Gamitrinib consists of a GA molecule, an inhibitor of the HSP90 ATPase pocket, connected through a linker to a mitochondriotropic moiety (88). The molecules caused cancer cells' death and inhibited the proliferation of xenografted human tumor cell lines in mice.

A recent study performed by Ghosh and coworkers (2010) revealed the involvement of another chaperone HSP60 in the formation of complex with CypD along with HSP90 and TRAP1 proteins. The authors demonstrated that HSP60 is a regulator of MPT, which inhibits CypD-dependent apoptosis (78). The exact mechanism of CypD association with chaperones is not known. The detailed structural studies of these complexes and the studies of the mechanism underlying the apoptotic signal

ISSN: 2249 –4820
Chemistry & Biology Interface, 2012, 2, 2, 59-75

triggered by the conformational switch of CypD would provide novel rationales for the development of anticancer strategies.

Conclusions

Proteins associated with activities occurring on mitochondrial membranes define the onset and the progression of a number of human diseases. Mutations in these proteins, their upregulation, or misfolding alter the network of signals processed by cell in its life cycle leading to the accumulation of abnormalities and consequently diseases. Remarkably, most of these numerous disorders commence in the same organelle. Understanding the biophysical processes occurring on mitochondrial membranes at a system level would provide a basis for the development of novel complex therapeutic strategies regulating cellular death processes.

Acknowledgements

The authors thank Dr. Arkadiy Bolshov for his help with the text of the manuscript. The authors also thank Ankit Bhatta, Sofia Cheliout Dasilva, and Baibhav Rawal for their help with figures.

Figures

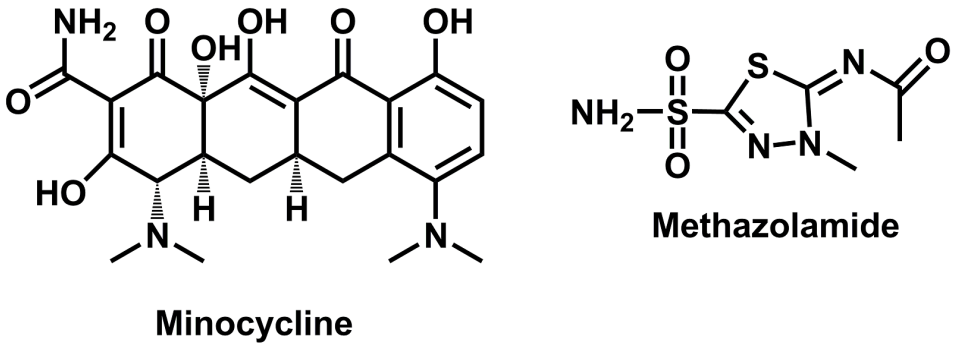
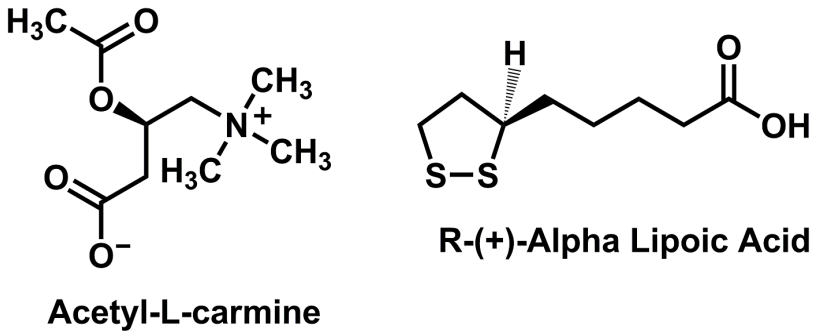


Figure 1A. The structures of two compounds found to inhibit the release of cytochrome c from mitochondria into the cytoplasm (36). The compounds inhibited the death of neuronal cells expressing a mutant huntingtin fragment, and thus are believed to be effective in the treatment of Huntington disease.



ISSN: 2249 –4820
Chemistry & Biology Interface, 2012, 2, 2, 59-75

Figure 1B. The structures of two antioxidants expected to be effective in the treatment of Altzheimer disease. The compounds were found to reduce the number of damaged mitochondria in animals (45).

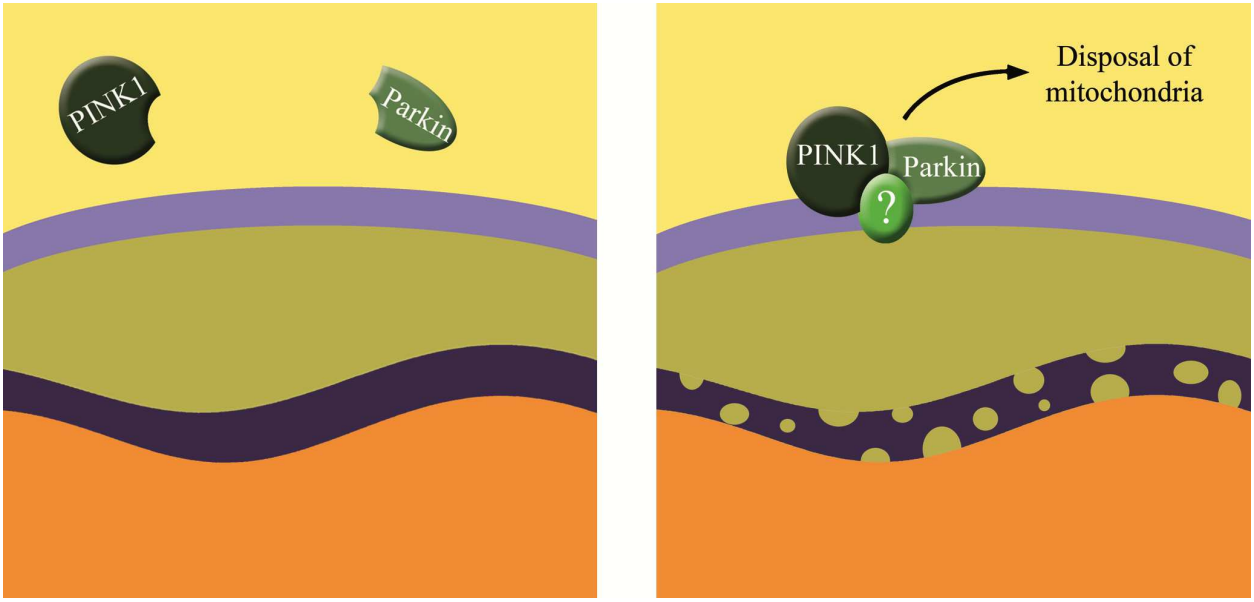


Figure 2A. Schematic presentation of the association of PINK1 and Parkin on the outer mitochondrial membrane. Left panel: a healthy mitochondrion, PINK1 does not bind to the outer membrane. Right panel: a damaged mitochondrion, PINK1 binds firmly to the membrane and recruits Parkin, which sends signals for the disposal of the mitochondrion.

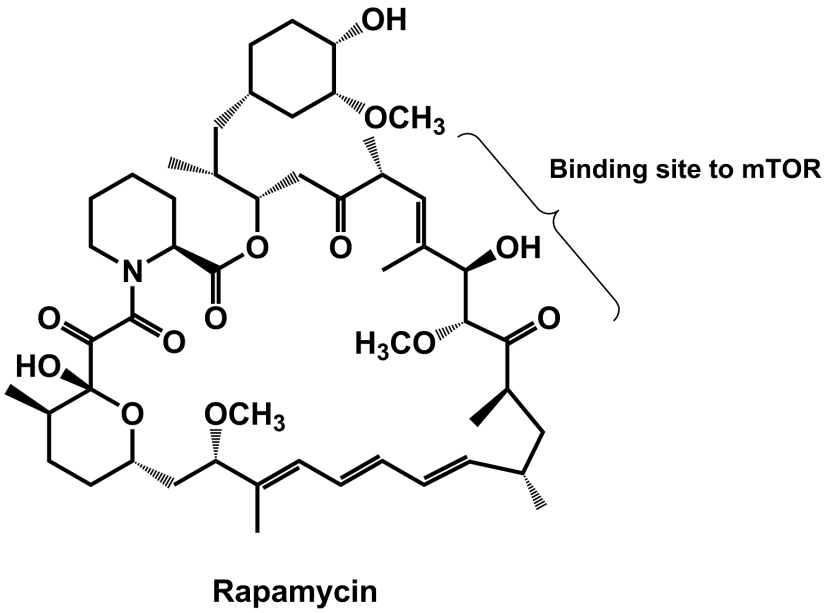


Figure 2B. The structure of rapamycin, an agent preventing neurodegeneration in Drosophila through the inhibition of mTOR, a negative regulator of 4E-BP gene, which reduces the viability of PARK1 and Parkin mutant (57).

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

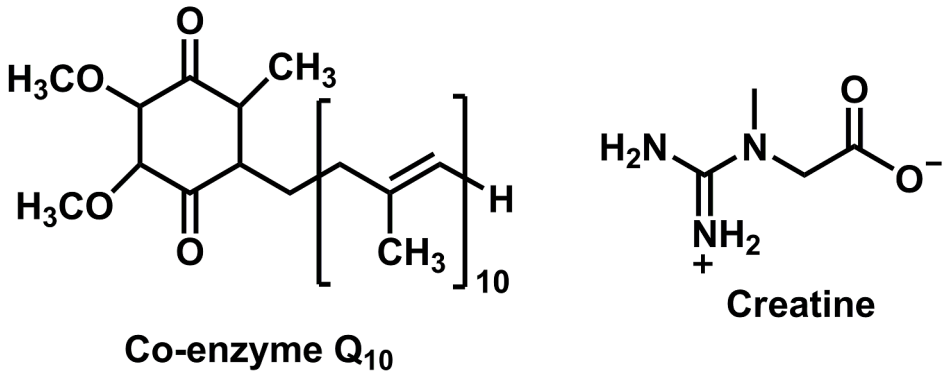


Figure 3A. The structures of co-enzyme Q_{10} and creatine. These molecules exhibit neuriprotective effects in mice and are promising therapeutic agents against Parkinson disease.

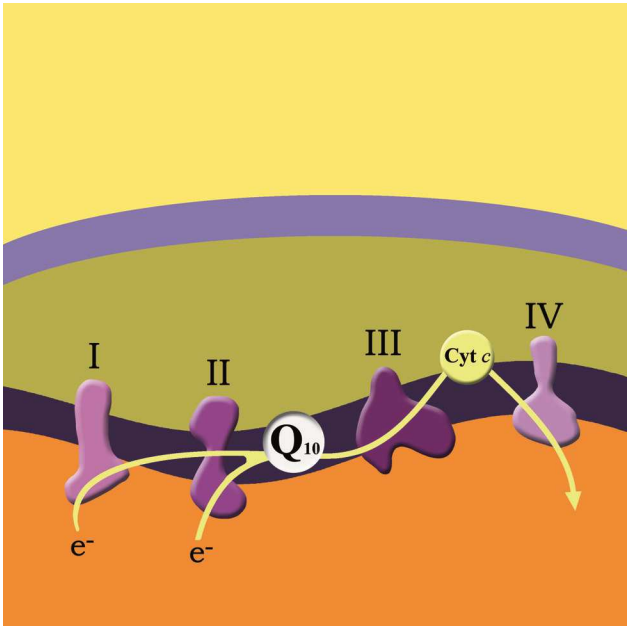
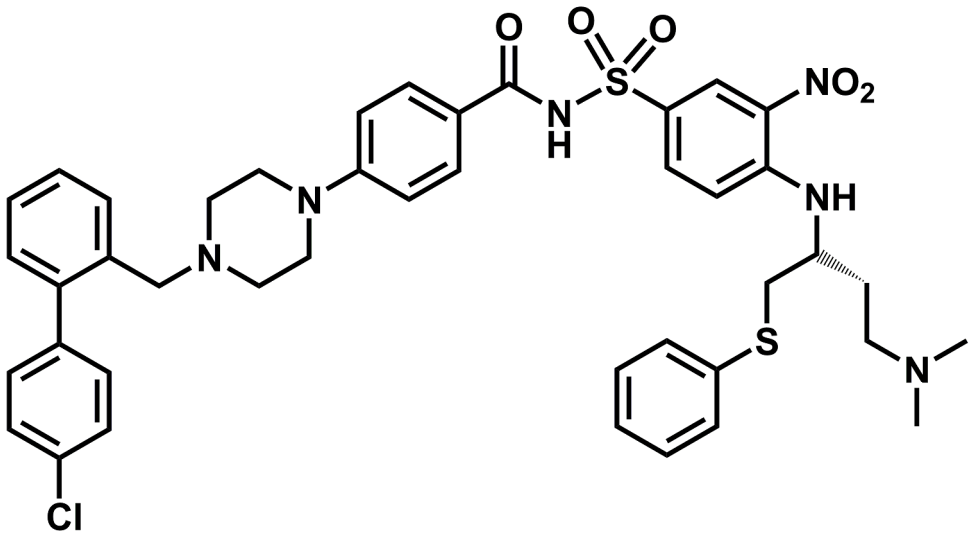


Figure 3B. Schematic presentation of a mitochondrial electron transport chain. Yellow – cytoplasm; light blue – outer mitochondrial membrane; light green – mitochondrial intermembrane space, dark blue – inner mitochondrial membrane; orange – mitochondrial matrix. Four protein complexes are embedded in the inner mitochondrial membrane. Co-enzyme Q10 shuttles electrons between complexes I, II, and III.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75



ABT -737

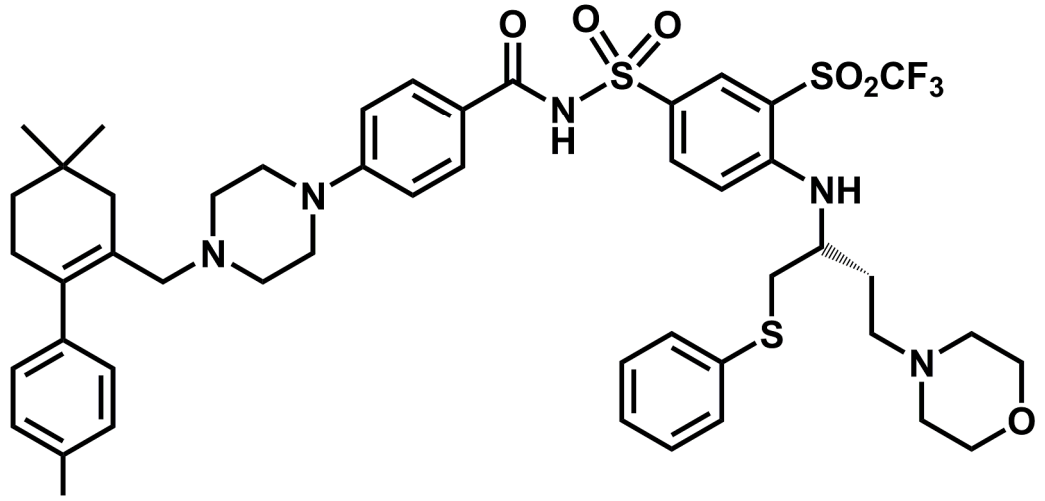


Figure 4. The structure of molecules inhibiting an antiapoptotic protein Bcl-2. ABT-737 prevents Bcl-2/Bcl-X_L - BH3 interactions by binding Bcl-2 groove. ABT-263 blocks interactions between Bcl-2/Bcl-X_L and a proapoptotic protein Bim.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

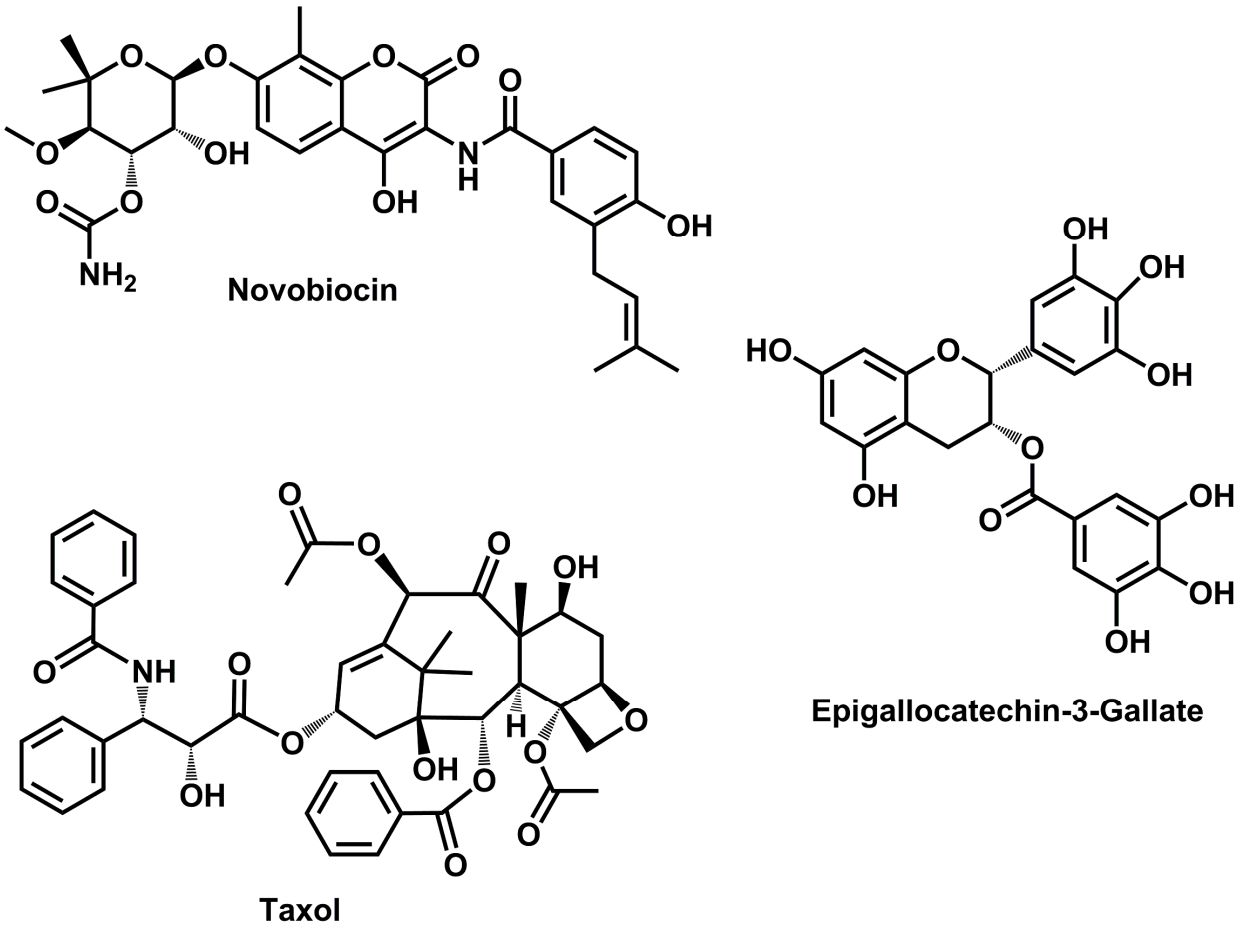


Figure 5A. The structures of antitumor agents selectively binding to a C-terminal ATPase pocket in HSP90 and inhibiting its ATPase activity.

ISSN: 2249 –4820
Chemistry & Biology Interface, 2012, 2, 2, 59-75

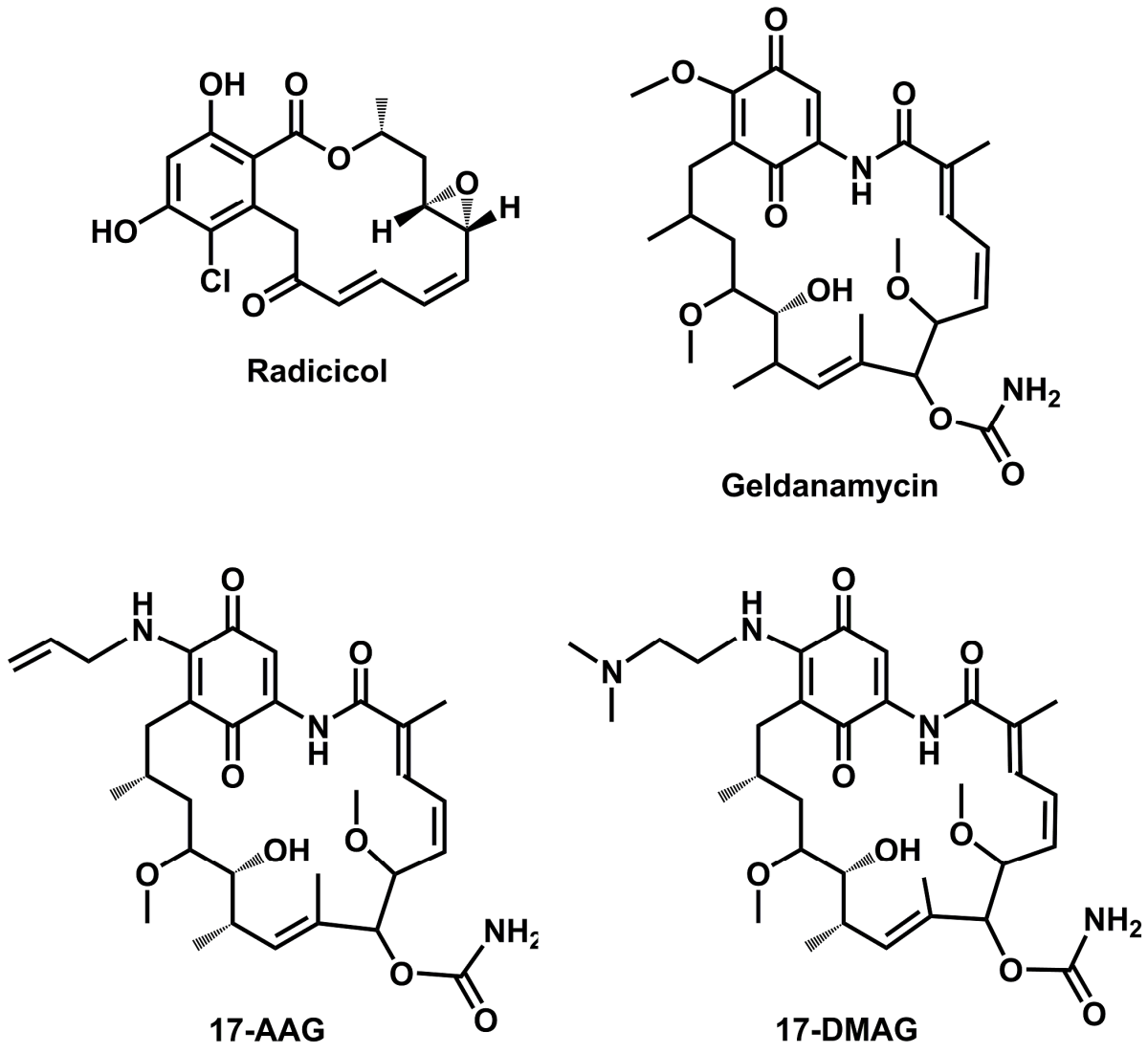


Figure 5B. The structures of the compounds inhibiting ATPase activity of HSP90 via binding to its N-terminal domain.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

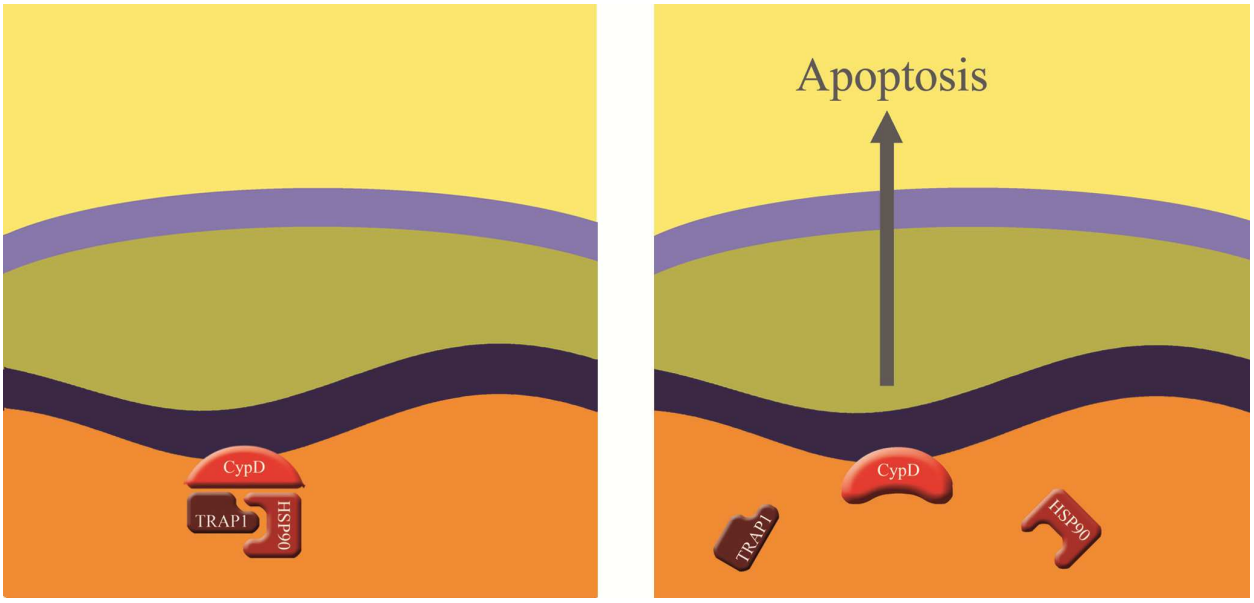


Figure 5C. Interactions between CypD, TRAP1, and HSP90 proteins in mitochondrial matrix. Left panel: chaperones TRAP1 and HSP90 form a complex with CypD antagonizing MPT. Right panel: a chaperone-CypD complex is disrupted resulting in a conformational switch in CypD, which in turn leads to MPT and apoptosis.

References

[1] M. Verma, J. Kagan, D. Sidransky, S. Srivastava, Nat Rev Cancer, **2003**, 3, 789-795.
[2] J. Kagan, S. Srivastava, Crit Rev Clin Lab Sci, **2005**, 42, 453-472.
[3] V. Gogvadze, S. Orrenius, B. Zhivotovsky, Trends Cell Biol, **2008**, 18, 165-173.
[4] B. Kadenbach, R. Ramzan, R. Moosdorf, S. Vogt, Mitochondrion, **2011**, 11, 700-706.
[5] P.H. Reddy, CNS Spectr, **2009**, 14, 8-18.
[6] X. Wang, S. Zhu, Z. Pei, et al., J Neurosci, **2008**, 28, 9473-9485.
[7] D.P. Narendra, S.M. Jin, A. Tanaka et al., Plos Biol, **2010**, 8, 1-21.
[8] J.A. Maassen, L.M. 'T Hart, E. Van Essen, et al., Diabetes, **2004**, 53, S103-S109.
[9] G.M Leinninger, J.L Edwards, M.J. Lipshaw, E.L. Feldman, Nat Clin Pract Neuro, **2006**, 2, 620-628.
[10] G. Serviddio, F. Bellanti1, J. Sastre, et al., Curr Med Chem, **2010**, 17, 2325-2337.
[11] K.M. Debatin, P.H. Krammer, Oncogene, **2004**, 23, 2950-2966.
[12] N. Imaizumi, Y. Aniya, Arch Biochem Biophys, **2011**, 516, 160-172.
[13] C.D. Williamson, R.L. De Biasi, A.M. Colberg-Poley. Infect Disord Drug Targets, **2012**, 12, 18-37.
[14] I. Kaddour-Djebbar, V. Choudhary, C. Brooks, T., et al., Int J Oncol., **2010**, 36, 1437-44.
[15] H.F. Lu, Y.J. Chie, M.S. Yang, et al., Hum Exp Toxicol, **2011**, 30, 1053-1061.
[16] M. Zbidah, A. Lupescu, K. Jilani, et al., J Agr Food Chem, **2012**, 60, 533-538.
[17] C. Feng, L.-Y. Zhou, T. Yu, et al., Int J Cancer, **2012**, DOI: 10.1002/ijc.27365.
[18] K. De Vos, V. Goossens, E. Boone, et al., J Biol Chem, **1998**, 273, 9673-9680.
[19] D.R. Green, G. Kroemer, Science, **2004**, 305, 626-629.
[20] H. Schägger, K. Pfeiffer, J Biol Chem, **2001**, 276, 37861-37867.
[21] B. Kadenbach, R. Ramzan, L. Wen, .S. Vogt, Biochim Biophys Acta, **2010**, 1800, 205-212.
[22] D. Acehan, X. Jiang, D. G. Morgan, et al., Mol Cell, **2002**, 9, 423-432.
[23] S. Yuan, X. Yu, M. Topf, et al., Structure, **2010**, 18, 571-583.
[24] T.F. Reubold, S. Wohlgemuth, S. Eschenburg, J Biol Chem, **2009**, 284, 32717-32724.
[25] N.N. Danial, S.J. Korsmeyer, Cell, **2004**, 116, 205-219.
[26] C.P. Baines, Front Physiol, **2010**, 1, 1-8.
[27] G. Kroemer, B. Dallaporta, M. Resche-Rigon. Annu Rev Physiol, **1998**, 60, 619-642.
[28] J.J. Lemasters, T. Qian, L. He, et al., Antioxid Redox Sign, **2002**, 4, 769-781.
[29] C.W. Davis, B.J. Hawkins, S. Ramasamy, et al., Free Radical Bio Med, **2010**, 48, 306-317.
[30] F. O Walker, Lancet, **2007**, 369, 218-228.
[31] M.E. MacDonald, C. M. Ambrose, M.P. Duyao, et al., Cell, **1993**, 72, 971-983.
[32] S.H. Li, X.J. Li, Neuroscientist, **2004**, 10, 467-475.

ISSN: 2249 –4820

Chemistry & Biology Interface, 2012, 2, 2, 59-75

[33] E.J. Slow, R.K. Graham, M.R. Hayden, Trends Genet, **2006**, 22, 408-411.
[34] M. Chen, V.O. Ona, M. Li, et al., Nat Med, **2000**, 6, 797-801.
[35] X. Wang, S. Zhu, M. Drozda, et al., P Natl Acad Sci USA, **2003**, 100, 18, 10483-10487.
[36] X. Wang, S. Zhu, Z. Pei, et al., J Neurosci, **2008**, 28, 9473-9485.
[37] B.G. Hoffstrom, A. Kaplan, R. Letso, et al., Nat Chem Biol. **2010**, 6, 900-906.
[38] D.A. Butterfield, T. Reed, Shelley F. et al., Free Radical Bio Med, **2007**, 43, 658-677.
[39] E. Aso, S. Lomoio, I. López-González, et al., Brain Pathol, **2012**, DOI: 10.1111/j.1750-3639.2011.00560.x.
[40] R. Sultana, Acta Neuropathol, **2009**, 118, 131-150.
[41] P.H. Reddy, CNS Spectr, **2009**, 14, 8-18.
[42] G. Aliev, H.H. Palacios, B. Walrafen, et al., Int J Biochem Cell B, **2009**, 41, 1989-2004.
[43] A. Colell, A. Fernández, J. C. Fernández-Checa, J Bioenerg Biomembr, **2009**, 41, 417-423.
[44] R. Sultana, D.A. Butterfield, J Bioenerg Biomembr, **2009**, 41, 441-446.
[45] G. Aliev, J. Liu, J.C. Shenk, et al., J Cell Mol Med, **2009**, 13, 320-333.
[46] J.C. Shenk, J. Liu, K. Fischbach, et al., J Neurol Sci, **2009**, 283, 199-206.
[47] I. Gulcin, Life Sci, **2006**, 78, 803-811.
[48] J. Bustamante, J.K. Lodge, L. Marcocci, et al., Free Radical Biol Med, **1998**, 24, 1023-1039.
[49] H. Moini, L. Packer, N.-E.L. Saris, Toxicol Appl Pharm, **2002**, 182**,** 84-90.
[50] K.P. Shay, R.F. Moreau, E.J. Smith, T.M. Hagen, IUBMB Life, **2008**, 60, 362-367.
[51] K.P. Shay, R.F. Moreau, E.J. Smith, et al., Biochim Biophys Acta, **2009**, 1790, 1149-1160.
[52] A.H. Schapira, Lancet Neurol, **2008**, 7: 97–109
[53] S. Shavali, H.M. Brown-Borg, M. Ebadi, J. Porter, Neurosci Lett, **2008**, 439, 125-128.
[54] E. Hattingen, J. Magerkurth, U. Pilatus, et al., Brain, **2009**, 132, 3285-3297.
[55] D.P. Narendra, S.M. Jin, A. Tanaka, et al., Plos Biology, **2010**, 8, 1-21.
[56] S. Geisler, K.M. Holmström, D. Skujat, et al., Nat. Cell Biol, **2010**, 12, 119-131.
[57] L.S. Tain, H. Mortiboys, R.N. Tao, et al., *Nat Neurosci*, ***2009***, 12, 1129-1135.
[58] D.E. Harrison, R. Strong, Z.D. Sharp, et al., Nature, **2009**, 460, 392-395.
[59] S. Faivre, G. Kroemer, E. Raymond, Nat Rev Drug Discov, **2006**, 5, 671-688.
[60] W.D. Parker, Jr., J.K. Parks, R.H. Swerdlow, Brain Res, **2008**, 1189, 215-218.
[61] O. Blin, C. Desnuelle, O. Rascol, et al., J Neurol Sci, **1994**, 125, 95-101.
[62] H. Yoshino, Y. Nakagawa-Hattori, T. Kondo, Y. Mizuno, J Neural Transm-Park, **1992**, 2, 27-34.
[63] T. Wallimann, M. Tokarska-Schlattner, U. Schlattner, Amino Acids, **2011**, 40, 1271-1296.
[64] L. Yang, N.Y. Calingasan, E.J. Wille, et al., J Neurochem, **2009**, 109, 1427-1439.
[65] G.G. Chen, P. Lai (Eds.), Apoptosis in Carcinogenesis and Chemotherapy, **2009**, Netherlands: Springer.
[66] J.M. Adams, S. Cory, Science, **1998**, 281, 1322-1326.
[67] Y. Tsujimoto, Bioscience Rep, **2002**, 22, 47-58.
[68] R.J. Youle, A. Strasser, Nat Rev Mol Cell Bio, **2008**, 9, 47-59.
[69] J. Ding, Z. Zhang, G.J. Roberts, et al., J Biol Chem, **2010**, 285, 28749-28763.
[70] Z. Jin, W.S. El-Deiry, Cancer Biol Ther, **2005**, 4, 139-163.
[71] A.M. Berghella, P. Pellegrini, I. Contasta, et al., Cancer Biother Radio, **1998**, 13, 225-236.
[72] J. Lieber, B. Kirchner, C. Eicher, et al., Pediatr Blood Cancer, 2010, 55, 1089-1095.
[73] T. Oltersdorf, S.W. Elmore, A.R. Shoemaker, et al., Nature, **2005**, 435, 677-681.
[74] C. Tse, A.R. Shoemaker, J. Adickes, et al., Cancer Res, **2008**, 68, 3421-3428.
[75] G. Lessene, P.E. Czabotar, P.M. Colman, Nat Rev Drug Discov, **2008**, 7, 989-1000.
[76] D.S. Dalafave, G. Prisco, Cancer Informatics, **2010**, 9, 169-177.
[77] B.H. Kang, J. Plescia, T. Dohi, et al., Cell, **2007**, 131, 257–270.
[78] J.C. Ghosh, M.D. Siegelin, T. Dohi, et al. Cancer Res, **2010**, 70, 8988-8993.
[79] M. Landriscina, M.R. Amoroso, A. Piscazzi, F. Esposito. Gynecologic Oncology, **2010**, 117, 177-182.
[80] D.C. Altieri, Oncotarget, **2011**, 2, 347-351.
[81] D.C. Altieri, G.S. Stein, J.B. Lian, L.R. Languino, Biochim Biophys Acta, **2012,** 1823,767-773.
[82] F.U. Hartl, A. Bracher, M. Hayer-Hartl , Nature, **2011**, 475, 324-332.
[83] A. Donnelly, B.S. Blagg, Curr Med Chem, **2008**, 15, 2702–2717.
[84] M. Breinig, E. Caldas-Lopes, B. Goeppert, et al., Hepatology, **2009**, 50, 102-112.
[85] G. Watanabe, K.E. Behrns, J.S. Kim, R.D. Kim. Cancer Chemother Pharmacol, **2009**, 64, 433-443.
[86] J. Plescia, W. Salz, F. Xia, et al., Cancer Cell, **2005**, 7, 457-468.
[87] E. Costantino, F. Maddalena, S. Calise, et al., Cancer Lett, **2009**, 279, 39-46.
[88] B.H. Kang, J. Plescia, H.Y. Song, et al., J Clin Invest, **2009**, 119, 454-464.