

# Mitochondria as targets for chemotherapy

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**Abstract** Mitochondrial malfunctioning is implicated in the pathogenesis of a variety of disorders, including cancer and multiple neurodegenerative diseases, such as Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, and Huntington's disease. Disturbance of mitochondrial vital functions, e.g., production of ATP, calcium buffering capacity, and generation of reactive oxygen species, can be potentially involved in disease pathogenesis. Neurological disorders caused by mitochondrial deterioration are often associated with cell loss within specific brain regions. In contrast, mitochondrial alterations in tumor cells and the "Warburg effect" might lead to cell survival and resistance of tumor cells to chemotherapy. This review is devoted to the role of mitochondria in neurodegeneration and tumor formation, and describes how targeting of mitochondria can be beneficial in the therapy of these diseases, which affect a large human population.

**Keywords** Mitochondria · Disease · Oxidative stress · Neurodegeneration · Cancer

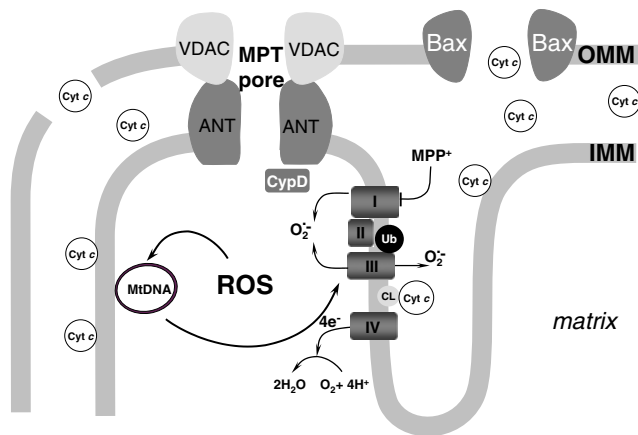
## Introduction

Observations made during the past decades have suggested that mitochondrial malfunctioning is implicated in the pathogenesis of a variety of human disorders, including cancer and multiple neurodegenerative diseases, such as Parkinson's disease (PD), Alzheimer's disease (AD),

amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD). For a long time, mitochondria were known mainly as cellular "power plants", although it soon became clear that their role in cellular physiology is not restricted to ATP production for metabolic demands. Hence, mitochondria were shown to be crucial for the regulation of intracellular  $\text{Ca}^{2+}$  homeostasis, especially under pathological conditions; they also produce reactive oxygen species (ROS), as well as reactive nitrogen species (RNS), which are involved in the regulation of various physiological processes, but might be harmful to the cell if produced excessively. In addition, mitochondria are key participants in several cell death pathways. All these functions are of critical importance for cell viability and physiology, and it is obvious that perturbation of mitochondrial functions might play an important role in the pathogenesis of various human diseases. The present review is devoted to two of them, namely, neurodegenerative disease and cancer, of which the former features enhanced cell death and neuronal loss in selective brain regions, whereas the latter is characterized by the suppression of cell death pathways. It is also widely believed that the pathogenesis of several neurodegenerative diseases involves elevated mitochondrial generation of ROS, which is commonly associated with malfunctioning of certain complexes of the respiratory chain (Fig. 1). ROS can damage mtDNA, which is poorly protected by histones in contrast to nuclear DNA, and cause mutations in the respiratory complexes, thereby stimulating a "vicious cycle" (i.e., ROS production, mtDNA mutations, malfunction of the mitochondrial respiratory chain, further stimulation of ROS production, etc.). The fact that the incidence of neurodegenerative disease increases dramatically in the elderly might be explained by the accumulation of mutations within the mitochondrial genome with age.

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**Fig. 1** Mitochondria produce ROS, which are involved in the regulation of various physiological processes, but might be harmful to the cell if produced excessively. ROS can damage mtDNA, which is poorly protected by histones in contrast to nuclear DNA, and cause mutations in the respiratory complexes, thereby stimulating a “vicious cycle” (i.e., ROS production, mtDNA mutations, malfunction of the mitochondrial respiratory chain, further stimulation of ROS production, etc.). In addition, mitochondria are key participants in different cell death pathways. Release of pro-apoptotic proteins from the intermembrane space of mitochondria is a “point of no return” in many models of cell death

In contrast to the situation in neurodegenerative disease, most tumor cells are characterized by predominant glycolytic production of ATP, which is often associated with silencing of mitochondrial activity and resistance to cell death triggers. Accordingly, the aim of targeting mitochondria in cancer cells is to destabilize them and sensitize them towards anticancer drugs.

### Neurodegenerative disorders and cell death

Neurological disorders caused by mitochondrial deterioration are frequently associated with cell loss within specific brain regions [1], although the mode of cell death is often unclear. The main types of neuronal cell death observed in neurodegenerative diseases are apoptosis, necrosis and autophagy (for review see [2]). Of these, necrosis has long been regarded as a form of uncontrolled cell death. It is characterized by cell swelling, disruption of the plasma membrane, as well as the membranes of intracellular organelles, chromatin digestion, extensive DNA hydrolysis and, finally, cell lysis. One of the most characteristic features of necrosis is a drastic drop in ATP level due to mitochondrial collapse, and, as a result thereof, a perturbation of intracellular  $\text{Ca}^{2+}$  homeostasis. Necrosis is known to play a prominent role in many pathological conditions, including ischemia/reperfusion injury (e.g., stroke and myocardial infarction), trauma and, possibly, some forms of neurodegeneration.

Autophagy is a regulated lysosomal pathway involved in the degradation and recycling of long-lived proteins and organelles within cells. Although the mechanism of autophagy-induced cell death remains unclear, some evidence favors mitochondrial involvement in this process [3]. In particular, mitochondrial permeability transition (MPT, see below) and subsequent permeabilization of the outer mitochondrial membrane (OMM) might be responsible for stimulation of mitochondrial autophagy, a process that is important for the removal of damaged mitochondria [4]. Autophagy can be a highly efficient inducer of cell death by excessive self-digestion, for instance in apoptosis-deficient cells which are exposed to various lethal agents, including radiation [5].

Like autophagy, but in contrast to pathological necrosis, apoptosis represents a regulated form of cell death, which is characterized by a set of morphological and biochemical features. The apoptotic cell death machinery needs ATP; hence, the mitochondria remain relatively intact during the early apoptotic process, although permeabilization of the OMM is responsible for the release of mitochondrial pro-apoptotic proteins in some models of apoptosis. Apoptosis is an evolutionarily conserved and genetically regulated process of critical importance for embryonic development and maintenance of tissue homeostasis in the adult organism, which also plays a significant role in tumor cell biology [6]. Thus, apoptosis might be involved in the spontaneous elimination of potentially malignant cells, as well as in therapeutically induced tumor regression, whereas defects in the apoptosis program of cancer cells contribute to tumor progression and resistance to treatment.

In addition to the types of cell death discussed above, other pathways of cell elimination have been described; in particular, caspase-independent programmed cell death with necrotic morphology [7]. This mode of cell death can be dependent on ROS [8], and it appears to be of therapeutic significance in the treatment of both apoptosis-resistant tumors and degenerating adult neurons [9]. Recent evidence suggests that cells can initiate their own death by necrosis in a manner that leads to both inflammatory and/or reparative responses in the host. By triggering these adaptive responses, programmed cell necrosis may serve to maintain tissue and organism integrity [10]. Interestingly, in many cases inhibition of apoptosis does not block cell death, but rather results in a switch from apoptotic to regulated caspase-independent cell death mechanisms with morphological features resembling necrosis. One such mechanism, called necroptosis, has recently been described [11]. Hence, in the absence of intracellular apoptotic signaling, stimulation of the Fas/TNFR receptor family triggered necroptosis and activation of autophagy. The authors demonstrated further that necroptosis was responsible for delayed ischemic brain injury in mice in vivo

through a mechanism distinct from that of apoptosis. A specific and potent small molecular inhibitor of necroptosis, necrostatin-1, was shown to block a critical step in necroptosis, offering new possibilities for therapeutic neuroprotection. Necroptosis is a gene-regulated process. Recently, a set of 432 genes that regulate necroptosis in apoptotic-deficient conditions has been identified. The expression of these genes is enriched in the immune and nervous systems, and cellular sensitivity to necroptosis is regulated by an extensive signaling network mediating innate immunity [12].

Among the different forms of cell death, apoptosis and pathological necrosis are probably those that occur most frequently in neurodegenerative diseases [13]. Thus, recent studies have shown that in an animal model of multiple sclerosis the activation of caspase- and calpain-mediated pathways contributed to apoptotic death of oligodendrocytes and neurons, promoting the pathological events leading to neurological deficits. Apoptosis is also involved in the disease-regulating as well as in the disease-promoting processes in experimental autoimmune encephalomyelitis (EAE) [12], a widely recognized animal model of multiple sclerosis [14]. Apoptotic cell death occurs in the course of HD, a devastating neurodegenerative disorder caused by a mutation of huntingtin, which is crucial for normal development and may be regarded as a cell survival gene, whereas mutant huntingtin results in neuronal death [15]. The huntingtin interacting protein (HIP-1) was identified by its altered interaction with mutant huntingtin. Although its function is poorly understood, HIP-1 has recently been identified as a pro-apoptotic protein. Hence, overexpression of HIP-1 resulted in rapid, caspase-3-dependent cell death [16], which was associated with permeabilization of the mitochondria and the release of cytochrome *c* and apoptosis-inducing factor (AIF) into the cytosol [17].

The interaction between the different forms of cell death is complex and still a matter of debate [18]. In fact, apoptosis and necrosis can occur simultaneously in tissues or cell cultures exposed to the same stimulus; often the intensity of the initial insult decides the subsequent mode of cell death. Intracellular energy levels and mitochondrial function are rapidly compromised in necrosis, but not in apoptosis of neuronal cells. ATP generation by glycolysis, or by mitochondrial oxidative phosphorylation, is required for the active execution of the final phase of apoptosis [19]. Indeed, during ischemic brain injury, glutamate accumulation leads to excessive stimulation of postsynaptic glutamate receptors resulting in intracellular  $\text{Ca}^{2+}$  overload and neuronal cell death, either via necrosis or delayed apoptosis. During, and shortly after, exposure to glutamate, a subpopulation of neurons was shown to die by necrosis [20]. If, however, neurons survived the early necrotic phase and recovered mitochondrial potential and energy levels,

they later underwent apoptosis with formation of apoptotic nuclei and chromatin degradation into high- and low-molecular weight fragments [20]. Hence, mitochondrial stability is of critical importance and can determine the mode of neuronal death.

### Mitochondria—key participants in the cell death machinery

Mitochondrial alterations, such as stimulation of ROS production, inhibition of respiratory complexes leading to decreased ATP synthesis, loss of membrane potential, and release of pro-apoptotic proteins from the mitochondrial intermembrane space, have been shown to be involved in, and often responsible for, various manifestations of cell death (reviewed in [21]). The release of proteins from the mitochondrial intermembrane space is a “point of no return” in many models of apoptosis. Once released, cytochrome *c* triggers the formation of the apoptosome complex in the cytosol and subsequent activation of the caspase cascade, which is primarily responsible for the cleavage of cellular proteins leading to the biochemical and morphological characteristics of apoptosis. Thus, permeabilization of the OMM is a critical step in apoptosis induction.

### Mechanisms of OMM permeabilization

There are currently several different mechanisms that might explain the OMM permeabilization. The firstly described pathway, which can be engaged in both necrotic and apoptotic cell death, involves the induction of MPT. This phenomenon was described some thirty years ago by Haworth and Hunter [22]. They showed that  $\text{Ca}^{2+}$  uptake by mitochondria stimulates drastic changes in mitochondrial morphology and functional activity due to the opening of a non-specific pore, commonly known as the MPT pore, in the inner mitochondrial membrane (IMM). This is followed by osmotic swelling of the mitochondria, loss of their membrane potential, and rupture of the OMM, causing the release of intermembrane space proteins, including cytochrome *c*, into the cytosol. This process can be facilitated by inorganic phosphate, oxidation of pyridine nucleotides, ATP depletion, low pH, and ROS [23]. The MPT pore machinery is generally believed to be a multi-meric complex, composed of the voltage dependent anion channel (VDAC) located in the OMM, the adenine nucleotide translocase (ANT), an integral protein of the IMM, and a matrix protein, cyclophilin D (CypD) (Fig. 2a). The pore is thought to form at contact sites between the OMM and the IMM, and other proteins may bind to the pore

complex, in particular kinases (e.g., hexokinase, creatine kinase) [24].

MPT was shown to be a key event in necrotic and some experimental models of apoptotic cell death. If permeability transition and subsequent uncoupling of mitochondria occur in a large subpopulation of these organelles, this would lead to destructive consequences for the cell. Indeed, under such circumstances, the mitochondria would start to hydrolyze cytosolic ATP (uncoupling-stimulated ATPase activity) and, as a result, the ATP content would drop leading to cytosolic  $\text{Ca}^{2+}$  overload. Subsequent activation of calcium-dependent catabolic enzymes (proteases, phospholipases, nucleases, etc.) might lead to necrotic cell death. In contrast, pore opening in only a small fraction of mitochondria could lead to cytochrome *c* release and apoptosome formation, without any significant effect on intracellular ATP content or calcium homeostasis. This would lead to apoptotic cell death.

Another mode of OMM permeabilization involves pro-apoptotic members of the Bcl-2 family of proteins. The first indication that genes and proteins, which play a role in tumorigenesis, might be involved in the negative regulation of cell death, came from the observation that the Bcl-2 protein is overexpressed as a result of a chromosomal translocation in B-cell lymphomas [25]. Overexpression of this protein was shown to inhibit cell death induced by a range of stimuli, such as IL-3 deprivation, chemotherapeutic agents and heat shock (reviewed in [26]). In an early publication, the Bcl-2 protein was shown to be localized to the mitochondria, although the precise mechanism of its action was still unclear [27]. Accumulation of Bcl-2 in mitochondria triggered a set of metabolic changes leading to the stabilization of this organelle. Cells overexpressing Bcl-2 demonstrated higher mitochondrial membrane potential than wild-type cells, which was thought to be a reason of the enhanced resistance towards TNF [28].

Today, more than thirty members of the Bcl-2 family and related proteins have been identified. They can be divided into three subgroups: Bcl-2-like survival factors, Bax-like, and BH3-only death factors [29]. There is accumulating evidence supporting a view that anti-apoptotic members of the Bcl-2 family act as oncogenes [30]. Thus, transgenic overexpression of Bcl- $X_L$  induced lymphomagenesis, or development of pancreatic B-cell tumors, and overexpression of Mcl-1 resulted in B-cell lymphomas. Bcl-w, which is expressed in almost all murine myeloid cell lines analyzed and in a wide range of tissues, is frequently overexpressed in colorectal adenocarcinomas and appears to play a role in the progression from adenoma to adenocarcinoma in the colorectal epithelium. Bcl-w is also expressed in a majority of infiltrative gastric adenocarcinomas.

Permeabilization of the OMM was shown to require the oligomeric form of the pro-apoptotic Bcl-2 family members, Bax or Bak (Fig. 2b). Oligomerization of Bax can result from its binding to the truncated form of the BH3-only, pro-apoptotic protein Bid (tBid) after cleavage by proteases, e.g., caspase-8 or caspase-2. Cells deficient in both Bax and Bak, but not cells lacking only one of these proteins, have been found to be resistant to tBid-induced cytochrome *c* release and apoptosis. Moreover, Bax- and Bak-deficient cells were also resistant to a variety of apoptotic stimuli that act through the mitochondrial pathway [31]. Thus, activation of a “multidomain”, pro-apoptotic Bcl-2 family member, Bax or Bak, appears to be a principal gateway to mitochondrial release of pro-apoptotic proteins required for cell death in response to diverse stimuli. Anti-apoptotic proteins, e.g., Bcl-2, Bcl- $X_L$ , Mcl-1, and Bcl-w, interact with the pro-apoptotic proteins, Bax and Bak, to prevent their oligomerization. A critical factor that regulates apoptosis induction is the balance between pro- and anti-apoptotic proteins in the OMM. It has been shown for many tumors that the mitochondrial pathway of apoptosis is suppressed due to a disproportion between anti- and pro-apoptotic mediators in favor of the former [32]. Thus, overexpression of Bcl-2 and Bcl- $X_L$  in cancer cells contributes to the drug resistance characteristic of various tumors. Disruption of the balance between anti- and pro-apoptotic Bcl-2 family members in favor of the latter can proceed by mechanisms involving BH3-only proteins that bind to and occupy the anti-apoptotic proteins, thereby liberating Bax and Bak, or change their conformational state to permit the formation of pores.

Recently, a new mode of OMM permeabilization was found, which occurs through the opening of a mitochondrial apoptosis-induced channel (MAC), large enough to allow the passage of intermembrane space proteins, in particular cytochrome *c*, into the cytosol [33]. Permeability of MAC can be regulated by Bcl-2 family proteins. Thus, depletion of Bax, which is an essential constituent of MAC in some systems, significantly diminished MAC activity, whereas overexpression of Bcl-2 blocked formation of MAC and cytochrome *c* release [34]. In contrast to MPT induction, opening of MAC does not affect the barrier properties of the IMM.

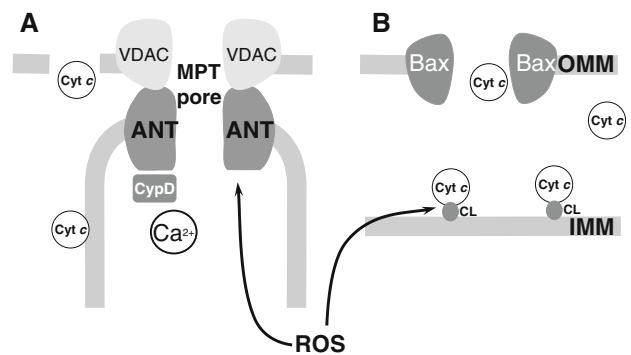
#### ROS facilitate OMM permeabilization

Mitochondria are thought to consume over 90% of the cellular oxygen in unstressed cells and are considered the major sites of aerobic ROS production [35]. In any cell, the majority of ROS are by-products of mitochondrial respiration. Approximately 2% of the molecular oxygen consumed during respiration are converted into the

superoxide anion radical, the precursor of most ROS. Normally, a four-electron reduction of  $O_2$ , resulting in the production of two molecules of water, is catalyzed by complex IV of the mitochondrial respiratory chain. However, the electron transport chain contains several redox centers (e.g., in complex I and III) that can leak electrons to molecular oxygen, serving as the primary source of superoxide production in most tissues. The one-electron reduction of oxygen is thermodynamically favorable for most mitochondrial oxidoreductases. Superoxide-producing sites and enzymes were recently analyzed in detail (for review see [36]). ROS, if not detoxified, oxidize cellular proteins, lipids, and nucleic acids and, by doing so, can cause cell dysfunction or death. A multitude of water- and lipid-soluble antioxidants and antioxidant enzymes suppress the harmful ROS activity. An imbalance that favors the production of ROS over antioxidant defenses, defined as oxidative stress, is implicated in a wide variety of pathologies, including malignant and neurodegenerative diseases. It should be mentioned that mitochondria are not only a major source of ROS, but are also a sensitive target for the damaging effects of oxygen radicals. ROS produced by mitochondria can oxidize proteins and induce lipid peroxidation, compromising the barrier properties of biological membranes. One of the targets of ROS is mitochondrial DNA (mtDNA), which encodes several proteins essential for the function of the mitochondrial respiratory chain and, hence, for ATP synthesis by oxidative phosphorylation. mtDNA, therefore, represents a crucial cellular target for oxidative damage, which might lead to mutations/deletions and lethal cell injury through the loss of respiratory chain activity and ATP generation. mtDNA is especially susceptible to attack by ROS, owing to its close proximity to the electron transport chain, the major site of superoxide production, and the lack of protective histones. For example, the level of oxidatively modified bases in mtDNA is 10- to 20-fold higher than that in nuclear DNA. Oxidative damage caused by endogenous ROS production is probably a major source of mitochondrial genomic instability, leading to respiratory dysfunction (Fig. 1).

Both MPT- and Bax/Bak-dependent cytochrome *c* release is facilitated by ROS. As mentioned above, ROS facilitate MPT induction; oxidative stress and thiol reagents can modify two thiol groups on the ANT, thereby stimulating pore opening [37] (Fig. 2a). Thus, through excessive ROS generation and self-directed induction of MPT, mitochondria might regulate their release of proteins inducing apoptosis.

Cytochrome *c* is bound to the outer surface of the IMM by both electrostatic and hydrophobic interactions with the unique mitochondrial phospholipid, cardiolipin. Oxidation of cardiolipin reduces its binding affinity for cytochrome *c*



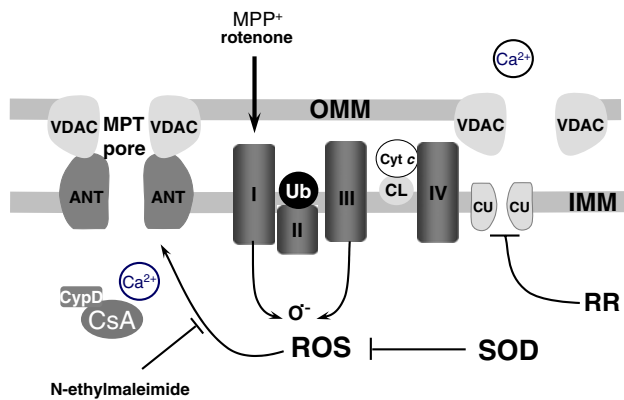
**Fig. 2** ROS facilitate OMM permeabilization. **a** Formation of ROS is a key factor facilitating MPT. It has been shown that oxidative stress and thiol reagents can modify two thiol groups on the ANT and thereby stimulate pore opening. **b** Stimulation of ROS and dissociation of cytochrome *c* from oxidized cardiolipin (CL) are critical events in cytochrome *c* release into the cytosol through pores formed by Bax/Bak

and increases the soluble pool of the hemoprotein in the intermembrane space. Hence, stimulation of ROS production and dissociation of cytochrome *c* from oxidized cardiolipin are critical early apoptotic events, promoting cytochrome *c* release into the cytosol through pores in the OMM formed by Bax/Bak [38] (Fig. 2b). This need for ROS during the early apoptotic process provides a reasonable explanation for the anti-apoptotic effects reported for multiple mitochondrial antioxidant enzymes [39].

### Involvement of mitochondria in the pathogenesis of neurodegenerative disorders

One of the most common neurodegenerative diseases is PD. The majority of PD cases involve the sporadic form of PD. Despite limited knowledge of the etiology of the sporadic form, mitochondrial dysfunction and oxidative stress are regarded as key players in its pathogenesis, although the causal relationship between oxidative damage and neurodegeneration is unclear.

The cause of dopaminergic cell death in PD presumably involves oxidative stress and mitochondrial complex I deficiency [40]. Complex I inhibitors, including 1-methyl-4-phenylpyridinium (MPP<sup>+</sup>), the active component of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which induces parkinsonism in man and various primates, are known to induce apoptosis in cell culture. It has further been shown that cyclosporin A and its non-immunosuppressant analog, *N*-methyl-4-valine cyclosporine, can suppress apoptosis induced by rotenone, MPP<sup>+</sup>, and tetrahydroisoquinoline in PC12 cells [41]. Similar protection was demonstrated by the inhibitor of the Ca<sup>2+</sup> uniporter, ruthenium red, the hydrophobic disulfide reagent,



**Fig. 3** Complex I inhibitors, rotenone or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine ( $MPP^+$ ), which induces parkinsonism in man and various primates, are known to trigger apoptosis in cell culture. Cyclosporin A suppresses apoptosis induced by rotenone or  $MPP^+$ . Similar protection was demonstrated by the inhibitor of the  $Ca^{2+}$  uniporter (CU), ruthenium red (RR), the hydrophobic disulfide reagent, *N*-ethylmaleimide, and the antioxidant enzymes, catalase and superoxide dismutase (SOD)

*N*-ethylmaleimide, butacaine, and the antioxidant enzymes catalase and superoxide dismutase [42] (Fig. 3). These results point to the involvement of  $Ca^{2+}$ -mediated MPT in the initiation of apoptosis, suggesting that novel neuroprotective strategies might be directed towards protection from mitochondrial dysfunction and apoptotic cell death.

Recently, induction of neurodegeneration has been associated with a perturbation of the regulation of mitochondrial fusion and fission [43]. In the cell, mitochondria form a highly dynamic network, which is maintained by continuous fusion and fission. During development, cell division, and under stress conditions the mitochondria within these networks can change in number and morphology. These mitochondrial dynamics are dictated by the equilibrium between fusion and fission of the organelles (for review, see [44]). Fusion is controlled by a complex regulatory system of multiple proteins that fuse both the OMM and the IMM in a coordinated manner, which maintains the integrity of both membranes as well as the intermembrane space and matrix compartments. Similarly, fission proceeds in a controlled way that prevents the leakage of soluble components from both the matrix and intermembrane space. Neurotoxins and/or oxidative stress, mutations in the mitochondrial fusion GTPases, mitofusin 2, a mitochondrial dynamin-related protein, and optic atrophy 1 (OPA1), can lead to perturbation of the cable-like morphology of the mitochondrial network and to impaired bioenergetics and mitochondrial migration that can trigger neurodegeneration.

The importance of fusion and fission in disease pathogenesis is emphasized by the observation that certain hereditary neurodegenerative disorders, such as autosomal

dominant optic atrophy and inherited motor neuropathy, Charcot-Marie-Tooth type 2A (CMT2A), are caused by mutations in mitofusin 2 and OPA1, respectively [45]. These mutations did not affect mitochondrial ultrastructure, mtDNA content, or respiratory capacity. Moreover, mitochondrial fusion occurred efficiently in CMT2A patient-derived fibroblasts [46]. Apparently, mitochondrial fusion can be maintained in cells that express mutant mitofusin 2 protein due to complementation by a second mitofusin, mitofusin 1 [47]. However, mutation in mitofusin 2 might still result in alteration of ER-mitochondria juxtaposition. Mitofusin 2 is enriched at the ER-mitochondria interface, which is important for their intercommunication during  $Ca^{2+}$  signaling. Ablation, or silencing, of mitofusin 2 in mouse embryonic fibroblasts and HeLa cells disrupts ER morphology and loosens ER-mitochondria interactions, thereby reducing the efficiency of mitochondrial  $Ca^{2+}$  uptake in response to stimuli that generate inositol-1,4,5-trisphosphate. Considering the role of mitochondria as cellular safety devices against cytosolic  $Ca^{2+}$  overload, mutation of mitofusin 2 might be responsible for  $Ca^{2+}$ -mediated neuronal cell death [48].

The discovery of the genes that are related to the rare familial form of PD clarified the molecular mechanisms involved in the pathogenesis of this disease. Thus, mutations in Parkin, PTEN-induced kinase 1 (pink1), and DJ-1 were found both in autosomal recessive forms and some sporadic cases of PD. Pink1 encodes a putative serine/threonine kinase with a mitochondrial targeting sequence. Removal of *Drosophila* Pink1 homologue (CG4523) function resulted in male sterility, apoptotic muscle degeneration, defects in mitochondrial morphology and increased sensitivity to multiple stresses, including oxidative stress [49]. Mutations in Parkin, which is implicated in the pathogenesis of autosomal recessive PD, caused marked alterations of mitochondrial structure and function and increased sensitivity to the mitochondrial stressors, rotenone and carbonyl cyanide 3-chlorophenylhydrazone. In addition, Parkin knockout muscle cells were more sensitive to the toxic effects of intracellular  $\beta$ -amyloid peptides ( $A\beta$ ) characteristic of AD, whereas Parkin expression in normal skeletal muscle cultures provided substantial protection against both mitochondrial toxins and overexpressed  $A\beta$  [50]. Further, the activity of mitochondrial respiratory enzymes in patients with Parkin gene mutations was markedly decreased [51]. In a variety of tissues with high-energy metabolism, such as germ line and adult flight muscle, Parkin mutant flies exhibited dramatic mitochondrial deteriorations [52]. Mitochondria were swollen and had severely fragmented cristae. Recently, a link has been established between pink1/Parkin and the mitochondrial fusion/fission machinery. Pink1/Parkin were shown to promote mitochondrial fission and/or inhibit

fusion by downregulating mitofusin 2 and Opa1, and/or upregulating drp1. Thus, interference with fusion and fission processes may represent a novel therapeutic strategy in PD [53].

Interestingly, in a murine disease model of multiple sclerosis, EAE mice lacking CypD, a key regulator of the MPT pore, developed EAE, but unlike wild-type mice, they partially recovered [54]. Moreover, examination of the spinal cords of CypD-knockout mice revealed a striking preservation of axons, despite a similar extent of inflammation. Furthermore, neurons prepared from CypD-knockout animals were resistant to ROS and RNS, which are responsible for axonal damage in multiple sclerosis. In addition, brain mitochondria lacking CypD sequestered substantially higher amounts of  $\text{Ca}^{2+}$ . Thus, opening of the MPT pore might be a key event in the axonal damage occurring during multiple sclerosis, and CypD could be a potential target for neuroprotective therapy of this disease.

In contrast to PD, the main mitochondrial target in AD is not Complex I, but cytochrome *c* oxidase. Analysis of the activity of this enzyme in platelet mitochondria isolated from patients with AD revealed a striking reduction of cytochrome oxidase activity, whereas other respiratory chain activities were not significantly different from control values [55]. As mentioned above, AD is characterized by excessive production of  $\text{A}\beta$ . Aggregation and extracellular deposition of this peptide cause deregulation of neuronal metabolism. Recent studies have shown that soluble forms of  $\text{A}\beta$  might be highly toxic. In particular, accumulation of  $\text{A}\beta$  in AD brain mitochondria was shown to precede extracellular deposition and to cause mitochondrial dysfunction and oxidative stress [56].

One of the reasons of mitochondrial deterioration in AD is the binding of  $\text{A}\beta$  to heme that triggers functional heme deficiency. This might in turn cause the release of oxidants (e.g., hydrogen peroxide) from mitochondria due to the loss of complex IV [57]. Indeed, hydrogen peroxide levels were found to be significantly increased in transgenic mice overexpressing amyloid precursor protein and correspondingly enhanced levels of soluble  $\text{A}\beta$ , suggesting that soluble  $\text{A}\beta$  could be responsible for the production of hydrogen peroxide in AD progression [58]. Cytochrome oxidase activity was found to be decreased, suggesting that mutant amyloid precursor protein and soluble  $\text{A}\beta$  might impair mitochondrial metabolism in the AD brain.

A recent study suggested that interaction of  $\text{A}\beta$  with one of the key components of the MPT pore, CypD, might be responsible for mitochondrial dysfunction in AD [59]. CypD-deficient mitochondria were resistant to  $\text{A}\beta$ -induced  $\text{Ca}^{2+}$ -mediated permeability transition, and CypD deficiency substantially improved learning and memory, as well as synaptic functions, in the AD mouse model. This

observation is in a good agreement with earlier findings that high CypD content of synaptic mitochondria results in increased vulnerability to permeability transition, and that lowering the level of CypD may be a protective mechanism [60, 61]. Although involvement of the pore machinery is a plausible explanation for the mitochondrial deterioration seen in AD brain mitochondria, there are currently no safe inhibitors of CypD, which are specific to the brain and could be used to treat AD in humans. Apparently, the functions of CypD, which is ubiquitously expressed in mammalian mitochondria, extend far beyond controlling the calcium sensitivity of the MPT pore [62].

### Therapeutic strategy

The mechanism of selective and age-dependent motor neuron degeneration in human ALS has not been defined, and the role of oxidants in motor neuron death remains largely unknown. Mutation of the antioxidant enzyme Cu/Zn-dependent superoxide dismutase (SOD1), a homodimeric metalloenzyme that catalyzes the dismutation of superoxide anions to  $\text{O}_2$  and  $\text{H}_2\text{O}_2$ , was thought to be responsible for at least some forms of ALS disease in humans [63]. The authors found eleven different SOD1 missense mutations in thirteen different ALS families. Further studies demonstrated that overexpression of mutant G93A-SOD1 gene, but not of the wild-type SOD1 gene, led to the clinical and pathological phenotype of ALS-like disease in a transgenic mouse model [64]. Transgenic mice overexpressing mutant SOD1 gene demonstrated mitochondrial dysfunction with elevated production of ROS and cytochrome *c* release that contributed to the death of motor neuron-like cells in vitro. Overexpression of mitochondrial antioxidant genes, MnSOD and GPX4 by stable transfection significantly increased cellular resistance to SOD1 mutation [65]. Thus, stimulation of mitochondrial antioxidant activity, or suppression of ROS production, can prevent motor neuron cell death induced by mutation in SOD1. Furthermore, treatment of mutant G93A-SOD1 transgenic mice with a spin trapping molecule, 5',5'-dimethylpyrroline-*N*-oxide, significantly delayed paralysis and increased survival.

Mutation in mitochondrial SOD1 (G93A-SOD1) in rat astrocytes caused inhibition of mitochondrial oxygen consumption, loss of ADP-dependent respiratory control, and decreased mitochondrial membrane potential [66]. Furthermore, superoxide radical formation in mitochondria was increased, and cells died by apoptosis. Similar defects were found in mitochondria isolated from the spinal cord of SOD1(G93A) rats. Motor neuron loss was prevented by preincubation of SOD1(G93A) astrocytes with antioxidants, or nitric oxide synthase inhibitors.

Hence, under the above experimental conditions, antioxidants selectively targeted to mitochondria had beneficial effects on cell function and survival. Recently, a novel antioxidant, named mitoQ, has been developed that selectively prevents mitochondrial oxidative damage. MitoQ is a ubiquinone derivative targeted to mitochondria by covalent attachment to a lipophilic triphenylphosphonium cation [67]. Accumulation of the cation is driven by the mitochondrial membrane potential, and ubiquinone inserted into the lipid phase of the IMM can be reduced by the respiratory chain. Further, similar to MitoQ, another mitochondria-targeting antioxidant, carboxy-proxyl nitroxide, also covalently coupled to a triphenylphosphonium cation (Mito-CP), prevented mitochondrial dysfunction, reduced superoxide production in SOD1(G93A) astrocytes, and restored motor neuron survival [66].

Recently, new mitochondria-targeting compounds, cationic plastoquinone derivatives, SkQs, containing positively charged phosphonium or rhodamine moieties coupled to plastoquinone by decane or pentane linkers have been developed. SkQs easily penetrate through planar, mitochondrial, and outer cell membranes, and reveal strong antioxidant activity in aqueous solutions, lipid micelles, liposomes, isolated mitochondria, and cells at low (nanomolar) concentrations. Although, at higher (micromolar) concentrations, SkQs demonstrate pronounced prooxidant activity, the “window” between the anti- and prooxidant concentrations is markedly wider than that for MitoQ [68]. SkQs were shown to prolong lifespan and prevent development of traits of senescence, to restore vision to blind animals, to inhibit tumor development, and to be beneficial in treatment of some ROS- and age-related diseases, such as heart arrhythmia, heart infarction, kidney ischemia, and stroke [69].

A novel class of cell-permeable small peptides (Szeto-Schiller peptides) was shown to selectively attack the IMM and to possess intrinsic mitoprotective properties. Studies with isolated mitochondrial preparations and cell cultures showed that these peptides can scavenge ROS, reduce mitochondrial ROS production, and inhibit MPT [70]. The peptides were very potent in preventing apoptosis and necrosis induced by oxidative stress, or by inhibition of the mitochondrial electron transporting chain, and they were also protective in animal models of ischemia-reperfusion, neurodegeneration, and renal fibrosis, being at the same time free of toxic adverse effects [71].

Attempts to find an efficient remedy against neurodegenerative disease brought promising results. A wide range of compounds were shown to protect against mitochondrial deterioration caused by MPP<sup>+</sup>, which causes cytotoxicity in dopaminergic neurons similar to that observed in PD patients. One such compound, Edaravone, a potent scavenger of hydroxyl radicals, has been demonstrated to be

beneficial for patients with acute ischemic stroke. Edaravone might also protect against MPP<sup>+</sup>-induced cytotoxicity in rat primary cultured astrocytes [72]. Edaravone prevented GSH depletion and downregulated mRNA expressions of NADPH oxidase membrane subunit gp91 and membrane-translocated subunit p47. In addition, it was shown to prevent MPP<sup>+</sup>-induced decrease in state 3 respiration and respiratory control ratio, and thereby inhibited ROS production evoked by MPP<sup>+</sup>. Further, Edaravone inhibited release of cytochrome *c* and AIF from the mitochondria. An antiepileptic drug, lamotrigine, similarly protected differentiated PC12 cells subjected to the complex I inhibitors, rotenone and MPP<sup>+</sup>, which otherwise caused formation of ROS and depletion of GSH, nuclear damage, and OMM permeabilization leading to cytochrome *c* release and caspase-3 activation [73]. Lamotrigine was suggested to exert its protective effects by suppression of oxidative stress and, as a result, inhibition of MPT. A similar effect was achieved by 5-hydroxydecanoate, a selective mitochondrial K(ATP) channel blocker, and glibenclamide, a cell surface and mitochondrial K(ATP) channel inhibitor. Both compounds prevented GSH depletion and suppressed ROS production in PC12 cells, thereby preventing MPT and subsequent release of pro-apoptotic proteins [74].

#### Coenzyme Q

A series of studies indicate that the neurodegenerative processes caused by mitochondrial dysfunction might be ameliorated by metabolic modifiers, such as coenzyme Q10, creatine, and L-carnitine, as well as by antioxidants, such as lipoic acid, vitamin E, and resveratrol. Compounds causing stabilization of the mitochondrial respiratory chain are actively being tested as therapeutic tools for suppression of mitochondrial deterioration in neurodegenerative diseases. Among these compounds is coenzyme Q10 (CoQ, ubiquinone), an electron carrier in the mitochondrial respiratory chain, shuttling electrons between complexes I and III. It has been shown that, in addition to this function, the reduced form of ubiquinone, ubiquinol, efficiently scavenges free radicals generated chemically within liposomal membranes. Ubiquinol-10 was about as effective in preventing lipid peroxidation as was vitamin E, which is considered the best lipid-soluble antioxidant in humans [75]. Thus, ubiquinol-10 is an important physiological lipid-soluble antioxidant. Indeed, CoQ was shown to be involved in ROS removal and prevention of oxidative stress-induced apoptosis in neuronal cells [76].

CoQ can modulate different pathways of cytochrome *c* release from mitochondria, thereby attenuating cell death. Some ubiquinone analogs were demonstrated to modulate MPT pore opening in isolated mitochondria and thought to

act through a common MPT pore-binding site rather than through oxidation–reduction reactions. It has been shown that the ubiquinone-binding site is directly involved in MPT pore regulation, and that quinones can stabilize the pore in the closed conformation [77]. Certainly, CoQ has been shown to efficiently reduce apoptotic cell death, attenuate ATP decrease, and block DNA fragmentation in response to serum deprivation, antimycin A, and ceramide [78]. Protection was accompanied by inhibition of mitochondrial depolarization, cytochrome *c* release, and caspase-9 activation. These results define a new structural class of pore inhibitors and open novel perspectives for the pharmacological modulation of the MPT pore in vivo. CoQ not only prevents MPT induction, but can also block the Bax-induced destabilization of mitochondria induced by oxidants. Thus, following a brief exposure of two human cell lines (fibroblasts and HEK293 cells) to H<sub>2</sub>O<sub>2</sub>, the intracellular level of ROS and the association of Bax with the mitochondria increased significantly, and the cells underwent apoptosis. However, 24 h pretreatment with CoQ10 successfully prevented both of these events. In the presence of CoQ10, cytochrome *c* remained within the mitochondria of H<sub>2</sub>O<sub>2</sub>-treated cells, indicating that the mitochondrial membranes were largely intact [79].

Considering the antioxidant potential of CoQ and its analogs, one can easily envision a role for these compounds in the protection from neurodegenerative diseases caused by oxidative stress and mitochondrial damage. Thus, orally administered CoQ10 markedly attenuated lesions produced by intrastriatal administration of malonate in rats [80]. Subsequent studies revealed that CoQ10 could also improve survival in transgenic mouse models of ALS and HD [81, 82]. Exogenous CoQ protected MC65 neuroblastoma cells from A $\beta$  protein precursor C-terminal fragment (APP CTF)-induced neurotoxicity in a concentration-dependent manner. Dietary supplementation of CoQ to C65/B16 mice for one month significantly suppressed brain protein carbonyl levels, markers of oxidative damage [83]. Similar protective effect was observed in the MPP<sup>+</sup> model of PD. Administration of various doses of two formulations of CoQ10 with food resulted in significant protection against loss of dopamine (DA) caused by MPP<sup>+</sup>, which was accompanied by a marked increase in the plasma concentration of CoQ10 [84]. CoQ10 also revealed a protective effect against DA depletion, loss of tyrosine hydroxylase neurons, and induction of  $\alpha$ -synuclein inclusions in the substantia nigra pars compacta in a chronic MPP<sup>+</sup> model (administration of MPP<sup>+</sup> for one month). These results provide further evidence that administration of CoQ10 might be a promising therapeutic strategy in PD. Indeed, recent clinical trials in PD, HD and Friedreich's ataxia suggest that supplemental CoQ can slow the

functional decline in these disorders, particularly in PD [85]. All these results indicate that orally administered CoQ might be a viable antioxidant strategy for neurodegenerative disease.

### Creatine

The mitochondrial decay observed in the different types of neurodegenerative disease results in a lowering of the level of ATP required for maintenance of ionic homeostasis and signaling activities of the cells in the central nervous system. Aerobic glycolysis, coupled to mitochondrial ATP synthesis via oxidative phosphorylation, is the primary pathway of ATP synthesis in the brain. However, the creatine kinase/phosphocreatine system provides an alternative source for rapid ATP synthesis [86]. In tissues and cells with high and fluctuating energy demands, ATP is continuously replenished from phosphocreatine by the action of the creatine kinase.

The loss of ATP is an early event in the neurotoxicity of glutamate and A $\beta$ . Creatine, the precursor of phosphocreatine, greatly reduced glutamate toxicity, as well as the toxicity of A $\beta$ , in embryonic neurons. Neurons from adult rats were also partially protected from a 24-h exposure to A $\beta$  by creatine, but protection was reduced in neurons from old animals [87]. Thus, fortified energy reserves are able to protect neurons against cytotoxic agents. Creatine also protected against MPP<sup>+</sup>-induced cell loss in cultures of rat embryonic ventral mesencephalic neurons [88]. Both creatine and phosphocreatine prevented death of cultured striatal and hippocampal neurons exposed to either glutamate or 3-nitropropionic acid. Apparently, this effect was due to enhancement of cytoplasmic high-energy phosphate levels rather than inhibition of MPT [89]. Thus, exogenous creatine supplementation might offer a tool for improvement of the survival of dopaminergic neurons.

### Carnitine

L-Carnitine is a natural nutrient essential for  $\beta$ -oxidation of long-chain fatty acids in mitochondria. Experiments with transgenic mice that overexpress a mutated form of human SOD1 and exhibit a phenotype similar to that observed in patients with ALS, revealed that administration of L-carnitine prior to disease onset significantly delayed the deterioration of motor activity and extended the life span of the animals [90]. A beneficial effect of the combined action of R- $\alpha$ -lipoic acid and acetyl-L-carnitine (LA/ALC) on mitochondrial functions was demonstrated in brain mitochondria from old rats. These mitochondria had significantly decreased endogenous antioxidant levels and SOD activity and exhibited oxidative damage to lipids and

proteins, as well as decreased activities of complex I, IV and V (ATP synthase) [91]. Feeding LA/ALC to old rats partially restored mitochondrial function to that observed in young rats, indicating that nutrients targeting mitochondria could ameliorate mitochondrial decay through prevention of oxidative damage. Thus, LA/ALC administration might benefit patients with neurodegenerative diseases.

### Cancer and mitochondria

In contrast to the aim of the therapeutic strategy in neurodegenerative disease, when the purpose of manipulation of mitochondrial functions is to prevent cell death, in cancer, the main goal of mitochondrial targeting is to kill malignant cells. One of the most important characteristics of tumor cells is a predominant glycolytic production of ATP, also under aerobic conditions, known as the “Warburg effect”. Hence, various cancer cell lines were shown to rely on glycolysis for ATP generation to different extents, but, typically, the most glycolytic tumor cells were also found to be the most aggressive ones [92]. In view of this peculiarity of tumor mitochondria, and the key role that they play in cell death pathways, they are a potential target for cancer chemotherapy. Exploitation of the Warburg effect could represent a novel and promising approach to overcome some of the limits of traditional radio- and chemotherapy, assuming that drugs suppressing glycolysis would display beneficial therapeutic activities.

The antitumor effect of glycolytic inhibitors is based predominantly on ATP depletion. Indeed, in a variety of cancer cells, inhibition of glycolysis with a non-metabolizable glucose analog, 2-deoxyglucose (2-DG) [93], or 3-bromopyruvate [94], caused a marked decrease in ATP level, especially in clones where mitochondrial ATP supply was compromised. ATP depletion led also to rapid dephosphorylation of the pro-apoptotic Bcl-2 family protein Bad, migration of Bax to the mitochondria, permeabilization of the OMM, and subsequent massive cell death [95]. Similar to the effects of inhibiting key steps in the glycolytic pathway, suppression of glucose transport might also sensitize tumor cells to anticancer agents. Thus, a glucose transporter inhibitor, phloretin, was reported to markedly enhance the anticancer effects of daunorubicin [96]. Combining inhibitors of glycolysis with conventional chemotherapeutic drugs might provide a novel therapeutic strategy to overcome drug resistance in hypoxia. Presently, several glycolytic inhibitors are in preclinical and clinical development. Thus, the cardiac drug digitoxin was shown to inhibit growth and induce apoptosis in cancer cells at concentrations commonly found in the plasma of cardiac patients treated with this

drug [97]. A key mechanism by which this natural product selectively targets cancer cells is inhibition of glycolysis.

### Induction of MPT

As mentioned above, overexpression of anti-apoptotic Bcl-2 family proteins in tumors might inhibit OMM permeabilization by Bax/Bak-mediated pore formation. If so, induction of MPT should overcome the resistance to OMM permeabilization by Bax/Bak. The anticancer effect of multiple conventional treatments (e.g., ionizing radiation, etoposide and arsenates) is based on their ability to stimulate ROS production. Anticancer drug-induced MPT may thus result from ROS-mediated modification of components of the MPT pore. For example, at higher doses the chemotherapeutic agent, arsenic trioxide, was found to cause oxidative modification of thiol groups in ANT and subsequent release of cytochrome *c* through MPT induction. However, at clinically achievable concentrations this drug stimulated cytochrome *c* release and apoptosis through a Bax/Bak-dependent mechanism [98]. Distinct, dose-dependent pathways of cytochrome *c* release were also shown for the DNA-damaging anticancer drug, etoposide. This drug was found to stimulate MPT induction and cytochrome *c* release at higher concentrations [99], but at low, therapeutic doses it affected mitochondria through activation of caspase-2. Apparently, both pathways might be relevant for its clinical effects.

Another component of the MPT pore is VDAC. Interaction of VDAC with hexokinase II, a key glycolytic enzyme that is usually upregulated in tumor cells, not only facilitates glucose phosphorylation at the expense of ATP produced by mitochondria, but also keeps VDAC in the open state, which counteracts OMM permeabilization. Another consequence of hexokinase-VDAC interaction is that it prevents binding of pro-apoptotic proteins to VDAC and thereby the induction of apoptosis [100]. A variety of compounds stimulate cell death via interaction with VDAC. Thus, avicins, pro-apoptotic, anti-inflammatory molecules with antioxidant effects both in vitro and in vivo, perturb mitochondrial functions and initiate apoptosis in tumor cells. Biophysical studies using lipid bilayers revealed that avicins target and close VDAC. Closure of VDAC would lead to an overall lowering of the cell energy metabolism, subsequently pushing these cells towards the apoptotic pathway by permeabilization of the OMM and release of cytochrome *c* [101]. This observation, however, is in contrast with the report that hexokinase-I, which binds to VDAC and causes its closure, inhibits cytochrome *c* release and protects against apoptotic cell death [102]. Apparently, the role of VDAC in apoptotic cell death is still obscure and needs to be further investigated.

### Neutralization of anti-apoptotic Bcl-2 family proteins

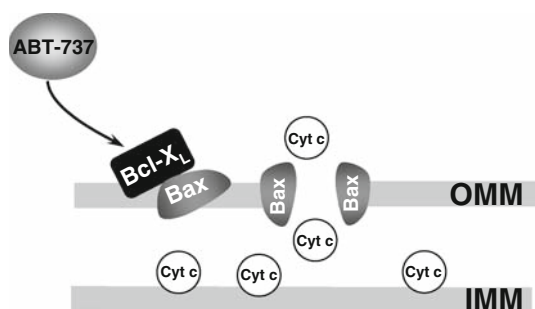
The anti-apoptotic proteins, Bcl-X<sub>L</sub> and Bcl-2, are over-expressed in many cancers and contribute to tumor initiation, progression and resistance to therapy. Localization of these proteins in the OMM stabilizes the membrane and prevents the release of cytochrome *c* and other pro-apoptotic proteins upon treatment with anticancer agents. Recently, ABT-737, a small molecule that binds to the anti-apoptotic proteins Bcl-2, Bcl-X<sub>L</sub> and Bcl-w, was synthesized. ABT-737 can disrupt intracellular Bcl-2 family protein-protein interaction. When released from the complex with Bcl-2 or Bcl-X<sub>L</sub>, Bax can form pores in the OMM, causing cytochrome *c* release (Fig. 4). Mechanistic studies revealed that ABT-737 does not directly initiate the apoptotic process, but that it enhances the effects of death signals and displays synergistic cytotoxicity with chemotherapeutics and radiation [103]. ABT-737 was found to augment TRAIL-induced cell killing by releasing Bim and Bak from their binding sites and to enhance Bax conformational changes induced by TRAIL in human pancreatic cancer cells [104]. Further, ABT-737 stimulated the activity of vincristine, L-ASP, and dexamethasone in lymphoblastic leukemia in vitro and in vivo [105]. In addition, ABT-737 was shown to induce apoptosis in chronic myeloid leukemia cells with diverse drug-resistance mechanisms [106], in lymphomas and small-cell lung carcinoma lines, as well as in primary patient-derived cells and in animal models [107]. ABT-737 was also shown to improve survival and initiate regression of established tumors in a high percentage of tumor-bearing mice [103].

Recently, it has been shown that ABT-737 not only disrupts pro- and anti-apoptotic Bcl-2 protein-protein interaction, but also induces IMM permeabilization, resulting in mitochondrial matrix swelling and rupture of

the OMM, thereby permitting the rapid efflux of cytochrome *c* from the mitochondrial intermembrane space [107]. This observation can be viewed as additional support for a link between Bcl-2 family proteins and MPT induction [108]. ABT-737 and a related orally active derivative, ABT-263, bind with high affinity to Bcl-2, Bcl-X<sub>L</sub> and Bcl-w, and both compounds promise to be useful tools for mechanistic studies. ABT-263 is in early clinical trials in lymphomas, small-cell lung cancer and chronic lymphocytic leukemia [109].

Another BH3-mimetic, 072RB, is localized to mitochondria and was shown to cause cell death in various cultured leukemic cells, as well as in cells derived from acute myeloid leukemia (AML) patients [110]. Intravenous administration of 072RB to xenografts of human AML cells in NOD/SCID mice caused a significant delay of leukemic cell growth with no evidence of toxicity to normal tissue. Thus, BH3 mimetics can be used as anticancer agents, both in mono- and combinatorial therapy.

Similar beneficial effects were achieved using non-peptide inhibitors of Bcl-2. Thus, antimycin A, a mitochondrial complex III inhibitor, which binds to Bcl-2 and Bcl-X<sub>L</sub> [111] was shown to stimulate mitochondrial swelling; and HA14-1 (ethyl 2-amino-6-bromo-4-(1-cyano-2-ethoxy-2-oxoethyl)-4H-chromene-3- carboxylate) was the first small molecular weight Bcl-2 inhibitor shown to induce apoptosis in several tumor cell lines [112]. Another pan-Bcl-2 inhibitor, GX15-070, was found to promote the release of cytochrome *c* from isolated leukemia cell mitochondria, and induced apoptosis in B cells from nine of the eleven chronic lymphocytic leukemia samples studied [113]. Despite encouraging results of in vitro experiments with compounds targeting Bcl-2 family proteins, the in vivo effects of these compounds need to be further investigated.



**Fig. 4** Localization of the pro-apoptotic proteins Bcl-X<sub>L</sub> and Bcl-2 in the OMM stabilizes the membrane and prevents the release of cytochrome *c* and other pro-apoptotic proteins upon treatment with anticancer agents. ABT-737, a small molecular inhibitor of the anti-apoptotic proteins Bcl-2, Bcl-X<sub>L</sub> and Bcl-w, can disrupt intracellular Bcl-2 family protein-protein interaction. When released from the complex with Bcl-2 or Bcl-X<sub>L</sub>, Bax can form pores in the OMM and cause cytochrome *c* release

### Concluding remarks

The involvement of mitochondrial malfunction in cancer and neurodegenerative disease etiology suggests that a novel strategy might be employed in fighting these diseases. Mitochondria are key participants in various cell death programs, and impairment of mitochondrial function as a result of inhibition of respiratory chain complexes occurs in multiple neurodegenerative diseases. This leads to abnormal production of ROS and/or RNS causing damage to mtDNA, mutations in mitochondrial genes that encode protein components of the respiratory chain, decreased respiration and ATP synthesis and, as a result thereof, further damage to the mitochondria. The destabilization of mitochondria facilitates mitochondrial collapse and release of death promoting proteins from their intermembrane space. Targeting the mitochondria represents a promising

**Table 1** Targeting of mitochondria as a therapeutic strategy

Drug or compound	Target	Desired effect	Reference
2-Deoxyglucose	Glucose uptake	Inhibition of glucose transport or of the initial steps of glycolysis	[93]
3-Bromopyruvate	Hexokinase (HK1 and HK2)	Inhibition of enzymatic activity and dissociation from mitochondria	[94]
Dichloroacetate (DCA)	Pyruvate dehydrogenase kinase 1 (PDK1)	Inhibition of PDK1, depolarization of mitochondria, activation of Kv1.5 channels	[114]
Somatostatin and its derivative TT-232	Pyruvate kinase (PK) isoenzyme PKM2	Translocation of PKM2 to the nuclei leads to apoptosis	[115]
siRNA	Lactate dehydrogenase A (LDHA)	Inhibition of LDHA leading to stimulation of mitochondrial respiration and decrease of mitochondrial membrane potential	[116]
Sorafenib	Acetyl-CoA carboxylase (ACC)	Inhibition of ACC leading to apoptosis or autophagy	[117]
Cell-permeable derivatives of $\alpha$ -ketoglutarate	HIF-1 $\alpha$ prolyl hydroxylase (PHDs)	Reversal of fumarate- or succinate-mediated inhibition of PHDs	[118]
Echinomycin	HIF1	Inhibition achieved by DNA binding	[119]
<i>N</i> -acetylcysteine (NAC)	ROS	Neutralization of ROS, leading to reduction of HIF1 function via PHDs and VHL	[120]
Tirapazamine	Hypoxia	Hypoxia-activated prodrug that leads to cytotoxic effect	[121]
Angiostatin	F <sub>1</sub> F <sub>0</sub> ATP synthase	Inhibition of proton pump	[122]
Oblimersen	Anti-apoptotic Bcl-2 family proteins	Downregulation of Bcl-2 and/or Bcl-X <sub>L</sub>	[123]
ABT737 (ABT263)	Anti-apoptotic Bcl-2 family proteins	Acts as BH3-only mimetic to induce release of mitochondrial pro-apoptotic proteins	[103]
SAHB (stabilized alpha helix of Bcl-2 domains)	Anti-apoptotic Bcl-2 family proteins	Acts as BH3-only mimetic to induce release of mitochondrial pro-apoptotic proteins	[124]
072RB	Anti-apoptotic Bcl-2 family proteins	Acts as BH3-only mimetic to induce release of mitochondrial pro-apoptotic proteins	[110]
HA14-1	Anti-apoptotic Bcl-2 family proteins	Acts as BH3-only mimetic to induce release of mitochondrial pro-apoptotic proteins	[112]
GX15-070	Anti-apoptotic Bcl-2 family proteins	Acts as BH3-only mimetic to induce release of mitochondrial pro-apoptotic proteins	[113]
Arsenic trioxide	Permeability transition pore	Inhibition of ANT and VDAC	[98]
Lonidamine	Permeability transition pore	Hexokinase and ANT inhibitor	[125]
2-Metoxystrobin	Redox balance	Inhibitor of manganese superoxide dismutase	[126]
$\alpha$ -TOS	Respiratory chain	Induces destabilization of mitochondria via accumulation of ROS	[127]
PRIMA-1	p53	Induces reactivation of p53, leading to expression of Bax and PUMA	[128]
MitoQ	Mitochondria	Prevents ROS-induced mitochondrial dysfunction	[67]
SkQ	Mitochondria	Prevents ROS-induced mitochondrial dysfunction	[68]
Szeto–Schiller peptides	IMM	Prevents MPT, efficient in animal models of ischemia-reperfusion, neurodegeneration, and renal fibrosis	[70]
Edaravone	Hydroxyl radicals	Scavenger of radicals, efficient for acute ischemic stroke	[72]
Lamotrigine	Complex I of respiratory chain	Protects from rotenone toxicity	[73]
Coenzyme Q10	Mitochondria	Scavenger of free radicals, prevents lipid peroxidation	[75]
Creatine	Mitochondria	Protects from neurotoxicity of glutamate and A $\beta$	[87]
L-Carnitine	Mitochondria	Essential for $\beta$ -oxidation of long-chain fatty acids in mitochondria, restores mitochondrial dysfunction	[90]

approach in fighting both cancer and neurodegenerative disease (Table 1). Stabilization of the mitochondria by antioxidants, or other compounds supporting the activity of

the mitochondrial respiratory chain, can protect mitochondria and also display therapeutically beneficial effects when administered to patients with neurodegenerative diseases. In

contrast, stabilization of mitochondria in tumor cells is responsible for resistance of a variety of tumors to treatment. Thus, stimulation of destructive processes in cancer cell mitochondria could be a useful approach, facilitating mitochondrial collapse and stimulating cell death processes.

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