Published in final edited form as:

Future Med Chem. 2013 January ; 5(1): 53–67. doi:10.4155/fmc.12.190.

Targeting cancer cell mitochondria as a therapeutic approach

Shijun Wen^{1,2}, Daqian Zhu^{1,2}, and Peng Huang^{*,1,3}

¹State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong, China

²School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, Guangdong, China

³Department of Molecular Pathology, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA

Abstract

Mitochondria are double membrane-enveloped organelles that play a central role in cellular metabolism, calcium homeostasis, redox signaling and cell fates. They function as main generators of ATP, metabolites for the construction of macromolecules and reactive oxygen species. In many cancer cells, mitochondria seem dysfunctional, manifested by a shift of energy metabolism from oxidative phosphorylation to active glycolysis and an increase in reactive oxygen species generation. These metabolic changes are often associated with upregulation of NAD(P)H oxidase. Importantly, the metabolic reprogramming in a cancer cell is mechanistically linked to oncogenic signals. Targeting mitochondria as a cancer therapeutic strategy has attracted much attention in the recent years and multiple review articles in this area have been published. This article attempts to provide an update on recent progress in identification of mitochondria-associated molecules as potential anticancer targets and the respective targeting compounds.

Mitochondria are special cellular organelles with their own genomic materials known as mitochondrial DNA (mtDNA), which may replicate independent of the nuclear DNA replication system. The well-characterized functions of mitochondria include energy metabolism, calcium homeostasis, redox regulation and apoptosis. As a main powerhouse of cells, mitochondria can use glucose, fatty acids, amino acids and other cellular materials to produce ATP through a series of biochemical processes known as oxidative phosphorylation. Electron transport through the mitochondrial respiratory chain is an essential requirement for oxidative phosphorylation, and this process is associated with the generation of **reactive oxygen species (ROS)**, which can be formed when electrons leaking from the respiratory complexes react with molecular oxygen to produce superoxide. Since a proper level of cellular ATP and redox balance are essential for cell viability and proliferation, mitochondrial dysfunction would cause major changes in cellular energy metabolism and ROS generation, thus, profoundly affecting cell fates and drug response. As such, targeting mitochondria using proper pharmacological agents is considered an attractive therapeutic strategy to kill cancer cells.

© 2013 Future Science Ltd

*Author for correspondence: Tel.: +1 713 834 6044 phuang@mdanderson.org.

Financial & competing interests disclosure

The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

Biochemical basis for therapeutic selectivity

Since cancer cells exhibit various degrees of mitochondrial dysfunctions such as change in energy metabolism, increased transmembrane potential and elevated ROS generation [1–3], these changes provide a possibility to preferentially target cancer cell mitochondria and improve therapeutic selectivity. Furthermore, it has been demonstrated that mtDNA is an important target of multiple anticancer drugs that interact with DNA, and that mitochondria determine the efficacy of this class of anticancer drugs [4]. Several approaches have been developed as possible means to selectively kill cancer cells based on their mitochondrial dysfunction and metabolic alterations.

The increase of ROS in cancer cells is associated with multiple changes in cellular functions such as cell proliferation, migration, differentiation and apoptosis. The increase in ROS generation in cancer cells with mitochondrial dysfunction may make them more vulnerable to further oxidative stress, compared with the normal cells with lower ROS output. For example, phenethyl isothiocyanate, a natural product found in cruciferous vegetables, has been shown to have a potent anticancer activity by disabling the glutathione (GSH) antioxidant system, resulting in severe ROS accumulation in cancer cells and consequently oxidative damage and cell death [1,2]. Interestingly, high levels of mitochondrial ROS generation in hypoxic cells seem to link to angiogenesis-related diseases such as cancer and ischemic disorders. In a phenotypic cell-based screening of a small-molecule library, an angiogenesis inhibitor YCG063 was identified to suppress mitochondrial ROS generation and inhibit *in vitro* angiogenic tube formation and cell invasion [5].

The difference between normal and cancer cells in their energy metabolism provides an important biochemical basis for development of new strategies and novel agents to selectively target cancer cells. In normal cells with competent mitochondria, the Krebs cycle generates key metabolic intermediates for the construction of biomolecules and NADH for utilization by the mitochondrial electron transport chain (METC) to fuel oxidative phosphorylation and generate the majority of ATP for cells. However, cancer cells seem to have certain mitochondrial dysfunction due to a variety of factors, such as oncogenic signals and mtDNA mutations, and thus, rely more on the glycolytic pathway in the cytosol to generate the metabolic intermediates and ATP. Such an increase in aerobic glycolysis in cancer cells is known as **the warburg effect**. Since cancer cells are more dependent on glycolytic metabolism, the key enzymes in this pathway have been considered as potential therapeutic targets. For example, HKII, a key glycolytic enzyme that catalyzes the phosphorylation of glucose and also plays a protective role in preservation of mitochondrial integrity by its association with certain mitochondrial molecules, is often overexpressed in cancer cells and has been considered as a potential target for anticancer agents. Indeed, induction of HKII dissociation from mitochondria by compounds such as 3-bromopyruvic acid and cerulenin leads to apoptosis of cancer cells [6,7]. A recent study demonstrated that 3-bromopyruvate propyl ester preferentially inhibited another key glycolytic enzyme, GAPDH, and exhibited potent activity in causing ATP depletion and cell death in colon cancer cells [8]. The same study also demonstrated that GAPDH is overexpressed in cancer cells compared with normal cells.

In addition to their critical roles in energy metabolism and redox regulation, mitochondria also play important roles in calcium homeostasis and regulation of apoptosis through their effectors such as cytochrome c, which induces apoptosis by activating caspases if released to cellular cytosol, and AIF, which causes caspase-independent apoptosis when it is translocated to the nucleus. Thus, mitochondrial dysfunction in cancer cells might render them more prone to induction of cytochrome c or AIF release, hence, using compounds to preferentially trigger the release of apoptotic factors from mitochondria is an attractive

strategy to selectively kill cancer cells. Importantly, mitochondria-targeted molecules may be able to kill drug-resistant cancer cells due to their ability to initiate mitochondrial outer membrane permeabilization independently of upstream signaling processes, which may be impaired in cancer cells [9].

The authors have previously reviewed many of the mitochondria-targeted compounds [10,11]. This article summarizes the most recently discovered mitochondria-associated proteins that could be potential targets of cancer therapeutics, and also provides an update on small molecules that directly or indirectly target the mitochondria-associated molecules that play critical roles in cancer cell growth and viability. Figure 1 illustrates some of the key pathways/targets and the compounds that affect these targets. The following sections provide more detailed descriptions of potential therapeutic targets associated with mitochondria and some of the targeting agents reported.

Potential therapeutic targets associated with mitochondria

NOX are enzyme complexes capable of oxidizing NADPH or NADH to NADP⁺ or NAD⁺, leading to generation of superoxide by a one-electron reduction of oxygen [12]. NOX enzyme activity is mainly associated with cellular membranes, and certain NOX isoforms are associated with the mitochondrial membranes. A recent study suggests that NOX is upregulated in cancer cells and that the NAD⁺ produced by NOX seems important to promote glycolysis in cancer cells [13]. As such, NOX may serve as a cancer therapeutic target. Indeed, Lu *et al.* demonstrated that inhibition of NOX could significantly reduce the source of NAD⁺ needed for active glycolysis in cancer cells with mitochondrial dysfunction, resulting in cancer cells death [13]. Upregulation of NOX was also consistently observed in cancer cells with compromised mitochondria due to the activation of oncogenic ras or the loss of tumor suppressor p53. Increase expression of the NOX components, such as p22, was observed in primary pancreatic cancer tissues. Suppression of NOX by diphenyleneiodium chloride or by genetic knockdown was shown to selectively impact cancer cells with mitochondrial dysfunction, leading to a decrease in cellular glycolysis, a loss of cell viability and a significant inhibition of cancer cell growth *in vivo*. These findings suggest that NOX is potentially a novel target for cancer treatment [13].

TSPO is a nuclear-encoded 18-kDa protein associated with the mitochondrial membrane that interacts with the mitochondrial multiprotein complex known as mitochondrial permeability transition pore, which includes voltage-dependent anion channel (VDAC) associated with the outer mitochondrial membrane and ANT associated with the inner mitochondrial membrane as the core components. TSPO can transport cholesterol into mitochondria for steroid synthesis. A recent study demonstrated that TSPO ligands might be valuable in the treatment of neurological and psychiatric disorders [14]. TSPO has also been found to be upregulated in human cancer cells including colorectal tumors. Ostuni *et al.* revealed that activation of TSPO induced a rise in cytosolic Ca²⁺ in human colon cancer cell HT-29, leading to the stimulation of Cl⁻ secretion [15]. Due to its overexpression in numerous cancer cells, the ligands of TSPO can be conjugated with signaling moieties for TSPO-targeted tumor imaging [16]. Since TSPO is associated with mitochondrial permeability transition pore, its interaction with VDAC plays a role in apoptotic cell death. TSPO was observed to mediate ROS generation which may provide a link between activation of TSPO and VDAC to stimulate the mitochondrial apoptosis pathway. The findings also implied that TSPO and VDAC may serve as targets to modulate apoptosis to treat diseases such as cancer [17]. High TSPO expression has been detected in approximately 80% of the patient-derived breast cancer mouse xenografts [18]. TSPO expression, nuclear localization and TSPO-mediated cholesterol transport into the nucleus are involved in regulating breast cancer cell proliferation and aggressive phenotype. Its expression seems to correlate with advanced

stages of this cancer. Significantly, treatment with high doses of TSPO ligand PK-11195 decreased cell proliferation, invasion and migration [19]. These recent results indicate that TSPO can be a biomarker of advanced stage breast cancer and a therapeutic target.

C1QBP, also known as p32, is a mitochondrial protein encoded by *C1QBP* gene. This molecule is often found upregulated in cancer cells. However, whether C1QBP has a tumor suppressive or tumorigenic function remains debatable. It has been reported that C1QBP seems required for cancer suppressor alternate reading frame to localize to mitochondria and to induce apoptosis, and that alternate reading frame mutations that disrupt p32 binding impair these functions [20,21]. C1QBP appears to act as an endogenous inhibitor of the mitochondrial permeability transition pore, most likely through its binding to cyclophilin D, and, thus, may protect cells from cytotoxicity induced by oxidative stress [22]. Interestingly, a knockdown of C1QBP expression in human cancer cells causes a significant decrease of mitochondrial oxidative phosphorylation, rendering the cells less tumorigenic *in vivo* [23]. C1QBP is highly expressed in prostate tumor samples and its expression is significantly associated with the Gleason score, pathological stage and disease relapse. Knocking down C1QBP by shRNA inhibited the growth of prostate cancer cell lines but not of a noncancerous cell line [24]. In fibroblasts, overexpression of C1QBP/p32 seems to protect the cells against staurosporine-induced apoptosis, and increase cell proliferation and cell migration in a 'wound-healing' assay. Consistently, C1QBP expression was found to be markedly elevated in human breast, lung and colon cancer cell lines, and a knockdown of C1QBP in MDA-MB-231 breast cancer cells suppressed cell proliferation and migration, and enhanced apoptosis induced by doxorubicin [25]. These recent findings suggest that C1QBP has a tumor-promoting function. As such, targeting this molecule may have an anticancer effect.

ANT2 are located on the inner mitochondrial membrane and mediates the exchange of ATP for ADP across the mitochondrial membrane. Thus, this molecule plays a critical role in maintaining the cellular energy supply to support cell growth and proliferation. Expression of ANT2 is activated by growth stimulation of quiescent cells and is downregulated when cells become growth-arrested. The expression of ANT2 was closely associated with the mitochondrial bioenergetics of tumors, and it might be exploited for the development of anticancer drugs [26,27]. ANT2 expression is upregulated in several hormone-dependent cancer cells, and a knockdown of ANT2 by shRNA provoked a minor increase in mitochondrial membrane potential (MMP) and ROS level, and reduced intracellular ATP concentration. ANT2 silence the facilitated MMP induction by the anti-tumor agent lonidamine. Further study suggested that ANT2 was an endogenous inhibitor of MMP and its inhibition could constitute a promising strategy of chemosensitization [28]. Treatment of MDA-MB-231 cells with ANT2 shRNA repressed cell growth and proliferation. In addition, cell cycle arrest, ATP depletion and apoptotic cell death were also detected in the ANT2 shRNA-treated breast cancer cells [29]. Knockdown of ANT2 by shRNA also downregulated oncoprotein HER2/neu by repressing the function of HSP90 and inhibited the PI3K/Akt signaling pathway, leading to suppressed migration and invasion of breast cancer cells [30]. ANT2 shRNA treatment sensitized cancer cell lines MCF7, T47 D and BT474 to TRAIL-induced apoptosis, implying ANT2 might be exploited to overcome TRAIL resistance in cancer [31]. Taken together, these studies have demonstrated that ANT is crucial for cancer survival and, thus, may be a promising therapeutic target in the mitochondria [32].

MCTs are a family of membrane-associated proteins that mediate the transport of several metabolites including lactate and pyruvate. MCTs contain a catalytic unit and an accessory subunit (CD147). An early study suggests that human MCT2 seems to function primarily as a pyruvate carrier and plays a special role in cancer cell metabolism [33]. In metastatic

cancer cells, the increased glucose consumption and metabolism via glycolysis provides a large amount of lactate as a end product, which must be transported out of the cells and MCTs play a key role in lactate efflux. Expression of MCT4 and CD147, an extracellular matrix metalloproteinase inducer, was found increased in highly metastatic breast cancer cell line MDA-MB-231. It was further found that MCTs and CD147 synergistically enhanced the metastatic potential of cancer cells through acidification of the tumor microenvironment and degradation of extracellular matrix partly via lactate efflux and induction of matrix metalloproteinase [34]. Another study demonstrated that MCT1 transported lactate to endothelial cells and triggered the phosphorylation and degradation of I κ B α and subsequently stimulated NF- κ B/IL-8 pathway to promote cancer cell migration and angiogenesis [35]. The mitochondria-associated MCTs are important for the transport of metabolic intermediates such as pyruvate, across the mitochondrial membranes to fuel the Krebs cycle and mitochondrial respiration. A recent paper demonstrated that supplementation of exogenous pyruvate was able to fuel mitochondrial oxygen consumption, leading to rapid proliferation of cancer cells [36]. Inhibition of cellular pyruvate uptake by a MCT inhibitor α -cyano-4-hydroxycinnamic acid impaired mitochondrial respiration and decreased cell growth, demonstrating the importance of mitochondrial metabolism in proliferative responses and a novel mechanism of action for MCT inhibitors through suppression of pyruvate-fuelled mitochondrial respiration.

Small molecules targeting mitochondria

Targeting mitochondrial membrane potential

Pancratistatin (Figure 2) is a natural alkaloid with potent apoptotic activity against a variety of cancer cells and low toxicity in noncancerous cell lines. This compound causes a decrease in mitochondrial membrane potential and induces apoptosis in colorectal carcinoma cell lines (HT-29 and HCT116). It also displays *in vivo* anticancer effect against human colorectal adenocarcinoma xenografts [37]. Interestingly, pancratistatin was not effective against mtDNA-depleted, respiration-defect cells (p^0), suggesting that the mechanism of action of this compound seems to require mitochondrial respiration.

Silver (I) complexes bearing a *N*-heterocyclic carbene (Figure 3) exhibit anticancer properties. The cytotoxic action of these silver-containing complexes seem to be mediated by inducing depolarization of the mitochondrial membrane potential, leading to translocation of AIF to the nuclei and activation of caspase-12 associated with endoplasmic reticulum [38]. A study using a fluorescent complex indicated that these compounds are localized in the mitochondria, although the precise target in the mitochondria remain unclear.

Rhodamine-123 (Figure 4) is well known for its ability to preferentially accumulate in the mitochondria in living cells and functions as a fluorescent chemical probe for mitochondria membrane potential. High retention of rhodamine-123 was often detected in various cancer cells [39] and such high accumulation of this chemical eventually disrupt mitochondria membrane potential. Kidney and breast cancer cells that retained rhodamine-123 longer than non-tumorigenic epithelial cells were highly sensitive to this compound [40]. The anticancer effect of rhodamine-123 could be enhanced by liposomal drug-delivery system. For instance, rhodamine-123 linked to amphiphilic PEG-phosphatidylethanolamine demonstrated better uptake by HeLa and B16F10 cells, leading to a higher in their mitochondria [41].

A naphthyridine derivative (Figure 5) 4-phenyl-2,7-di(piperazin-1-yl)-1,8-naphthyridine can cause peculiar changes in mitochondrial membrane potential. It first induces an increase in mitochondrial membrane potential, followed by a loss of the potential leading to the release

of apoptotic factors and consequent cancer cell death [42]. The precise mechanism responsible for the dual effects of this naphthyridine derivative on MMP remains unclear.

2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone (RH1; Figure 6) is a bio-reductive agent that can be activated by NAD(P)H quinone oxidoreductase 1. Treatment of NAD(P)H quinone oxidoreductase 1-overexpressing cells with RH1 caused rapid disruption of mitochondrial membrane potential, leading to translocation of AIF and endonuclease G from mitochondria to nuclei and an eventual cell death in a caspase-independent fashion [43]. This mitochondria-mediated apoptosis can be suppressed by inhibition of c-JNK pathway.

PMT7 (Figure 7) is a novel redox-active quinone phloroglucinol derivative capable of inducing cytotoxicity in cancer cells. Its anticancer activity seems due in part to the induction of mitochondria membrane potential loss and the subsequent loss of mitochondrial metabolic activity [44]. Mitochondrial respiration-defective p^0 cells were less sensitive to this compound compared with corresponding wild-type cells.

A recent study demonstrated that the combination of sodium selenite (Figure 8) and docetaxel exhibited synergistic effect in inhibiting the proliferation of metastatic prostate cancer cells (PC3) and inducing cell death mainly by apoptosis. This effect seems to correlate with the loss of mitochondria membrane potential [45].

1-*O*-octadecyl-2-*O*-methyl-racglycero-3-phosphocholine, (edelfosine, **Figure 9**) is a lipid raft-targeting agent with anti-tumor activity. This compound promotes a redistribution of lipid rafts from the plasma membrane to mitochondria, induces loss of mitochondrial membrane potential and apoptosis in human cervical carcinoma HeLa cells [46].

Targeting METC

α -tocopheryl succinate (α -TOS), a vitamin E analogue is a selective inducer of apoptosis in cancer cells. Inhibiting succinate dehydrogenase activity of METC complex II via a interaction with the proximal and distal ubiquinone binding site. It is also well established that α -TOS involves the accumulation of ROS [47]. In the attempts to enhance mitochondria-targeted delivery and improve the anticancer effect of α -TOS, a variety of modified vitamin E succinates with various alkyl chain lengths linked to a charged triphenylphosphonium group (TPP⁺) have been developed and tested [48]. One such derivative, MitoVES (Figure 10) with an 11-carbon linker, the mitochondrially targeted form of α -TOS (with higher anticancer activity), was found to referentially localize to mitochondria with a 1–2 log enhancement of anticancer activity compared with the parental α -TOS [49]. Similar to α -TOS, MitoVES was able to target the METC complex II and affect its function, leading to ROS generation and cell death due to depolarization of the mitochondria and activation of apoptotic cascades [39]. Using genetically modified cells, it was demonstrated that MitoVES caused apoptosis and generation of ROS in complex II proficient malignant cells but not in the complex II-mutated counterparts [48], suggesting that METC complex II is the key target of the vitamin E analogs.

Arsenic trioxide (Figure 11) has been used in traditional Chinese medicine to treat various diseases. This compound is particularly effective in the clinical treatment of acute promyelocytic leukemia [50]. Several studies demonstrated arsenic trioxide caused ROS generation and induced a decrease of mitochondria membrane potential and subsequent apoptosis [51,52], likely due to its inhibition on the METC leading to electron leakage and formation of superoxide [53]. A recent study demonstrated that combination of arsenic trioxide and a PDK inhibitor dichloroacetate had synergistic effect in inducing cell death [54]. Synergistic effect was associated with strong suppression of c-Myc and HIF-1 α .

expression, and reduced Bcl-2 expression. In the same study, arsenic trioxide was found to suppress mitochondrial function by the inhibiting cytochrome c oxidase, a critical component of the METC complex IV.

Photodynamic therapy (PDT) kills tumor cells by irradiating photosensitizers such as porfimer sodium with light to cause damage to the cancer cells. A recent study demonstrated that low-dose PDT induced intracellular ROS production by targeting the METC [55]. The increase in ROS induced by PDT seems to contribute to the increase in apoptosis. In another study, it was found that the hypocrellin-B derivative SL017 (Figure 12) targeted mitochondria and functioned as a sonosensitizer, causing generation of ROS, loss of mitochondrial membrane potential, and eventually mitochondrial fragmentation [56].

In addition to the compounds that inhibit METC complexes such as MitoVES and arsenic trioxide described above, several other compounds have recently been found to exert their anticancer activity by, at least in part, inhibiting METC. For instance, compound 11 β (CAS 865070-37-7; Figure 13), consisting of a DNA-damaging aniline mustard linked to an androgen receptor (AR) ligand, is expected to form covalent DNA adducts and induce apoptosis in AR-positive prostate cancer cells. Unexpectedly, 11 β also exhibited strong activity against AR-negative cancer cells *in vitro* and *in vivo* [57]. Mechanistic study revealed that this compound was able to inhibit complex I and cause ROS generation.

Aspirin (Figure 14), a widely used anti-inflammatory drug through inhibition of cyclooxygenase and suppression of prostaglandin, was recently found to inhibit METC complex I, complex IV and aconitase, leading to mitochondrial dysfunction, mitochondrial permeability transition and inhibition of ATP synthesis in human hepatoma HepG2 cells [58]. This mode of action was associated with an increase in ROS generation, a decrease in cellular GSH and induction of apoptosis.

The anticancer agent doxorubicin has been shown to cause mitochondrial depolarization, elevated matrix calcium levels, ROS generation, inhibition of mitochondrial respiration, and ATP depletion associated with cell cycle arrest and death [59]. This effect seems to be dependent on type p53 function, but cancer cells with p53 mutation or loss of function are resistant to doxorubicin. Ellipticine (5,11-dimethyl-6H-pyrido[4,3-b]carbazole; Figure 15) is able to reactivate certain mutant p53, enhances p53 mitochondrial translocation, inhibits the activity of METC complex I and restore sensitivity to doxorubicin [60].

Targeting mitochondrial apoptotic pathway

Curcumin (Figure 16) from the tumeric spice has long been used as an alternative medicine to treat a variety of diseases and disorders. This compound is able to induce cancer cell death via mitochondria-mediated apoptosis. A recent study suggests that curcumin may induce an Apaf-1-dependent caspase activation leading to apoptosis [61]. This study demonstrated that treatment of cancer cells with curcumin activated caspase 3, which could be antagonized by Apaf-1 silencing, and that the absence of p21-blocked cytochrome c release and caspase activation in curcumin-treated cancer cells. Upregulation of pro-apoptotic proteins, such as Bax, Bak, Bid and Bim was also observed. These findings seem to imply that curcumin induces cancer cell death through the mitochondria-mediated apoptotic pathway, perhaps by inducing the release of Apaf-1 or activation of the mitochondria-associated pro-apoptotic proteins. Curcumin also induced apoptosis in malignant melanoma A375 cells and the drug-resistant G361 cells at higher concentrations [62]. The combinational treatment with curcumin and tamoxifen, an estrogen receptor inhibitor that also affects mitochondrial respiratory function, resulted in a synergistic induction of apoptosis in chemoresistant melanoma cells, and displayed a significant increase in mitochondria depolarization and ROS generation.

Resveratrol (Figure 17) is a naturally occurring phytoalexin and is able to induce apoptosis in multiple cancer cell types. Resveratrol disturbs the interaction between Bax and the X-linked inhibitor of apoptosis protein in the cytosol and promotes Bax mitochondrial translocation and its oligomerization on the mitochondrial membranes, which leads to cytochrome c release and activation of apoptotic cascades [63]. Recent evidence has also revealed an interesting link between metabolism and apoptosis. For instance, suppression of the conversion of glutamate to α -ketoglutarate can antagonize resveratrol-induced cell death in castration-resistant human prostate cancer C4-2 cells. A similar effect was also observed by reducing extracellular glutamine concentration in the culture medium, implying that resveratrol-induced cell death is dependent on glutamine metabolism, a process frequently dysregulated in cancer [64]. Like MitoVES, resveratrol derivatives with a membrane permeable lipophilic TPP⁺ cation also can enhance the accumulation of resveratrol in mitochondria. Moreover, the derivatives selectively displayed cytotoxic effects on fast-growing cells [65].

Berberine (Figure 18) is a natural alkaloid, which was first isolated from *Mahonia swaseyi* approximately 70 years ago [66]. Berberine exhibited anticancer effects against a variety of cancer cell types including HeLa (cervical cancer), L1210 (murine leukemia), U937 (human leukemia) and B16 (murine melanoma) *in vitro* [67]. However, the mechanism of its anticancer activity remains elusive. A recent study demonstrated that berberine decreased colon tumor colony formation in agar, and induced cell death and LDH release in colon cancer cells, apparently by inducing the release of AIF from the mitochondria and its translocation to the nuclei in a ROS-dependent manner, leading to caspase-independent cell death [68].

Cerulenin (Figure 19) is an antibiotic that can be found in the culture filtrate of *Cephalosporium caerulens* [69]. The antifungal biological activity of this compound may be attributed in part to its inhibition of steroid and fatty acids biosynthesis [70]. However, the anticancer activity of cerulenin seems to be associated with induction of mitochondrial apoptotic pathway involving Bax upregulation and cytochrome c release [71]. More recently, cerulenin was found to disrupt the physical interaction between HKII and AIF in the mitochondria, causing AIF-mediated eventual cell death, which can be further potentiated by inhibition of PI3K [6].

Certain plant extracts have also been shown to interact with a multiple cellular targets including those associated with mitochondria, and influence a variety of biochemical and molecular cascades. For example, the decoction of *Hemidesmus indicus*, a plant found in South Asia, is able to cause an increase in the Bax/Bcl-2 ratio and a loss of mitochondrial membrane potential, leading to tumor cell death [72]. *Hemidesmus indicus* extract also causes a significant increase of Ca²⁺ through the mobilization of intracellular Ca²⁺ stores. Another study demonstrated that dandelion root extract (DRE) is able to induce ROS generation in isolated mitochondria and effectively cause apoptosis in human melanoma cells [73]. Since DRE directly stimulates ROS generation in isolated mitochondria, the potential target of DRE is likely located in mitochondria.

It was recently reported that two synthetic ruthenium complexes *trans,cis,cis*-[RuCl₂(DMSO)₂(H₂biim)] (Figure 20A) and *mer*-[RuCl₃(DMSO)(H₂biim)] (Figure 20B) exhibited cell growth inhibitory effect, which seemed to be mediated by inducing cell cycle arrest at G0/G1 phase and triggering mitochondria-mediated apoptosis [74]. In addition, *mer*-[RuCl₃(DMSO)(H₂biim)] exerts potent inhibitory effects on cell adhesion and migration of human cancer cells comparable to that of NAMI-A ([imidazole H][*trans*-[RuCl₄(imidazole)(DMSO-S)]]); Figure 20c).

The expression or activation status of certain mitochondria-associated proapoptotic proteins such as PUMA, BMF, BIM and NOXA in cancer cells may set the mitochondria at the stage close to the threshold of apoptosis: a property called mitochondrial priming. Such mitochondrial priming status seems to determine the sensitivity of cancer cells to anticancer agents and is correlated with clinical response to chemotherapy with cytotoxic agents [75]. Thus, manipulation of mitochondria-associated proapoptotic proteins is an attractive novel strategy to affect cancer cell response to drug treatment. For instance, exogenous BH3 domain peptides derived from proapoptotic proteins BID and BIM may enhance drug sensitivity in cancer cells, even in drug-resistant cells, and small-molecule inhibitor of Bcl-2, Obatoclax™, was found to cause loss of cancer cell viability regardless platinum resistance status [76].

A recent study demonstrated that an ATAP, a protein from a bifunctional Bcl-2 family member exhibited strong ability to induce apoptosis by causing permeabilization of the mitochondrial outer membrane [77]. This study further revealed that ATAP was specifically localized to mitochondria, and its pro-apoptotic activity seemed independent of expression of Bcl-2 family proteins. These findings suggest that ATAP a potential anticancer agent targeting mitochondria and may be used to overcome the intrinsic drug resistance.

Similarly, the membrane-associated segment of Bax was found to directly induce the release of mitochondrial apoptotic factors and cause tumor cell death independent of the expression of endogenous Bcl-2 proteins [78]. Based on this observation, a synthetic peptide mimicking the Bax pore-forming domain (Bax 106–134) was tested and found to induce cytochrome c release and trigger caspase-dependent apoptosis. The Bax-derived peptide also exhibit *in vivo* therapeutic activity in a nude mouse xenograft model. The findings suggest that the peptides derived from the pore-forming segment of Bax can effectively target the mitochondrial outer membrane and may serve as scaffold for anticancer drug development [78].

Targeting mitochondrial HSP90

Gamitrinib (Figure 21) is an inhibitor of mitochondrial HSP90. A recent study demonstrated that this compound triggers acute mitochondrial dysfunction, loss of transmembrane potential and release of cytochrome c in mouse prostate cancer cells. Gamitrinib displayed preclinical activity and favorable tolerability in mouse models of localized and metastatic prostate cancer in immunocompetent mice. These findings suggest that to selectively target mitochondrial HSP90 could serve as a novel strategy to treat advanced prostate cancer [79].

Celastrol (Figure 22) is a quinine methide triterpenoid and is an active ingredient of a traditional Chinese medicinal plant *Tripterygium wilfordii hook F* used as an antirheumatic. A recent study suggests that celastrol may function as an inhibitor of HSP90 and exhibit significant inhibitory activity against various cancer cells [80]. It was found that this compound caused cell cycle arrest at G0/G1 at 400 nM and induced cell death at 1 μ M in human monocytic leukemia cell line U937. In another study, celastrol inhibited cell proliferation in androgen-independent prostate cancer cell lines PC-3, DU145 and CL1 with IC₅₀ in a range of 1–2 μ M, possibly through inhibition of NF- κ B and modulating of Bcl-2 family proteins, thus, affecting mitochondrial stability [81]. Interestingly, a recent study demonstrated that it potently inhibited the activity of METC complex I and induced ROS-mediated cytotoxicity in cancer cells [82].

Modulating AMPK

The antidiabetic agent metformin (Figure 23) has chemopreventive effect against certain cancer. This compound seems to exert its pharmacological effect by inhibiting the

mitochondrial electron transport and cause an increase in AMP and decrease in ATP, resulting a substantially increase in the AMP/ATP ratio, which in turn activates AMPK to promote catabolism [83].

Another novel AMPK activator OSU-53 (Figure 24) also exhibit anticancer activity. This compound seems to affect a spectrum of cellular metabolic activities and pathways [84]. However, it remains unclear if OSU-53 directly targets mitochondrial.

Targeting PDH & PDK

CPI-613 (Figure 25) is an analog of lipoic acid capable of disrupting mitochondrial metabolism and seems to exhibit selective effect against cancer cells *in vitro* and *in vivo* [85]. Mechanistic studies demonstrated that CPI-613 seemed to cause mitochondrial metabolic disruption by targeting PDK leading to inactivation of PDH and, thus, suppressing the flux of pyruvate into the mitochondrial metabolism, such as the TCA cycle [85]. Interestingly, this seems to be in contrast to the action of another anticancer agent dichloroacetate that activates PDH via inhibition of PDK.

Targeting mitochondrial translation

Tigecycline (Figure 26) is a glycylcycline antimicrobial originally found to be effective against a broad spectrum of bacteria including methicillin-resistant *Staphylococcus aureus*, penicillin-resistant *Streptococcus pneumoniae* and *Acinetobacter baumannii* [86]. Recently, it was demonstrate that this compound has anticancer effects in human AML cell lines in a chemical screen [87]. The same study also demonstrated that tigecycline selectively killed leukemia stem and progenitor cells but not normal hematopoietic cells. Mechanistic studies using a genome-wide screen in yeast identified inhibition of mitochondrial translation as a key mechanism by which tigecycline induces cytotoxicity in cancer cells [87].

Targeting mitochondrial Trx2

The mitochondrial Trx2 system is critical for maintaining redox balance and cell viability. Certain cationic triphenylmethanes such as brilliant green (Figure 27) are found to show anti-tumor activity at nanomolar concentrations. Brilliant green accumulates in mitochondria and rapidly causes a dramatic decrease in mitochondrial Trx2 protein by causing oxidation of both Trx1 and Trx2, followed by the release of cytochrome c and AIF from the mitochondria into the cytosol [88].

Future perspective

Recent studies suggest that targeting mitochondria is an attractive strategy for cancer therapy [89]. The term **mitocans** has been used to classify mitochondria-targeted anticancer drugs with various modes of action [89,90]. Some of the compounds recently identified to target mitochondria with anticancer activity and their modes of action are listed in Table 1. While mitocans have promising potential to be used in cancer treatment, one significant concern associated with targeting mitochondria is the issue of therapeutic selectivity. Since the mitochondrial functions are essential for normal cells, a compound that attacks one of these critical functions can be harmful to the normal cells and cause a significant toxic side effect. Thus, it is high desirable and also challenging to develop agents that preferentially target the mitochondrial abnormalities in cancer cells without toxic impact on normal cells. For instance, the increase in ROS generation resulting from mitochondrial dysfunctions in cancer cells may render them highly dependent on the GSH antioxidant system to maintain redox balance, and become highly vulnerable to pharmacological abrogation of the glutathione system using small molecules, such as phenethyl isothiocyanate [91,92]. Therefore, it is of high importance to study the differences between normal and cancer cells

in their mitochondrial structures and functions and the associated metabolic alterations that are unique in cancer cells as a basis to improve therapeutic selectivity [93]. This will enable a more effective design and development of cancer therapeutics with high selectivity against cancer cells. In addition, enhancing mitochondria-targeted delivery of the anticancer agents could be another strategy to increase their cytotoxic effects against cancer cells. The improved anticancer activity of MitoVES by tagging a triphenylphosphonium group TPP⁺ is an excellent example [49]. Furthermore, combination of mitochondria-targeted agents with traditional chemotherapeutic drugs may be a good potential to enhance anticancer activity and selectivity, and to overcome drug resistance in cancer cells that are insensitive to standard anticancer drugs.

Acknowledgments

This work was supported in part by a grant from the major science and technology project of the National Basic Research Program of China (973 Program grant 2012CB967004), and grants CA085563 and CA100428 from the NIH, USA.

No writing assistance was utilized in the production of this manuscript.

References

1. Trachootham D, Zhang H, Zhang W, et al. Effective elimination of fludarabine-resistant CLL cells by PEITC through a redox-mediated mechanism. *Blood*. 2008; 112(5):1912–1922. [PubMed: 18574029]
2. Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug Discov.* 2009; 8(7):579–591. [PubMed: 19478820]
3. Lu W, Ogasawara MA, Huang P. Models of reactive oxygen species in cancer. *Drug Discov. Today Dis. Models*. 2008; 4(2):67–73. [PubMed: 18591999]
4. Hara K, Kasahara E, Takahashi N, et al. Mitochondria determine the efficacy of anticancer agents that interact with DNA but not the cytoskeleton. *J. Pharmacol. Exp. Ther.* 2011; 337(3):838–845. [PubMed: 21421738]
5. Kim KH, Park JY, Jung HJ, Kwon HJ. Identification and biological activities of a new antiangiogenic small molecule that suppresses mitochondrial reactive oxygen species. *Biochem. Biophys. Res. Commun.* 2011; 404(1):541–545.
6. Jeong NY, Yoo YH. Cerulenin-induced apoptosis is mediated by disrupting the interaction between AIF and hexokinase II. *Int. J. Oncol.* 2012; 40(6):1949–1956. [PubMed: 22426850]
7. Chen Z, Zhang H, Lu W, Huang P. Role of mitochondria-associated hexokinase II in cancer cell death induced by 3-bromopyruvate. *Biochim. Biophys. Acta*. 2009; 1787:553–560. [PubMed: 19285479]
8. Tang Z, Yuan S, Hu Y, et al. Over-expression of GAPDH in human colorectal carcinoma as a preferred target of 3-bromopyruvate propyl ester. *J. Bioenerg. Biomembr.* 2012; 44(1):117–125. [PubMed: 22350014]
9. Fulda S, Kroemer G. Mitochondria as therapeutic targets for the treatment of malignant disease. *Antioxid. Redox Sign.* 2011; 15(12):2937–2949.
10. Chen G, Wang F, Trachootham D, Huang P. Preferential killing of cancer cells with mitochondrial dysfunction by natural compounds. *Mitochondrion*. 2010; 10:614–625. [PubMed: 20713185]
11. Wang F, Ogasawara MA, Huang P. Small mitochondria-targeting molecules as anti-cancer agents. *Mol. Aspects Med.* 2010; 31:75–92. [PubMed: 19995573]
12. Griendling KK, Sorescu D, Ushio-Fukai M. NAD(P)H oxidase: role in cardiovascular biology and disease. *Circ. Res.* 2000; 86:494–501. [PubMed: 10720409]
13. Lu W, Hu Y, Chen G, et al. Novel role of NOX in supporting aerobic glycolysis in cancer cells with mitochondrial dysfunction and as a potential target for cancer therapy. *PLoS Biol.* 2012; 10(5):e1001326. [PubMed: 22589701]

14. Rupprecht R, Papadopoulos V, Rammes G, Baghai TC, et al. Translocator protein (18 kDa) (TSPO) as a therapeutic target for neurological and psychiatric disorders. *Nat. Rev. Drug. Discov.* 2010; 9(12):971–988. [PubMed: 21119734]
15. Ostuni MA, Ducroc R, Péranzi G, et al. Translocator protein (18 kDa) ligand PK 11195 induces transient mitochondrial Ca^{2+} release leading to transepithelial Cl^- secretion in HT-29 human colon cancer cells. *Biol. Cell.* 2007; 99(11):639–647. [PubMed: 17561806]
16. Bai M, Rone MB, Papadopoulos V, Bornhop DJ. A novel functional translocator protein ligand for cancer imaging. *Bioconjug. Chem.* 2007; 18(6):2018–2023. [PubMed: 17979225]
17. Veenman L, Shandalov Y, Gavish M. VDAC activation by the 18 kDa translocator protein (TSPO), implications for apoptosis. *J. Bioenerg. Biomembr.* 2008; 40(3):199–205. [PubMed: 18670869]
18. Zheng J, Boisgard R, Siquier-Pernet K, Decaudin D, Dollé F, Tavitian B. Differential expression of the 18 kDa translocator protein (TSPO) by neoplastic and inflammatory cells in mouse tumors of breast cancer. *Mol. Pharm.* 2011; 8(3):823–832. [PubMed: 21417480]
19. Mukherjee S, Das SK. Translocator protein (TSPO) in breast cancer. *Curr. Mol. Med.* 2012; 12(4):443–457. [PubMed: 22348612]
20. Itahana K, Zhang Y. Mitochondrial p32 is a critical mediator of ARF-induced apoptosis. *Cancer Cell.* 2008; 13(6):542–553. [PubMed: 18538737]
21. Itahana K, Clegg HV, Zhang Y. ARF in the mitochondria. the last frontier? *Cell Cycle.* 2008; 7(23):3641–3646. [PubMed: 19033735]
22. McGee AM, Baines CP. Complement 1q-binding protein inhibits the mitochondrial permeability transition pore and protects against oxidative stress-induced death. *Biochem. J.* 2011; 433(1):119–125. [PubMed: 20950273]
23. Fogal V, Richardson AD, Karmali PP, et al. Mitochondrial p32 protein is a critical regulator of tumor metabolism via maintenance of oxidative phosphorylation. *Mol. Cell Biol.* 2010; 30(6):1303–1318. [PubMed: 20100866]
24. Amamoto R, Yagi M, Song Y, et al. Mitochondrial p32/C1QBP is highly expressed in prostate cancer and is associated with shorter prostate-specific antigen relapse time after radical prostatectomy. *Cancer Sci.* 2011; 102(3):639–647. [PubMed: 21205079]
25. McGee AM, Douglas DL, Liang Y, Hyder SM, Baines CP. The mitochondrial protein C1qbp promotes cell proliferation, migration and resistance to cell death. *Cell Cycle.* 2011; 10(23):4119–4127. [PubMed: 22101277]
26. Luciakova K, Barath P, Poliakova D, Persson A, Nelson BD. Repression of the human adenine nucleotide translocase-2 gene in growth-arrested human diploid cells. the role of nuclear factor-1. *J. Biol. Chem.* 2003; 278(33):30624–30633. [PubMed: 12777383]
27. Chevrollier A, Loiseau D, Reynier P, Stepien G. Adenine nucleotide translocase 2 is a key mitochondrial protein in cancer metabolism. *Biochim. Biophys. Acta.* 2011; 1807(6):562–567. [PubMed: 20950584]
28. Le Bras M, Borgne-Sanchez A, Touat Z, et al. Chemosensitization by knockdown of adenine nucleotide translocase-2. *Cancer Res.* 2006; 66(18):9143–9152. [PubMed: 16982757]
29. Jang JY, Choi Y, Jeon YK, Kim CW. Suppression of adenine nucleotide translocase-2 by vector-based siRNA in human breast cancer cells induces apoptosis and inhibits tumor growth *in vitro* and *in vivo*. *Breast Cancer Res.* 2008; 10(1):R11. [PubMed: 18267033]
30. Jang JY, Jeon YK, Kim CW. Degradation of HER2/neu by ANT2 shRNA suppresses migration and invasiveness of breast cancer cells. *BMC Cancer.* 2010; 10:391. [PubMed: 20650008]
31. Jang JY, Jeon YK, Choi Y, Kim CW. Short-hairpin RNA-induced suppression of adenine nucleotide translocase-2 in breast cancer cells restores their susceptibility to TRAIL-induced apoptosis by activating JNK and modulating TRAIL receptor expression. *Mol. Cancer.* 2010; 9:262. [PubMed: 20875141]
32. Sharaf el dein O, Mayola E, Chopineau J, Brenner C. The adenine nucleotide translocase 2, a mitochondrial target for anticancer biotherapy. *Curr. Drug Targets.* 2011; 12(6):894–901. [PubMed: 21269262]

33. Lin RY, Ver JC, Chaganti RSK, Golde DW. Human monocarboxylate transporter 2 (MCT2) is a high affinity pyruvate transporter. *J. Biol. Chem.* 1998; 273(44):28959–28965. [PubMed: 9786900]
34. Gallagher SM, Castorino JJ, Wang D, Philp NJ. Monocarboxylate transporter 4 regulates maturation and trafficking of CD147 to the plasma membrane in the metastatic breast cancer cell line MDA-MB-231. *Cancer Res.* 2007; 67:4182–4189. [PubMed: 17483329]
35. Vegran F, Boidot B, Michiels C, Sonveaux P, Feron O. Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF- κ B/IL-8 pathway that drives tumor angiogenesis. *Cancer Res.* 2011; 71:2550–2560. [PubMed: 21300765]
36. Diers AR, Broniowska KA, Chang CF, Hogg N. Pyruvate fuels mitochondrial respiration and proliferation of breast cancer cells. Effect of monocarboxylate transporter inhibition. *Biochem. J.* 2012; 444(3):561–571. [PubMed: 22458763]
37. Griffin C, Karnik A, McNulty J, Pandey S. Pancratistatin selectively targets cancer cell mitochondria and reduces growth of human colon tumor xenografts. *Mol. Cancer Ther.* 2011; 10(1):57–68. [PubMed: 21220492]
38. Eloy L, Jarrousse AS, Teyssot ML, et al. Anticancer activity of silver-*N*-heterocyclic carbene complexes. Caspase-independent induction of apoptosis via mitochondrial apoptosis-inducing factor (AIF). *ChemMedChem.* 2012; 7(5):805–814. [PubMed: 22383263]
39. Summerhayes IC, Lampidis TJ, Bernal SD, et al. Unusual retention of rhodamine 123 by mitochondria in muscle and carcinoma cells. *Proc. Natl Acad. Sci. USA.* 1982; 79:5292–5296. [PubMed: 6752944]
40. Bernal SD, Lampidis TJ, Summerhayes IC, Chen LB. Rhodamine-123 selectively reduces clonal growth of carcinoma cells *in vitro*. *Science.* 1982; 218:1117–1119. [PubMed: 7146897]
41. Biswas S, Dodwadkar NS, Sawant RR, Koshkaryev A, Torchilin VP. Surface modification of liposomes with rhodamine-123-conjugated polymer results in enhanced mitochondrial targeting. *J. Drug Target.* 2011; 19(7):552–561. [PubMed: 21348804]
42. Capozzi A, Mantuano E, Matarrese P, et al. A new 4-phenyl-1,8-naphthyridine derivative affects carcinoma cell proliferation by impairing cell cycle progression and inducing apoptosis. *Anticancer Agents Med. Chem.* 2012; 12(6):653–662. [PubMed: 22263796]
43. Park MT, Song MJ, Oh ET, et al. The anti-tumour compound, RH1, causes mitochondria-mediated apoptosis by activating c-Jun N-terminal kinase. *Br. J. Pharmacol.* 2011; 163(3):567–585. [PubMed: 21250978]
44. Broadley K, Larsen L, Herst PM, Smith RAJ, Berridge MV, McConnell MJ. The novel phloroglucinol PMT7 kills glycolytic cancer cells by blocking autophagy and sensitizing to nutrient stress. *J. Cell. Biochem.* 2011; 112(7):1869–1879. [PubMed: 21433059]
45. Freitas M, Alves V, Sarmiento-Ribeiro AB, Mota-Pinto A. Combined effect of sodium selenite and docetaxel on PC3 metastatic prostate cancer cell line. *Biochem. Biophys. Res. Commun.* 2011; 408(4):713–719. [PubMed: 21549092]
46. Mollinedo F, Fernandez M, Hornillos V, et al. Involvement of lipid rafts in the localization and dysfunction effect of the anti-tumor ether phospholipid edelfosine in mitochondria. *Cell Death Dis.* 2011; 2(5):e158. [PubMed: 21593790]
47. Dong LF, Low P, Dyason JC, et al. α -tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II. *Oncogene.* 2008; 27(31):4324–4335. [PubMed: 18372923]
48. Dong LF, Jameson VJA, Tilly D, et al. Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anti-cancer activity via mitochondrial complex II. *J. Biol. Chem.* 2011; 286(5):3717–3728. [PubMed: 21059645]
49. Dong LF, Jameson VJA, Tilly D, et al. Mitochondrial targeting of α -tocopheryl succinate enhances its pro-apoptotic efficacy. A new paradigm for effective cancer therapy. *Free Radical Biol. Med.* 2011; 50(11):1546–1555. [PubMed: 21402148]
50. Shen ZX, Chen GQ, Ni JH, et al. Use of arsenic trioxide (As₂O₃) in the treatment of acute promyelocytic leukemia (APL). II. Clinical efficacy and pharmacokinetics in relapsed patients. *Blood.* 1997; 89:3354–3360. [PubMed: 9129042]

51. Chen YC, Lin-Shiau SY, Lin JK. Involvement of reactive oxygen species and caspase 3 activation in arsenite-induced apoptosis. *J. Cell Physiol.* 1998; 177:324–333. [PubMed: 9766529]
52. Wang TS, Kuo CF, Jan KY, Huang H. Arsenite induces apoptosis in Chinese hamster ovary cells by generation of reactive oxygen species. *J. Cell Physiol.* 1996; 169:256–268. [PubMed: 8908193]
53. Pelicano H, Feng L, Zhou Y, et al. Inhibition of mitochondrial respiration, a novel strategy to enhance drug-induced apoptosis in human leukemia cells by a reactive oxygen species-mediated mechanism. *J. Biol. Chem.* 2003; 278:37832–37839. [PubMed: 12853461]
54. Sun RC, Board PG, Blackburn AC. Targeting metabolism with arsenic trioxide and dichloroacetate in breast cancer cells. *Mol. Cancer.* 2011; 10:142. [PubMed: 22093145]
55. Zhao H, Xing D, Chen Q. New insights of mitochondria reactive oxygen species generation and cell apoptosis induced by low dose photodynamic therapy. *Eur. J. Cancer.* 2011; 47(18):2750–2761. [PubMed: 21741231]
56. El-Sikhry HE, Miller GG, Madiyalakan MR, Seubert JM. Sonodynamic and photodynamic mechanisms of action of the novel hypocrellin sonosensitizer, SL017. Mitochondrial cell death is attenuated by 11, 12- epoxyeicosatrienoic acid. *Invest. New Drugs.* 2011; 29(6):1328–1336. [PubMed: 20676746]
57. Fedeles BI, Zhu AY, Young KS, et al. Chemical genetics analysis of an aniline mustard anticancer agent reveals complex I of the electron transport chain as a target. *J. Biol. Chem.* 2011; 286(39): 33910–33920. [PubMed: 21832047]
58. Raza H, John A, Benedict S. Acetylsalicylic acid-induced oxidative stress, cell cycle arrest, apoptosis and mitochondrial dysfunction in human hepatoma HepG2 cells. *Eur. J. Pharmacol.* 2011; 668(1–2):15–24. [PubMed: 21722632]
59. Kuznetsov AV, Margreiter R, Amberger A, Saks V, Grimm M. Changes in mitochondrial redox state, membrane potential and calcium precede mitochondrial dysfunction in doxorubicin-induced cell death. *Biochim. Biophys. Acta.* 2011; 1813(6):1144–1152. [PubMed: 21406203]
60. Wang F, Liu J, Robbins D, et al. Mutant p53 exhibits trivial effects on mitochondrial functions which can be reactivated by ellipticine in lymphoma cells. *Apoptosis.* 2011; 16(3):301–310. [PubMed: 21107702]
61. Gogada R, Amadori M, Zhang H, et al. Curcumin induces Apaf-1-dependent, p21-mediated caspase activation and apoptosis. *Cell Cycle.* 2011; 10(23):4128–4137. [PubMed: 22101335]
62. Chatterjee SJ, Pandey S. Chemo-resistant melanoma sensitized by tamoxifen to low dose curcumin treatment through induction of apoptosis and autophagy. *Cancer Biol. Ther.* 2011; 11(2):216–228. [PubMed: 21088500]
63. Gogada R, Prabhu V, Amadori M, Scott R, Hashmi S, Chandra D. Resveratrol induces p53-independent, X-linked inhibitor of apoptosis protein (XIAP)-mediated bax protein oligomerization on mitochondria to initiate cytochrome c release and caspase activation. *J. Biol. Chem.* 2011; 286(33):28749–28760. [PubMed: 21712378]
64. Freeman MR, Kim J, Lisanti MP, Di Vizio D. A metabolic perturbation by U0126 identifies a role for glutamine in resveratrol induced cell death. *Cancer Biol. Ther.* 2011; 12(11):966–977. [PubMed: 22108021]
65. Biasutto L, Mattarei A, Marotta E, et al. Development of mitochondria-targeted derivatives of resveratrol. *Bioorg. Med. Chem. Lett.* 2008; 18:5594–5597. [PubMed: 18823777]
66. Greathouse GA, Rigler NE. Isolation of the Alkaloids, Berberine and Berbamine, from *Mathonia Swaseyi*. *Plant Physiol.* 1940; 15(3):563–564. [PubMed: 16653655]
67. Letasiová S, Jantová S, Cipák L, Múcková M. Berberine-antiproliferative activity *in vitro* and induction of apoptosis/necrosis of the U937 and B16 cells. *Cancer Lett.* 2006; 239(2):254–262. [PubMed: 16229943]
68. Wang L, Liu L, Shi Y, et al. Berberine induces caspase-independent cell death in colon tumor cells through activation of apoptosis-inducing factor. *PLoS ONE.* 2012; 7(5):e36418. [PubMed: 22574158]
69. Arison BH, Omura S. Revised structure of cerulenin. *J. Antibiot. (Tokyo).* 1974; 27:28–30. [PubMed: 4858397]

70. Nomura S, Horiuchi T, Omura S, Hata T. The action mechanism of cerulenin. I. Effect of cerulenin on sterol and fatty acid biosynthesis in yeast. *J. Biochem.* 1972; 1(5):783–796. [PubMed: 4561339]
71. Heiligt SJ, Bredehorst R, David KA. Key role of mitochondria in cerulenin-mediated apoptosis. *Cell Death Differ.* 2002; 9(9):1017–1025. [PubMed: 12181752]
72. Fimognari C, Lenzi M, Ferruzzi L, et al. Mitochondrial pathway mediates the antileukemic effects of hemidesmus indicus, a promising botanical drug. *PLoS ONE.* 2011; 6(6):e21544. [PubMed: 21738701]
73. Pandey S, Chatterjee SJ, Ovadjee P, Mousa M, Hamm C. The efficacy of dandelion root extract in inducing apoptosis in drug-resistant human melanoma cells. *Evid. Based Complement. Alternat. Med.* 2011; 129045. [PubMed: 21234313]
74. Tan C, Hu S, Liu J, Ji L. Synthesis, characterization, antiproliferative and anti-metastatic properties of two ruthenium-DMSO complexes containing 2,2'-biimidazole. *Eur. J. Med. Chem.* 2011; 46(5): 1555–1563. [PubMed: 21354673]
75. Chonghaile TN, Sarosiek KA, Vo TT, et al. Pretreatment mitochondrial priming correlates with clinical response to cytotoxic chemotherapy. *Science.* 2011; 334(6059):1129–1133. [PubMed: 22033517]
76. Crawford N, Chacko AD, Savage KI, et al. Platinum resistant cancer cells conserve sensitivity to BH3 domains and obatoclax induced mitochondrial apoptosis. *Apoptosis.* 2011; 16(3):311–320. [PubMed: 21107700]
77. Ko JK, Choi KH, Peng J, He F, et al. Amphipathic tail-anchoring peptide and Bcl-2 homology domain-3 (BH3) peptides from Bcl-2 family proteins induce apoptosis through different mechanisms. *J. Biol. Chem.* 2011; 286(11):9038–9048. [PubMed: 21189256]
78. Valero JG, Sancey L, Kucharczak J, et al. Bax-derived membrane-active peptides act as potent and direct inducers of apoptosis in cancer cells. *J. Cell Sci.* 2011; 124(4):556–564. [PubMed: 21245196]
79. Kang BH, Tavecchio M, Goel HL, et al. Targeted inhibition of mitochondrial Hsp90 suppresses localised and metastatic prostate cancer growth in a genetic mouse model of disease. *Br. J. Cancer.* 2011; 104(4):629–634. [PubMed: 21285984]
80. Peng B, Xu L, Cao F, et al. HSP90 inhibitor, celastrol, arrests human monocytic leukemia cell U937 at G0/G1 in thiol-containing agents reversible way. *Mol. Cancer.* 2010; 9:79. [PubMed: 20398364]
81. Dai Y, DeSano J, Tang W, et al. Natural proteasome inhibitor celastrol suppresses androgen-independent prostate cancer progression by modulating apoptotic proteins and NF-kappaB. *PLoS ONE.* 2010; 5(12):e14153. [PubMed: 21170316]
82. Chen G, Zhang X, Zhao M, et al. Celastrol targets mitochondrial respiratory chain complex I to induce reactive oxygen species-dependent cytotoxicity in tumor cells. *BMC Cancer.* 2011; 11:170. [PubMed: 21569548]
83. Duque JE, Velez J, Samudio I, Lai E. Metformin as a novel component of metronomic chemotherapeutic use. a hypothesis. *J. Exp. Clin. Med.* 2012; 4(3):140–144.
84. Lee KH, Hsu EC, Guh JH, et al. Targeting energy metabolic and oncogenic signaling pathways in triple-negative breast cancer by a novel adenosine monophosphate-activated protein kinase (AMPK) activator. *J. Biol. Chem.* 2011; 286(45):39247–39258. [PubMed: 21917926]
85. Zachar Z, Marecek J, Maturo C, et al. Nonredox-active lipoate derivatives disrupt cancer cell mitochondrial metabolism and are potent anticancer agents *in vivo*. *J. Mol. Med.* 2011; 89(11): 1137–1148. [PubMed: 21769686]
86. Betriu C, Rodríguez-Avil I, Sánchez BA, et al. *In vitro* activities of tigecycline (GAR-936) against recently isolated clinical bacteria in Spain. *Antimicrob. Agents Chemother.* 2002; 46(3): 892–895. [PubMed: 11850282]
87. Škrtić M, Sriskanthadevan S, Jhas B, et al. Inhibition of mitochondrial translation as a therapeutic strategy for human acute myeloid leukemia. *Cancer Cell.* 2011; 20(5):674–688. [PubMed: 22094260]

88. Zhang X, Zheng Y, Fried LE, et al. Disruption of the mitochondrial thioredoxin system as a cell death mechanism of cationic triphenylmethanes. *Free Radical Biol. Med.* 2011; 7:811–820. [PubMed: 21215310]
89. Neuzil J, Dong LF, Rohlena J, Truksa J, Ralph SJ. Classification of mitocans, anti-cancer drugs acting on mitochondria. *Mitochondrion*. 2012 doi:10.1016/j.mito.2012.07.112. Epub ahead of print.
90. Neuzil J, Wang XF, Dong LF, Low P, Ralph SJ. Molecular mechanism of `mitocan'-induced apoptosis in cancer cells epitomizes the multiple roles of reactive oxygen species and Bcl-2 family proteins. *FEBS Lett.* 2006; 580(22):5125–5129. [PubMed: 16979626]
91. Trachootham D, Zhou Y, Zhang H, et al. Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. *Cancer Cell*. 2006; 10(3):241–252. [PubMed: 16959615]
92. Chen G, Chen Z, Hu Y, Huang P. Inhibition of mitochondrial respiration and rapid depletion of mitochondrial glutathione by β -phenethyl isothiocyanate. mechanisms for anti-leukemia activity. *Antioxid. Redox Signal.* 2011; 15(12):2911–2921. [PubMed: 21827296]
93. Hammoudi N, Ahmed KB, Garcia-Prieto C, Huang P. Metabolic alterations in cancer cells and therapeutic implications. *Chin. J. Cancer*. 2011; 30(8):508–525. [PubMed: 21801600]

Executive summary

- Mitochondria are important cellular organelle and play essential roles in energy metabolism, calcium homeostasis, redox regulation and apoptosis.
- Cancer cells often exhibit some degrees of mitochondrial dysfunctions such as alterations in energy metabolism, increased transmembrane potential and elevated reactive oxygen species generation. These changes provide a biochemical basis for preferentially targeting cancer cell to improve therapeutic selectivity.
- Multiple proteins associated with mitochondria are potential cancer therapeutic targets.
- Small molecules targeting mitochondria exhibit promising anticancer activity.

Key Terms

Reactive oxygen species

Oxygen-containing chemical species with a highly reactive potential. In the cells, reactive oxygen species can be generated from mitochondria when oxygen captures the leaked electrons from mitochondrial electron transport chain.

The warburg effect

Metabolic change observed in cancer cells manifested by an increase in aerobic glycolysis. It is postulated that this metabolic alteration is closely related to mitochondrial dysfunction and plays an important role in supporting cancer cell viability and proliferation. Targeting the glycolytic pathway is considered as an attractive strategy to preferentially kill cancer cells.

Key Term**Mitocans**

Mitochondria-targeted compounds with anticancer activity. This family of compounds can be further classified into subgroups according to their mechanisms of action.

Figure 1. Mitochondria-associated molecules and pathways as potential targets for anticancer agents

The putative targets and relevant pathways are shown in ovals, while the small-molecular-weight compounds are shown in red boxes.

GSH: Glutathione; METC: Mitochondrial electron transport chain; ROS: Reactive oxygen species; VDAC: Voltage-dependent anion channel.

Figure 2.
Pancratistatin

Figure 3. Silver carbene
X = Cl, Br, I; R₁, R₂ = alkyl or aryl.

Figure 4.
Rhodamine-123.

Figure 5.
Naphthyridine derivative.

Figure 6.
2,5-diaziridinyl-3-(hydroxymethyl)-6-methyl-1,4-benzoquinone (RH1).

Figure 7.
PMT7.

Figure 8.
Sodium selenite.

Figure 9.
Edelfosine.

Figure 10.
MitoVES.

Figure 11.
Arsenic trioxide.

Figure 12.
SL017.

Figure 13.
11β (CAS 865070-37-7).

Figure 14.
Aspirin.

Figure 16.
Curcumin.

Figure 17.
Resveratrol.

Figure 18.
Berberine.

Figure 19.
Cerulenin.

Figure 20. Ruthenium complexes

(A) $[\text{RuCl}_2(\text{DMSO})_2(\text{H}_2\text{biim})]$; (B) $\text{mer-}[\text{RuCl}_3(\text{DMSO})(\text{H}_2\text{biim})]$; (C) NAMI-A.

Figure 21.
Gamitrinibs. $n = 4, 2, 0$.

Figure 22.
Celastrol.

Figure 23.
Metformin.

Figure 24.
OSU-53.

Figure 25.
CPI-613.

Future Med Chem. Author manuscript; available in PMC 2013 November 01.

Figure 27.
Brilliant green.

Table 1

Compounds targeting mitochondria-associated molecules and pathways.

Putative targets/modes of action	Compounds	Ref.
Mitochondrial membrane potential	Pancratistatin	[37]
	Silver complex	[38]
	Rhodamine-123	[39–41]
	Naphthylidine compounds	[42]
	RH1	[43]
	PMT7	[44]
	Sodium selenite	[45]
	Edelfosine	[46]
Mitochondrial electron transport chain	MitoVES	[48–49]
	Arsenic trioxide	[50–54]
	SL017	[56]
	11 β	[57]
	Aspirin	[58]
	Ellipticine	[60]
	Celastrol	[82]
	Metformin	[83]
Mitochondrial apoptotic pathway	Curcumin	[61–62]
	Resveratrol	[63–65]
	berberine	[67–68]
	Cerulein	[6,69–71]
	Hemidesmus indicus	[72]
	Ruthenium complex	[74]
	Bcl-2 BH3-derived peptide	[75,77–78]
	Celastrol	[81]
Mitochondrial HSP90	Gamitinib	[79]
	Celastrol	[80]
AMPK	Metformin	[83]
	OSU-53	[84]
PDH and PDK	CPI-63	[85]
Mitochondrial translation	Tigecycline	[86–87]
Mitochondrial thoredoxin 2	Brilliant green	[88]

Note: Some compounds may act on multiple targets.