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Small mitochondria-targeting molecules as anti-cancer agents

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Abstract

Alterations in mitochondrial structure and functions have long been observed in cancer cells. Targeting mitochondria as a cancer therapeutic strategy has gained momentum in the recent years. The signaling pathways that govern mitochondrial function, apoptosis and molecules that affect mitochondrial integrity and cell viability have been important topics of the recent review in the literature. In this article, we first briefly summarize the rationale and biological basis for developing mitochondrial-targeted compounds as potential anticancer agents, and then provide key examples of small molecules that either directly impact mitochondria or functionally affect the metabolic alterations in cancer cells with mitochondrial dysfunction. The main focus is on the small molecular weight compounds with potential applications in cancer treatment. We also summarize information on the drug developmental stages of the key mitochondria-targeted compounds and their clinical trial status. The advantages and potential shortcomings of targeting the mitochondria for cancer treatment are also discussed.

Keywords

Mitochondria; Warburg effect; Glycolysis; Reactive oxygen species; Apoptosis; Anticancer agents; Therapeutic selectivity

1. Introduction

As an important organelle in the cells, mitochondria not only play a central role in energy metabolism and calcium homeostasis (Clapham, 2007), but also are essential components of the apoptotic machinery and sites of reactive oxygen species (ROS) generation (Green and Reed, 1998). Under physiological conditions, mitochondria are considered as the main powerhouse of the cells, since this organelle can use glucose, fatty acids, and certain amino acids as the fuel sources to generate ATP through oxidative phosphorylation. The Krebs cycle within the mitochondrial provides an essential metabolic platform for effective conversion of various metabolic intermediates and the production of NADH as the substrate (electron donor) for the mitochondrial respiratory chain, through which electrons are transported in an orderly

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manner and transmembrane potential is established. The energy stored in the form of transmembrane potential across the inner mitochondrial membrane is then used for ATP generation in complex V. In addition to energy production, the important roles of mitochondria in calcium uptake/homeostasis and in lipid metabolism are essential for normal cellular function and survival. Importantly, mitochondria also contain potentially lethal molecules, which when released from this organelle, may cause the death of the host cells. For instance, cytochrome *c* is a normal component of the mitochondrial respiratory chain and plays an essential function in electron transport. However, when cytochrome *c* is released from the mitochondria to the cytosol, it binds to the cytosolic protein Apaf-1 in the presence of dATP to form a protein complex known as apoptosome, which activates the caspase protease cascade leading to cell destruction. Another mitochondrial protein known as apoptosis-inducing factor (AIF) can cause caspase-independent apoptosis when it is translocated to the nucleus. Many factors, including exogenous and endogenous stimuli, can induce the change of mitochondrial membrane permeability and cause the release of apoptotic factors.

Because of their important functions in energy production and in regulation of cell death, mitochondria have been considered to be a potentially important target for anticancer drug development, and this strategy has recently gained momentum (Fantin et al., 2002; Toogood, 2008). Interestingly, numerous notable differences in the structure and function of mitochondria between cancer cells and normal cells have been reported (Modica-Napolitano and Singh, 2004). For instance, there are various changes in the size, shape and number of the mitochondria in liver cancer cells when compared to the corresponding normal cells. It has also been observed that certain fast growing tumor cells seemed to have fewer and smaller mitochondria than slowly growing tumors, while certain relatively benign tumors (such as oncocytic adenomas) exhibited large numbers of mitochondria and high levels of oxidative enzymes (Maximo and Sobrinho-Simoes, 2000; Modica-Napolitano and Singh, 2002). Alterations of the inner mitochondrial membrane proteins have also been observed in hepatoma cells (Chang et al., 1971; Irwin et al., 1978). Consistent with these structural changes, mitochondrial dysfunction have been observed in various types of cancer cells, evident by a compromised capacity in mitochondrial ATP generation and an abnormal increase in ROS generation (Chen, 2007; Pelicao, 2004). It should be pointed out, however, that the mitochondrial morphological changes and functional alterations observed in one cancer type or cell line should not be generalized as common mitochondrial abnormalities in all cancer cells. Although mitochondrial dysfunction is often observed in cancer, it is likely that the specific alterations may vary depending in the cancer types, tissue origins, disease stages, proliferation and differentiation states, and microenvironment such as hypoxia.

A prominent metabolic alteration in cancer cells is that they exhibit a substantial increase in aerobic glycolysis and seem to rely more on this non-oxidative glucose metabolism for generation of ATP and for production of other molecules for cell growth and proliferation. This phenomenon, known as the Warburg effect, has been observed in a variety of cancer types including solid tumors and leukemia (Warburg, 1956). The successful use of positron emission tomography (PET) imaging in clinical diagnosis of cancer, based on the increased uptake of glucose in tumor tissues, is an excellent example of the clinical relevance of the Warburg theory. Although the underlying mechanisms responsible for the Warburg effect remain to be defined, it has been postulated that mitochondrial dysfunction (respiration injury) may be a key event that compromises the cancer cell's ability to generate ATP through oxidative phosphorylation, and thus forces them to increase glucose fermentation to compensate the energy supply (Warburg, 1956). Mitochondrial mutations, oncogenic signals, and metabolic stress are possible factors that can lead to mitochondrial dysfunction (Chen et al., 2007). In fact, electron transport chain complex activities in mitochondria have been found to be decreased in certain cancer cells such as hepatoma (Chan and Barbour, 1983; Sun et al., 1981), and tumor cells (Morris hepatomas and ascites tumor cells) showed markedly reduced

ATPase activity when compared with that of the normal cells(Pedersen and Morris, 1974). Mitochondrial DNA (mtDNA, which encodes for 13 key electron transport components) mutations were detected in various cancers, and certain mutations seemed to be associated with specific cancers (Modica-Napolitano and Singh, 2004). Moreover, some studies showed that cancer cells have increased mitochondrial transmembrane potential (Bernal et al., 1982; Johnson et al., 1981). In addition, mitochondria are major sites of ROS generation in the cells, and increased ROS generation in cancer cells may reflect an increased leakage of electrons from the transport complexes (complex I and complex III). The capture of an electron by molecular oxygen may lead to a formation of superoxide, which can then be further converted to hydrogen peroxide. Thus, elevated ROS generation in cancer cells may be considered as an indication of mitochondrial dysfunction, although it should be noted that ROS generation can also occur by other mechanisms outside the mitochondria, such as reactions catalyzed by NAD (P)H oxidase (NOX) complex and xanthine oxidase.

These differences between normal cells and cancer cells in their mitochondrial structure and functions may provide a biological basis to preferentially target cancer cells using agents that either directly interact with mitochondria or functionally impact the metabolic alterations as the consequences of mitochondrial dysfunction in cancer cells. As illustrated in Fig 1, the increase of mitochondrial transmembrane potential in cancer cells may provide a possibility of utilizing compounds such as rhodamine123 with certain biochemical properties that can be selectively taken up by the cancer mitochondria and preferentially disturb cancer cell metabolism or activate the apoptotic cell death process. Similarly, the structural/functional mitochondrial alterations in cancer cells may render them more vulnerable to damage by certain pharmacological agents leading to release of apoptotic factors. For instance, targeting Bcl-2 family proteins may cause the opening of mitochondrial permeability pore and release of apoptotic factors. Furthermore, the increased dependence on glycolysis as a consequence of mitochondrial dysfunction in cancer cells may serve as a biochemical basis to preferentially kill malignant cells using proper glycolytic inhibitors such as 3-bromopyruvate (3-BrPA) and lonidamine. The phenomenon of mitochondrial dysfunction, the signaling pathways that mitochondrial affect function and regulate apoptosis, and agents that affect mitochondrial integrity and cell viability have been reviewed previously (Armstrong, 2006;Dias and Bailly, 2005;Galluzzi et al., 2006;Morrison et al., 2009;Ralph and Neuzil, 2009). In this article, we summarize the recent advances in this research area and review key examples of small molecules that preferentially target mitochondria in cancer cells directly or preferentially interfere with cancer metabolism, with a focus on those compounds that may have potential utility in cancer treatment. The chemical structure these compounds and their status in drug development will also be indicated when such information is available.

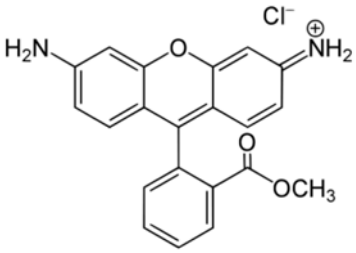
2. Small molecules that directly target mitochondria

2.1 Compounds targeting mitochondria based on altered mitochondrial transmembrane potential (Δψm)

It has been known for sometime that mitochondria of cancer cells and transformed cells have significantly higher transmembrane potential than normal cells (Dairkee and Hackett, 1991; Davis et al., 1985; Johnson et al., 1980; Modica-Napolitano and Aprille, 1987; Summerhayes et al., 1982). This biological property has been used as a basis to develop compounds that may preferentially accumulate within the mitochondria of cancer cells. A group of molecules known as delocalized lipophilic cations (DLCs), with highly hydrophobic structures and positive charge, seem to be able to accumulate in the tumor mitochondria of tumor due to the highly negatively-charged microenvironment within the mitochondrial matrix (Modica-Napolitano and Aprille, 2001). Several members of DLCs have been found to exhibit some degree of efficacy in killing cancer cells or inhibiting their growth. Although increased transmembrane potential in cancer cells is necessary for DLCs to achieve selective cytotoxictiy, the overall

effectiveness of these compounds depends on other factors. For example, cytoplasmic characteristics are involved in the kinetics of uptake and retention of these compounds (Modica-Napolitano and Singh, 2002) as well as the ability of these agents to disrupt mitochondrial function once they have accumulated within the organelle. Although elevation of mitochondrial transmembrane potential has been observed in various types of cancer cells, it is important to compare cancer cells with the normal cells of same tissue origins and at similar developmental stages for their transmembrane potential and cytotoxic responses to these compounds. This would be particularly important for the evaluation of the anticancer selectivity of compounds whose action is membrane potential-dependent, including DLCs, rhodamine-123, and MKT-077 (see below).

Rhodamine-123—

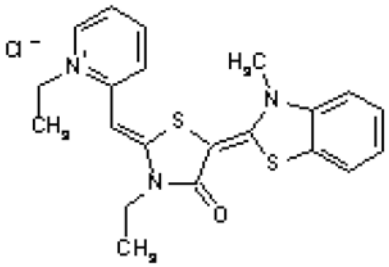


Studies in the early 1980s showed that Rhodamine-123 could function as a specific chemical probe for the localization of mitochondria in living cells. After cells were incubated with Rhodamine-123, mitochondria were preferentially stained and revealed as clusters of organelle in the perinuclear region of src-transformed cells (Chen et al., 1982; Johnson et al., 1980). The accumulation of Rhodamine-123 in mitochondria depends on its lipophilic and cationic properties, which helps it cross the double mitochondrial membranes and stay within the mitochondrial matrix where the microenvironment is negatively charged (Johnson et al., 1981; Lampidis et al., 1984). By depolarizing the plasma membrane, it was revealed that the uptake of Rhodamine-123, driven mainly by the mitochondrial membrane potential, was greater in cancer cells than in normal cells (Davis et al., 1985; Lampidis et al., 1985). High retention of Rhodamine-123 in mitochondria was observed in transitional cell carcinoma, adenocarcinoma, chemical carcinogen-transformed epithelial cell lines, and squamous cell carcinoma lines. Interestingly, the mitochondria of these cancer cells retained Rhodamine-123 for 2 to 5 days, whereas normal cells released Rhodamine-123 within a few hours (Summerhayes et al., 1982).

Kidney and breast cancer cells that retained Rhodamine-123 longer than non-tumorigenic epithelial cells were highly sensitive to this compound, as evidenced by significant inhibition of colony formation (Bernal et al., 1982). Rhodamine-123 also showed selective anticancer activity in animal tumor models, and this selective toxicity could be further potentiated by addition of 2-deoxyglucose, an inhibitor of glycolysis (Arcadi, 1986; Bernal et al., 1983; Herr et al., 1988). A phase I clinical trial of Rhodamine-123 was carried in hormone refractory prostate cancer patients to determine the maximum tolerated dose (MTD) and its safety/toxicity profile (Jones et al., 2005). The MTD of Rhodamine-123 was estimated at 96 mg/m^2 and that this compound could be safely administered at monthly intervals. This drug administration schedule resulted in preferential drug retention in prostatic tumor tissue without detectable drug accumulation in serum. Therapeutic efficacy, as assessed by the lengthening of PSA doubling time, was observed in this clinical trial but the data did not reach statistical significance. Thus, monthly administration of Rhodamine-123 at a dose of 96 mg/m^2 seems not an effective therapeutic schedule. Dose and schedule optimization and combination of Rhodamine-123 with other therapeutic modalities may improve therapeutic efficacy. Since a decrease in mitochondrial production of ATP may be compensated by an increase in glycolysis

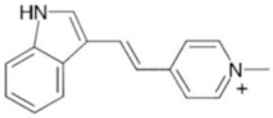
in the cytosol to generate ATP, a combination of rhodamine-123 and glycolytic inhibitors such as 2-deoxyglucose seems to be a logical strategy. It should be noted that some cancer cells may use fatty acids and amino acids such as glutamine as alternative energy sources for ATP generation (Vander Heiden et al, 2009), inhibition glycolysis alone may not be sufficient to cause ATP deprivation. A combination of a glycolytic inhibitor and compounds that disrupt mitochondria or suppress the alternative energy metabolic pathways in the mitochondria would be more effective in killing these cancer cells.

MKT-077—



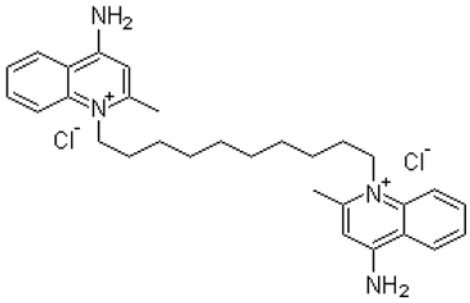
The rhodacyanine analogue MKT-077 (also known as FJ-776) is localized in the mitochondria after the compound enters the cells. Like other lipophilic cations, MKT-077 accumulates preferentially in the mitochondria of tumor cells due to a higher mitochondrial membrane potential. A previous study showed that MKT-077 selectively damages mitochondria of carcinoma cells, as evidenced by a 4-fold higher drug concentration required to obtain a similar inhibition of respiration in normal cells (Modica-Napolitano et al., 1996). It was also observed that carcinoma cells exposed to MKT-077 led to a preferential damage to mitochondrial DNA without a significant loss of nuclear DNA. Interestingly, MKT-077 exhibited a growth inhibitory effect in human breast, ovary, endometrial, colon and non-small cell lung cancer cell lines, but not in normal epithelial cells (Petit et al., 1999). In vivo, MKT-077 was able to inhibit the growth of implanted human renal carcinoma and prostate carcinoma in mice, and prolonged the animal survival (Koya et al., 1996). A phase I study has been conducted to evaluate the safety and pharmacokinetics of MKT-077 in advanced solid tumors. This study showed that MKT-077 was well tolerated in this group of patients with a recommended dose of 126 mg/m^2/week. The main toxicity was hypomagnesemia due to renal loss of magnesium, which was manageable by administration of magnesium (Britten et al., 2000).

F16—



Through a cell-based high throughput screening of a chemical library, Fantin et al identified a novel molecule designated as F16 that specifically inhibited the proliferation of Her2-overexpressing mammary epithelial cells. This molecule exhibited low binding to the mitochondrial membranes and high accumulation in the mitochondrial matrix, resulting in mitochondrial damage, opening of the permeability transition pore, cytochrome c release, cell cycle arrest, and cell death. Besides the apoptosis effect triggered by F16, this compounds also induced necrosis in tumor cells (Fantin et al., 2002).

Dequalinium—

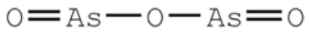


Dequalinium is a lipophilic compound with two positive charges and a C-10 aliphatic side chain. Clinically, this compound has been used as a topical antimicrobial agent for half a century (Babbs et al., 1956). Like other lipophilic cationic compounds, dequalinium mainly localized to the mitochondria of tumor cells. Prolonged exposure to dequalinium drastically altered the morphology of the mitochondria, causing globular change and perinuclear distribution (Weiss et al., 1987). This compound also inhibits the ability of calmodulin, a calcium-binding protein, to activate phosphodiesterase and thereby suppresses cell proliferation (Bodden et al., 1986). Furthermore, dequalinium selectively targeted cancer cells, impaired carcinoma cell proliferation, migration and invasion and prolonged survival of mice with implanted bladder and colon cancer (Bleday et al., 1986; Helige et al., 1992; Weiss et al., 1987).

2.2 Compounds that target cancer mitochondrial respiration

Similar to that observed in embryos or neonates, mitochondria in tumor cells seem to maintain a low electron transfer activity at approximately 20–30% of that seen in normal quiescent organelles (Galli et al., 2003). In colorectal cancer, NADH-cytochrome c reductase complex I and cytochrome oxidase, part of complex IV in the mitochondrial electron transport chain were observed to be decreased in tumor and peritumor tissues. The enzyme activity of superoxide dismutase 1 (SOD1), an antioxidant which converts superoxide to hydrogen peroxide, decreased while the mitochondrial nitric oxide synthase (mtNOS) activity increased in tumor tissue in advanced stage as compared with the initial stage (Sanchez-Pino et al., 2007). The relatively lower mitochondrial electron transport chain activity in cancer cells seems to reflect dysfunction of oxidative phosphorylation of cancer mitochondria. Because the electron transport chain is a major site of reactive oxygen species (ROS) generation due to capture of electrons by molecular oxygen, compounds that interfere with the respiratory chain may promote leakage of electrons and thus, increase the production of ROS, leading to mitochondrial damage and activation of apoptosis in cancer cells. The killing of cancer cells by ROS-mediated mechanisms is considered to have therapeutic selectivity against malignant cells due to their intrinsic high ROS generation (Trachootham et al., 2009).

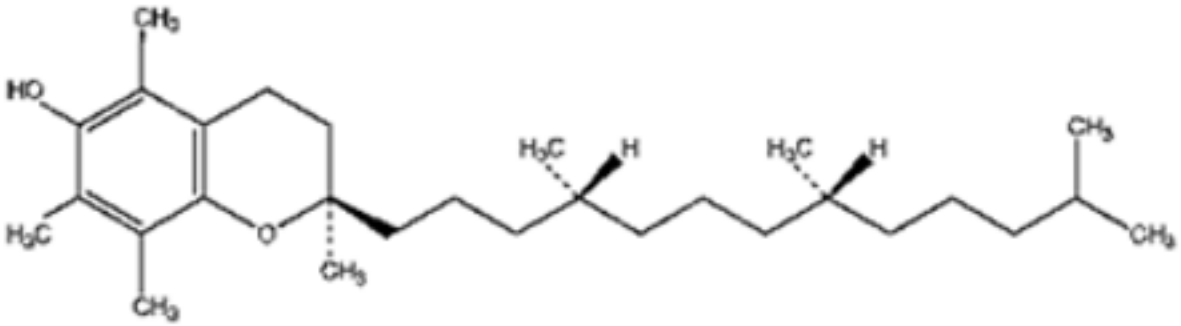
Arsenic trioxide—



Arsenic trioxide (As_2O_3) has long been used in traditional Chinese medicine to treat a variety of diseases. In the 1970s, As_2O_3 was introduced for the treatment of acute promyelocytic leukemia (APL) and showed a striking therapeutic effectiveness, with complete remission rates ranging from 65.6% to 84% in clinical studies conducted in the northeastern region of China (Shen et al., 1997). Interestingly, As_2O_3 exerted a dose dependent dual effect on APL cell lines in vitro, triggering apoptosis at high concentrations (0.5–2.0umol/L) while inducing differentiation at lower concentrations (0.1–0.5umol/L). Several studies showed As_2O_3 caused generation of superoxide and hydrogen peroxide, leading to a decrease of mitochondria membrane potential and apoptosis (Chen et al., 1998; Iwama et al., 2001; Wang et al., 1996).

A recent study showed that interfering with the mitochondrial electron transport chain may be an important mechanism by which As_2O_3 promoted ROS generation (Pelicano et al., 2003). In leukemia cells, As_2O_3 was capable of inhibiting mitochondrial respiration, resulting in a substantial decrease of oxygen consumption as early as 3 hours, concurrent with the increase of ROS generation. Since cell death was detected after 24 h of drug treatment, inhibition of respiration seemed to be a primary event rather than the consequence of cell death. Moreover, in the presence of the mitochondrial complex I inhibitor rotenone, As_2O_3 caused further inhibition which suggested they may act in series. It appears that by interfering with electron transport in the mitochondria, As_2O_3 may cause an increase in electron leakage and therefore promotes ROS generation. Furthermore, a study using respiration-deficient cells revealed that the rho-0 cells were resistant to As_2O_3, confirming that the respiratory chain activity is important for the cytotoxic action of this drug (Pelicano et al., 2003).

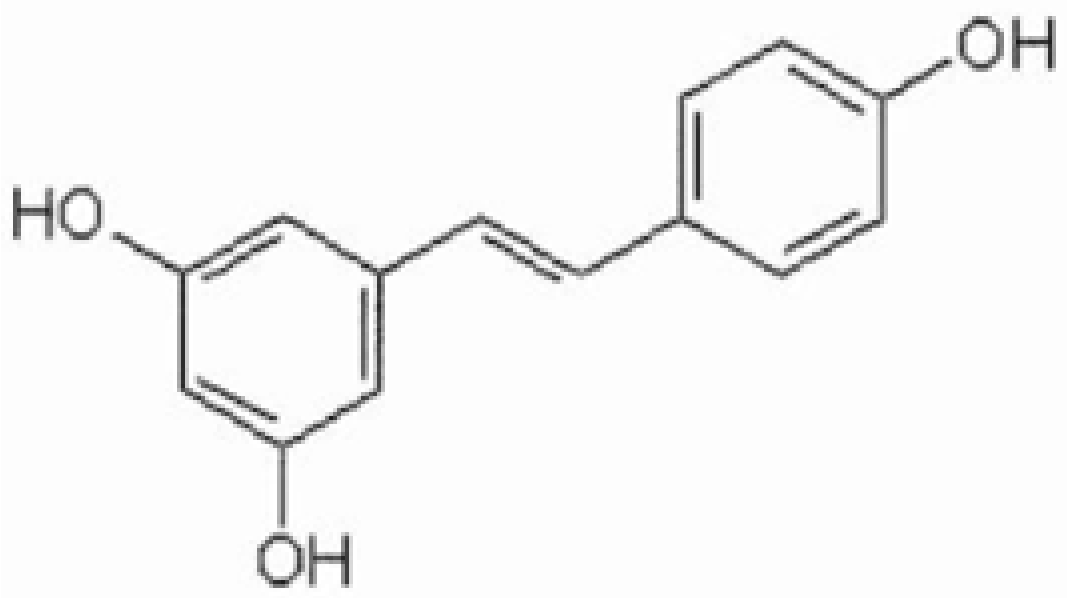
Vitamin E analogues—



(α-Tocopheryl succinate)

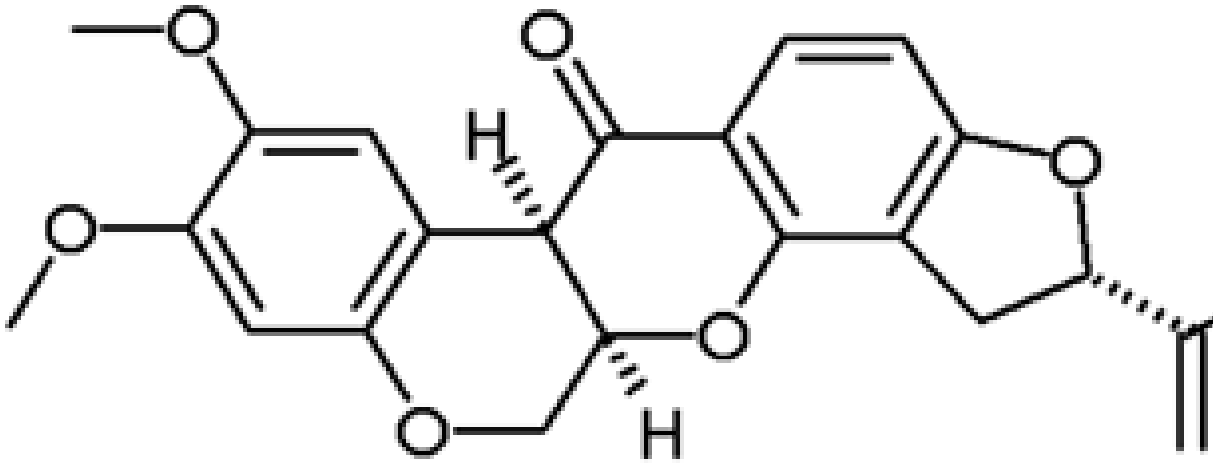
Alpha-tocopheryl succinate (α-TOS), a redox-inactive vitamin E analogue, exhibited strong pro-apoptotic and anticancer activity by causing rapid production of ROS (Stapelberg et al., 2005; Wang et al., 2005; Weber et al., 2003).α-TOS also seems to be able to inhibit the anti-apoptotic function of Bcl-xl and Bcl-2 by disrupting their binding by the Bak BH3 peptide (Shiau et al., 2006). The molecular target of α-TOS was recently identified (Dong et al., 2008). Dong et al revealed that this compound is a competitive inhibitor of the UbQ sites (Qp and QD) in Complex II thus inhibiting succinate dehydrogenase (SDH) activity in the mitochondrial electron transport chain. Knockdown of CybL made cancer cells resistant to α-TOS mediated killing, and addition of MitoQ overcame the α-TOS-mediated inhibition of MTT reduction driven by succinate. Reconstitution of a functional complex II in the CybL mutant cells led to normalization of SDH activity and reestablished sensitivity to α-TOS (Dong et al., 2008). In addition, Vitamin E analogues selectively inhibited a diverse range of malignant cells including melanomas, colon cancer and breast cancer in experimental animals (Dong et al., 2009; Neuzil et al., 2007). Higher esterase activities in normal cells can hydrolyze α-TOS into α-TOC (alpha-tocopheryl) and attenuate their activity. The selectivity of Vitamin E analogues against cancer cells seems to depend on their ability to target and down-regulate certain abnormally activated pathways such as PI3K/AKT and NF-kB (Constantinou et al., 2008).

Resveratrol—



Resveratrol (3,5,4′-trihydroxystilbene) is a natural compound found in certain fruits, particularly in some grapes and blueberries, and has been suggested to have cancer chemopreventive properties. This compound seems to inhibit cellular events associated with tumor initiation, promotion, and progression (Jang et al., 1997). It was found that the mitochondrial respiratory chain (complexes I–III) was inhibited by resveratrol in mice. By competing with DUQH2, resveratrol decreased complex III activity and lowered ROS levels (Zini et al., 1999). Though considered as an antioxidant, resveratrol was shown to induce apoptosis through the mitochondrial pathway (Juan et al., 2008; Vetvicka et al., 2007). Interestingly, resveratrol seems to interfere with important signaling pathways such as PI3K/AKT, JAK/STAT, and the MAPK cascade (Filomeni et al., 2007; Madan et al., 2008; Roy et al., 2009). Moreover, this compound attenuated HIF-1α and VEGF expression induced by lysophosphatidic acid (Park et al., 2007).

Rotenone—



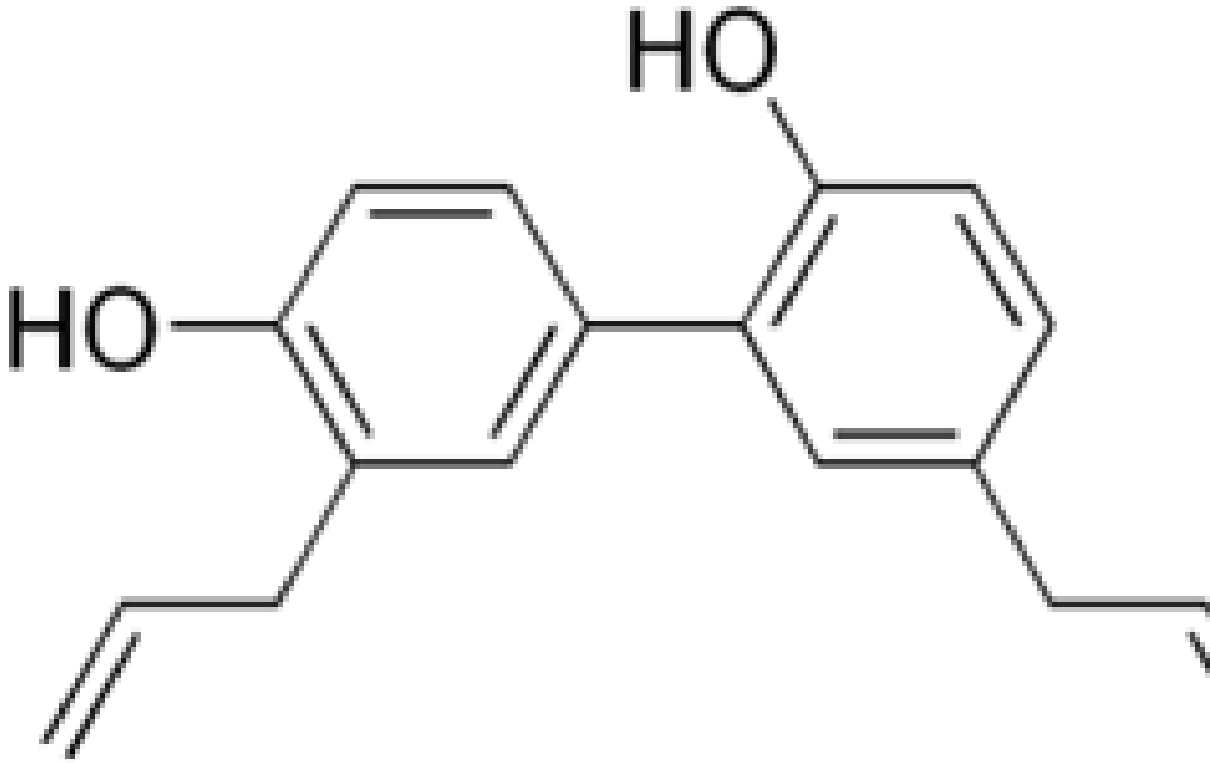
Rotenone, a strong inhibitor of mitochondria complex I (Chance et al., 1963), is often used as a pesticide and insecticide. Interestingly, this compound at the concentration which blocks

electron flow through complex I, did not significantly affect cell viability or growth in B lymphoma cells (Armstrong et al., 2001), suggesting ATP generated outside the mitochondria might be sufficient to support cell survival in these lymphoma cells. It has been observed that chronic rotenone treatment could result in increased superoxide production, leading to mitochondrial alteration (Koopman et al., 2005). However, reports on the effect of rotenone on cellular ROS levels have been conflicting, with both increase and decrease of ROS observed in cells treated with rotenone (Deshpande et al., 2000; Torres-Roca et al., 2000; Vrablic et al., 2001 ; Armstrong et al., 2001; Kitamura et al., 2002; Koopman et al., 2005; Pelicano et al., 2003). The reasons for these opposite results are unclear. Differences in the cellular genetic backgrounds, mitochondrial functional states and different rotenone concentrations used in these studies might possibly contribute to the variations in ROS observed. While rotenone is a potent and specific inhibitor of complex I and has been commonly used as a biochemical tool, its utility as an anticancer agent is unclear, although its cancer preventive potential has been suggested (Yoshitani et al., 2001).

2.3 Compounds affecting cancer mitochondrial membrane permeability

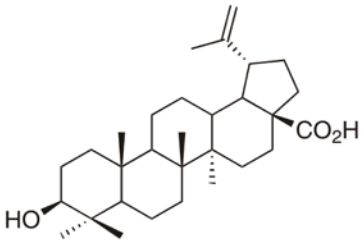
A traditional model for mitochondrial membrane permeability transition pore (MPTP) was a multi-protein structure between the inner and outer mitochondrial membranes comprised of several proteins such as adenine nucleotide transporter (ANT), voltage dependent anion channel (VDAC) and cyclophilin D (Chipuk et al., 2006). As a voltage-dependent, cyclosporine-A-sensitive and high-conductance inner membrane channel, MPTP can depolarize the mitochondrial potential by its opening. Abnormal opening of MPTP may result in the collapse of mitochondrial membrane potential and the release of apoptotic factors from the mitochondria to cytosol, leading to cell death. Some studies also found certain differences in MPT between normal and malignant cells. For instance, in mitochondria of certain hepatoma cells, the adenine nucleotide exchange function of ANT was decreased and the sensitivity to bongkrekic acid (an inhibitor of adenine nucleotide exchange and formation of MPTP) was also diminished. The expression of ANT2, a gene that is usually repressed in quiescent cells was up-regulated in several tumor cells (Modica-Napolitano and Singh, 2002). These differences may provide an opportunity to preferentially target MPTP in cancer cells for therapeutic purpose. However, it should be noted that the absence of one or more ANT or VDAC isoforms in animal models by gene knockout did not inactivate the mitochondrial pore, suggesting that what would constitute the necessary components for the standard model of MPTP complexes might need to be reconsidered and further characterized (Baines et al., 2007; Berridge et al., 2009; Kokoszka et al., 2004; Krauskopf et al., 2006). Regardless of the putative roles of ANT or VDAC as the components of MPTP, several compounds have been shown to effectively induce changes in mitochondrial membrane permeability and exhibit anticancer activity.

Honokiol—



Extracted from *Magnolia officinals*, honokiol has been used for the treatment of thrombotic stroke, anxiety and gastrointestinal diseases for a long time. Honokiol can induce not only cancer cell death through caspase-dependent and independent apoptosis but also necrosis through mitochondrial permeability transition pore (Filomeni et al., 2007; Ishitsuka et al., 2005). Honokiol also exhibited an antioxidant effect and inhibited lipid peroxidation in the mitochondria (Haraguchi et al., 1997; Lo et al., 1994). Recently, it was found that honokiol could selectively kill esophageal adenocarcinoma cells which expressed higher levels of antioxidant molecules and seemed well adapted to tolerate ROS stress (Chen et al., 2009a). Mechanistically, honokiol was able to cause a decrease in mitochondrial transmembrane potential, which could be inhibited by pretreatment with cyclosporine A, suggesting that the cyclosporine-sensitive MPTP is a possible target of Honokiol. Although treatment of esophageal cells with honokiol led to increased ROS generation, this seemed not the critical event in honokiol cytotoxicity, since antioxidants didn't affect honokiol induced cell death. Cyclophilin D seems to play a key role in mediating the cytotoxicity of honokiol, since a knockdown of cyclophilin D by siRNA partially suppressed honokiol-induced cell death (Chen et al., 2009a).

Betulinic acid—

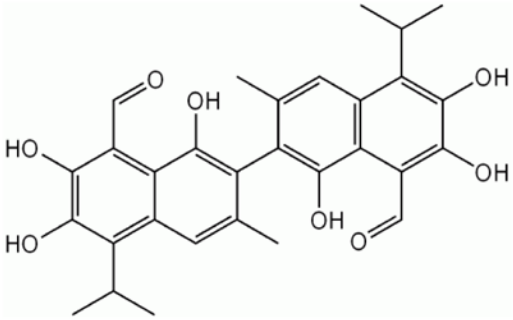


3β-Hydroxy-lup-20(29)-en-28-oic acid (Betulinic acid), a plant-derived pentacyclic triterpenoid, possesses some intriguing pharmacological effects including anti-inflammation, anti-HIV-1, and anticancer effects (Cichewicz and Kouzi, 2004). Betulinic acid was reported to exhibit selective cytotoxicity against several types of tumors including neuroectodermal tumor, colon cancer, and melanoma (Fulda and Debatin, 2000; Fulda et al., 1999; Jung et al., 2007; Pisha et al., 1995). A study found that betulinic acid triggered an apoptotic pathway independent of CD95 ligand/receptor interaction. The cytotoxic action of this compound appeared to be p53-independent. Moreover, betulinic acid showed high efficacy in neuroblastoma cells resistant to CD95 and doxorubicin (Fulda et al., 1997). Using isolated mitochondria in vitro, Fulda et al demonstrated that betulinic acid could directly trigger mitochondrial permeability transition without the involvement of a Z-VAD-fmk-inhibitable caspase (Fulda et al., 1998). Another study also found that betulinic acid induced a significant mitochondrial depolarization independent of caspase activation, while cyclosporine A (CsA) completely prevented mitochondrial depolarization caused by cyclophilin D (Mullauer et al., 2009). Upon mitochondrial permeability transition, apoptogenic factors such as cytochrome c and AIF were released into the cytosol and caspase 8 was cleaved (Fulda et al., 1998).

Bcl-2 inhibitors—Bcl-2 is an integral inner mitochondrial membrane protein, the overexpression of which blocks apoptosis (Hockenbery et al., 1990). In some tumors like non-Hodgkin's lymphoma, Bcl-2 is overexpressed or deregulated, which is correlated with drug resistance and poor prognosis. Antisense oligonucleotides specific for Bcl-2 RNA sequences have been shown to reduce Bcl-2 protein level and specifically suppressed proliferation of cancer cells or enhanced their sensitivity to chemotherapeutic drugs (Campos et al., 1994; Kitada et al., 1994). Antisense oligonucleotides form specific DNA:RNA duplexes leading to RNA degradation and causing cell death (Manion and Hockenbery, 2003). The apoptotic effect of antisense Bcl-2 was mediated by caspase 9, caspase 3 and cytochrome c release from mitochondria. Pretreatment of cancer cells with antisense Bcl-2 potentiated apoptosis induced by dexamethasone, adenovirus-mediated delivery of p53, or paclitaxel in drug resistant myeloma cells (Dong et al., 2008). Mechanistically, a decrease of Bcl-2 protein would release Bad from the Bcl-2/Bad complex, leading to association of Bad with the MPTP component and opening of the pore. G3139, also known as oblimersen sodium, is an 18-mer synthetic phosphorothioate oligodeoxynucleotide with a sequence complementary to a portion of human Bcl-2 mRNA (O'Brien et al., 2005). Combination of G3139 with doxorubicin exhibited a synergistic effect in a breast cancer xenograft model (Lopes de Menezes et al., 2000). G3139 has now entered clinical trails in combination with chemotherapeutic agents in a variety of tumors such as leukemia, small cell lung cancer, multiple myeloma and prostate cancer (Badros et al., 2005; Iwama et al., 2001; O'Brien et al., 2005; Pepper et al., 1999; Rudin et al., 2004).

Small molecular weight compounds have been developed as potent inhibitors of Bcl-2 family proteins. Gossypol, a natural compound initially identified as an antifertility agent for male in China, is the prototype of BH3 interacting small molecule that showed inhibition of Bcl-2, Bcl-XL and Mcl-1 (Kang and Reynolds, 2009). Gossypol seems to be able to directly act upon Bcl-2 molecules present on the mitochondrial outer membrane. It can block Bcl-XL heterodimerization with Bax or Bad, and promote caspase-3 activation and cytochrome c release in Bcl-2 and Bcl-XL overexpressing cells (Meng et al., 2008; Oliver et al., 2005; Shiau et al., 2006). Since gossypol exhibited potent killing in multiple cancer cells (Meng et al., 2007; Vetvicka et al., 2007), it has been introduced into clinical trials in CLL, hormone refractory prostate cancer, and advanced breast cancer (Politzer, 2008; Stein et al., 1992; Van Poznak et al., 2001). Several gossypol derivatives are currently under development as potential anticancer agents. Another small molecule, ABT-737 was identified by using nuclear magnetic resonance (NMR)-based screening. ABT-737 binds with high affinity to Bcl-XL, Bcl-2, and Bcl-w, and antagonizes their anti-apoptotic effect, leading to sensitization of cancer cells to chemotherapy and radiation (Oltersdorf et al., 2005). Besides inducing the classic features of

apoptosis such as caspase activation and cytochrome c release, ABT-737 also induced mitochondrial inner membrane permeabilization (MIMP) leading to outer mitochondrial membrane rupture and matrix swelling in leukemia and lymphoma cells (Vogler et al., 2008). The apoptotic function of ABT-737 was also found to be associated with diminishing intracellular GSH and increased ROS (Howard et al., 2009). Moreover, the combination of ABT-737 and CPT-11 or bortezomib can upregulate pro-apoptotic molecule Noxa expression in colorectal cancer cells and melanoma cells (Miller et al., 2009; Okumura et al., 2008).

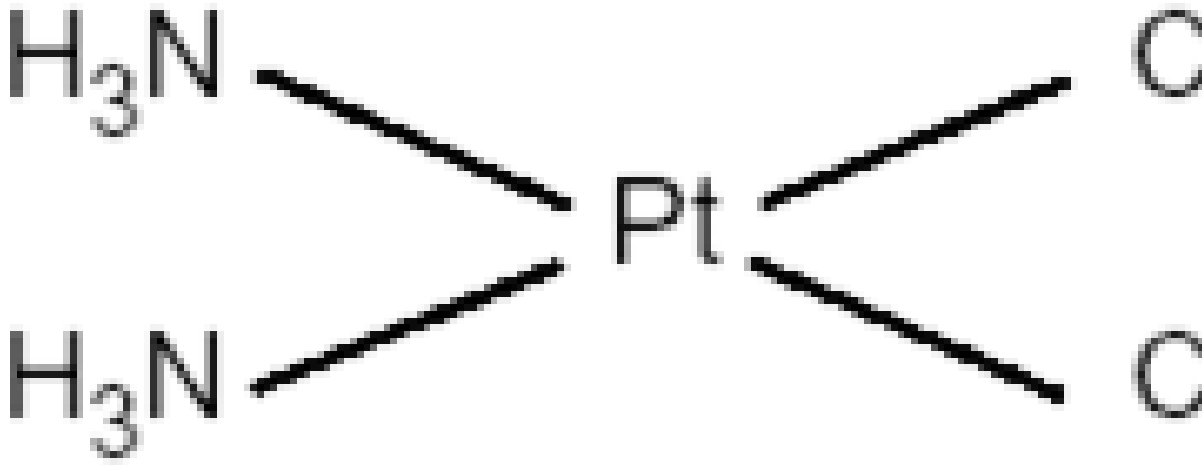


(Gossypol)

2.4 Compounds targeting mtDNA

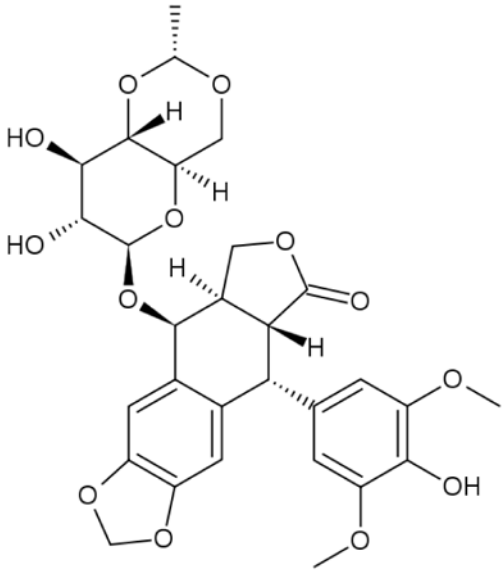
The human mitochondrial genome contains 16.5 kb DNA which encodes 13 respiratory chain subunits. The lack of protection by histones and the relatively weak DNA repair capacity in mitochondria seems to make mitochondrial DNA more vulnerable to damage. By comparing mtDNA from primary bladder, head and neck, and lung cancers with the mtDNA from the blood samples in all cases, and the corresponding normal tissues where available, Fliss et al revealed a high frequency of mtDNA mutations in tumor cells (Fliss et al., 2000). Most of the mutations were T-to-C and G-to-A base transitions, which might be related to ROS derived mutagens. A majority of the mutations were homoplasmic which implies the mtDNA mutation gain substantial replicative advantage. Seventy percent of colorectal cancer cell lines were found with mtDNA mutations, most of which were transitions at purines, again consistent with a ROS related derivation (Polyak et al., 1998). MtDNA also plays a role in affecting cellular response to chemotherapy drugs (Singh et al., 1999). Due to the important role of respiratory chain in mitochondrial ATP generation, compounds that target mtDNA are likely to significantly affect cellular energy metabolism and cell viability.

Cisplatin—



Cisplatin (CDDP) was found to preferentially bind to mtDNA about 50 times more than to nuclear DNA, resulting in NADH-ubiquinone reductase inhibition and decreased ATP generation (Murata et al., 1990). After exposure to one dose of cisplatin during gestation, mice showed higher cisplatin-mtDNA adduction levels than corresponding genomic DNA adduct levels in maternal and fetal brain and liver tissues. The high levels of cisplatin adduction in mtDNA may impair its function and lead to cell death (Giurgiovich et al., 1997). Since CDDP binds to nuclear DNA and mtDNA, both mechanisms of action likely contribute to the anticancer activity of this compound.

Topoisomerase inhibitors—

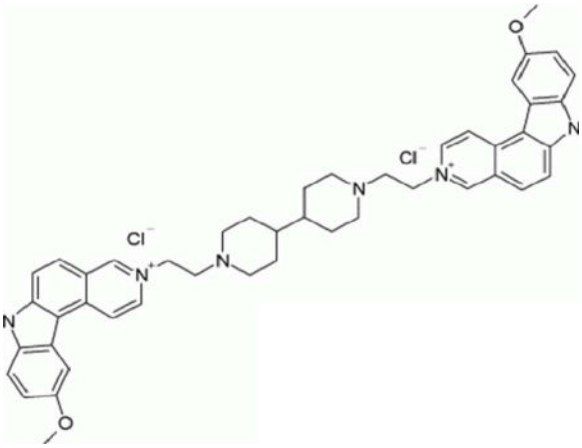


(Etoposide)

The 4-quinolone antibiotics nalidixic acid and ciprofloxacin, inhibitors of the bacterial type II topoisomerase, caused a time-dependent decrease in mtDNA content associated with a reduction in mitochondrial respiration and an increase of lactate production due to upregulation of glycolysis (Petit et al., 1999). DNA topoisomerase II isolated from calf thymus mitochondria

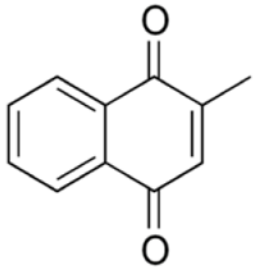
was inhibited *in vitro* by amsacrine (m-AMSA), etoposide (VP-16) and teniposide (VM-26) (Lin and Castora, 1991). Etoposide, a topoisomerase II poison widely used in cancer treatment, can induce cytochrome c release in a caspase-independent manner. Low concentrations of etoposide predominantly caused nuclear DNA damage, while higher concentrations result in a direct effect on the mitochondria through mitochondrial pore transition (Robertson et al., 2000). The contribution of mtDNA damage induced by topoisomerase inhibitors to their anticancer activity is unclear. Interference of nuclear DNA processing is considered the main mechanism by which this class of compounds exerts their anticancer activity. It should also be pointed out that mitochondrial damage has been associated with cardiotoxicity of doxorubicin.

Ditercalinium—



Ditercalinium (NSC 335153) is a bis-intercalating agent which accumulates mainly in the mitochondria (Fellous et al., 1988). Treating mouse and human cells with ditercalinium caused a specific elimination of mtDNA and inhibited its replication (Okamaoto et al., 2003). Ultrastructural studies showed a complete loss of mitochondrial cristae and depletion of mtDNA after ditercalinium treatment (Segal-Bendirdjian et al., 1988). This compound exhibits in vivo antitumor activity in animal models.

Vitamin K3—



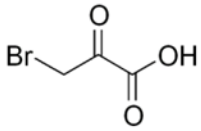
Although vitamin K3 (menadione) does not target mitochondrial DNA directly, this compound exhibited specific inhibitory affect on DNA polymerase γ (pol γ), the mitochondrial enzyme responsible for mtDNA replication. A recent study showed that at the concentration of 30 μM, Vitamin K3 inhibited pol γ activity by 80% without affecting other DNA polymerases, and led to mitochondrial dysfunction, ROS generation, and apoptosis (Sasaki et al., 2008). Vitamin K3 also induced mitochondria-related apoptosis in breast cancer cells (Akiyoshi et al., 2009; Marchionatti et al., 2009). Some recent studies have found its inhibitory effect on pancreatic cancer cells (Osada and Yoshida, 2009). The effect of Vitamin K3 on blood coagulation and

its clinical usefulness have long been recognized, but its use as a potential anticancer agent still remains to be evaluated.

3. Compounds that target altered metabolisms associated with mitochondrial dysfunction

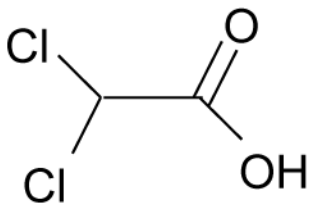
In cancer cells with mitochondrial dysfunction, one important metabolic alteration is the increase of glycolytic activity, which provides ATP as well as other metabolic intermediates for cancer cells to survive and proliferate. Altered expression and activities of enzymes in glycolysis and the tricarboxylic acid (TCA) cycle have been observed in various cancers (Kroemer and Pouyssegur, 2008). These enzyme changes contribute to or promote the preference of cancer cells to aerobic glycolysis and thus have prompted researchers to test whether targeting these enzymes will have any therapeutic benefit. Some of these compounds described below have indeed been shown to be useful in targeting cancer cells with high ‘glycolytic phenotype’ and also enhance their sensitivity towards other chemotherapeutic agents.

3-Bromoyruvate



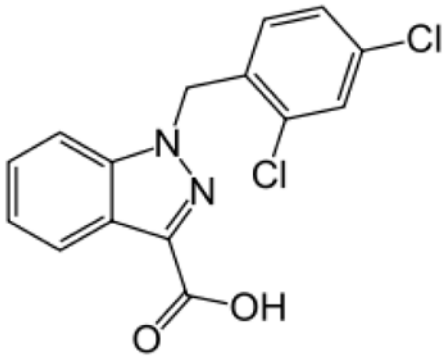
Hexokinase (HXK) (D-hexose 6-phosphotransferase) is the first enzyme in the glycolytic pathway and converts glucose to glucose-6-phosphate via transfer of a phosphate group from ATP to the 6-carbon of glucose. Four isoforms of HXK exist (I–IV) and are expressed in various tissues ((Katzen and Schimke, 1965)). HXKII has been found to bind onto the mitochondria (Robey and Hay, 2006) and has been shown to have elevated activity in H-91 hepatoma cells compared to the control liver samples (Bustamante and Pedersen, 1977). Known for high glucose catabolism ((Shatton et al., 1969; Weinhouse et al., 1972) a hepatocellular carcinoma model, generated via liver implantation of the rabbit VX2 tumor, was tested with 3-bromopyruvate (3BrPA) (Ko et al., 2004). Isolated tissue samples exhibited greater lactate production and higher hexokinase activity in implanted VX2 tumors compared to normal liver samples isolated from the same animal. In addition, the VX2 tumors showed greater distribution of HXK activity to the mitochondria compared to the normal liver tissue. Total mitochondrial HXK activity and mitochondrial respiration was inhibited with 3BrPA (Ko et al., 2004). Recent work by Chen et al found 3BrPA to cause a covalent modification of mitochondria-bound HXKII, leading to disruption of interaction between AIF (Apoptosis Inducing Factor) and HXKII (Chen et al., 2009b). HXKII and AIF released from the mitochondria leads to induction of cell death, revealing another mechanism of action of 3BrPA (Chen et al., 2009b). In addition, 3BrPA exhibited greater potency against lymphoma and colon cancer cells cultured under hypoxic conditions, and was very effective in killing cancer cells with defective mitochondria (Xu et al., 2005).

Dichloroacetate



A key mechanism of action of dichloroacetate (DCA) is via inhibition of pyruvate dehydrogenase kinase (PDK), leading to activation of pyruvate dehydrogenase (PDH). PDH is responsible for the conversion of pyruvate into acetyl-CoA thereby allowing it to enter the TCA cycle. Phosphorylation of PDH by PDK results in inhibition of the pyruvate dehydrogenase activity. Bonnet et al showed DCA was able to reduce hyperpolarized mitochondrial membrane potential in glioblastoma, non-small-cell lung and breast cancer cells but not in noncancerous cell lines (Bonnet et al., 2007). Analysis of metabolic parameters upon DCA treatment in A549 lung cancer cells showed it was able to reduce both glycolysis and fatty acid oxidation while increasing glucose oxidation. In addition, DCA was able to induce apoptosis, elevate H_2O_2 production and activate the Kv1.5 potassium channel in A549 cells (Bonnet et al., 2007). Treatment of head and neck squamous cell carcinomas by DCA showed a dose dependent reduction in phosphorylated PDHα in primary UM-22A cells (McFate et al., 2008). However, such reduction was not seen in the metastatic UM-22B cell line which exhibited a higher ratio of phosphorylated PDHα to total PDHα, greater basal PDK1 levels and lactate. Interestingly, the UM-22B cells were more sensitive to DCA than the UM-22A cells.

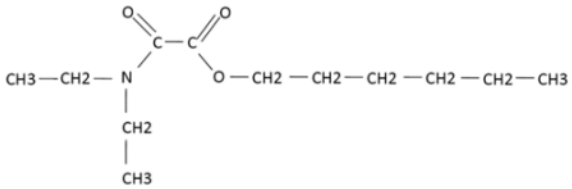
Lonidamine



Lonidamine (LND) derived from indazole-3-carboxylic acid, exhibits both antispermatogenic and antineoplastic properties. LND exhibits a plethora of effects on cells, its primary effect being an energy modulator in cancer cells. LND has been shown to inhibit glycolysis due to inhibition of hexokinase II (Floridi et al., 1981a) which is often found elevated in cancers (Bustamante and Pedersen, 1977). Use of LND was found to deplete ATP, inhibit oxygen consumption in Ehrlich's ascites tumor cells and lactate production under aerobic and anaerobic conditions (Floridi et al., 1998; Floridi et al., 1981a; Floridi et al., 1981b). Minimal apoptosis was observed with individual LND or radiation treatment but was significantly increased upon their combination in radio-resistant malignant melanoma cells (Miyato and Ando, 2004). LND was also found to enhance the cytotoxicity of several chemotherapeutic agents including doxorubicin ((Floridi et al., 1998; Zupi et al., 1986), cisplatin (Raaphorst (Pratesi et al., 1996; Raaphorst et al., 1991) and others such as carmustine (BCNU) and 4-hydroperoxycyclophosphamide (4-HC) (Rosbe et al., 1989). Multiple clinical trials of

lonidamine with or without other chemotherapy drugs have been carried on in non small cell lung cancer, breast cancer, ovary cancer and glioblastoma (Berruti et al., 2002; De Lena et al., 2001; De Marinis et al., 1999; Oudard et al., 2003; Portalone et al., 1999).

Oxamate



Oxamate inhibits lactate dehydrogenase (LDH), an NADH-dependent enzyme responsible for the conversion of pyruvate to lactate. LHD-A activity was shown to be elevated in several mouse tumor mammary epithelial cell lines in comparison to immortalized, non-transformed mammary epithelial cells (Fantin et al., 2002). Goldberg et al showed that the glycolytic rate was reduced, via measurement of lactate production, in HeLa S3 cells treated with oxamate (Goldberg and Colowick, 1965). Furthermore, cell growth and glucose uptake were also inhibited by this compound. This study also supported LDH as a target of oxamate by using α-ketobutyrate as a substitute for pyruvate. Addition of α-ketobutyrate reversed the inhibition of growth and glucose uptake in the presence of oxamate in HeLa cells. However, α-ketobutyrate by itself inhibited cell growth. Generation of recombinant human LDH-A showed oxamate competitively inhibited the enzyme, and was also confirmed in vitro (Thornburg et al., 2008). Both in vitro and in vivo growth inhibition was seen with oxamate treatment in MDA-MB-231 breast adenocarcinoma cells. Depletion of ATP in HeLa cells by oxamate also appears to contribute to its growth inhibition and enhance doxorubicin-mediated cytotoxicity (Hamilton et al., 1995). Additionally, oxamate was shown to inhibit the activity of human recombinant aspartate aminotransferase (AAT) ((Rej, 1979; Thornburg et al., 2008). AAT functions with malate dehydrogenase, a TCA enzyme which oxidizes malate to oxaloacetate, to help shuttle the two metabolites between the mitochondria and cytosol.

4. Summary

Conventional chemotherapy using cytotoxic agents tends to damage both tumor and normal cells and cause significant toxic side effects, which compromise the therapeutic outcomes. The emergence of targeted therapy which is based on the concept of specifically targeting critical molecules unique to cancer cells may provide promising means of selectively killing malignant cells. However, cancer may be caused by multiple genetic alterations and environmental factors. As such, there are many potential targets in cancer cells but no single critical target can be readily identified in most cancer types, perhaps with an exception of Bcr-Abl in CML. This situation makes the development of targeted therapy a challenging task (Green, 2004). Mitochondria structural and functional alterations associated with malignant transformation seem to be a common phenomenon observed in many types of cancers. Utilizing these differences to preferentially target mitochondria of cancer cells may be a logical strategy to achieve therapeutic selectivity. Several classes of small molecules that directly target mitochondria at specific sites or indirectly impact the metabolic alterations in cancer cells with dysfunctional mitochondria seem to exhibit promising anticancer activity. Since many of these small molecules have mainly been tested in cancer cell lines and in animal tumor models, clinical trials are needed to further test their potential for use in clinical treatment of cancer patients. Table 1 provides a list of compounds that target mitochondria or impact the metabolic alterations associated with mitochondrial dysfunction in cancer cells. The modes of action of these compounds and their clinical trial status are also indicated. It is important to recognize

that cancer cells may be able to tolerate inhibition of mitochondrial function by upregulation of glycolysis and other survival mechanisms. As such, a combination of mitochondria-targeted agents with glycolytic inhibitors and other chemotherapeutic drugs may be required to achieve maximum efficacy. However, caution must be exercised to prevent potential increase in toxic side effects. A comprehensive understanding of mitochondrial biology in cancer cells and the interaction between cellular metabolism and drug action is essential in developing mitochondrial-targeted agents for cancer treatment.

Abbreviations

PET	positron emission tomography
ATP	adenosine triphosphate
mtDNA	mitochondrial DNA
DLCs	delocalized lipophilic cations
SOD1	superoxide dismutase1
mtNOS	mitochondrial nitric oxide synthase
ROS	reactive oxygen species
As_2O_3	arsenic trioxide
APL	acute promyelocytic leukemia
NAC	n-acetyl cysteine
α-TOS	alpha-tocopheryl succinate
UbQ	ubiquinone
Cyb	cytochrome B
SDH	succinate dehydrogenase
α-TOC	alpha tocopherol
dUQH2	decyl-ubiquinol
Mn-SOD	mitochondria SOD
MPTP	mitochondrial membrane permeability transition pore
ANT	adenine nucleotide transporter
VDAC	voltage dependent anion channel
MMP	mitochondrial inner membrane permeabilization
mt	mitochondrial
m-AMSA	amsacrine
etoposide	VP-16
teniposide	VM-26
TCA	tri-carboxylic acid
HXK	hexokinase
3BrPa	3-bromopyruvate
DCA	dichloroacetate
PDK	pyruvate dehydrogenase kinase

PDH	pyruvate dehydrogenase
H_2O_2	hydrogen peroxide
LND	lonidamine
BCNU	carmustine
4-HC	4-hydroperoxycyclophosphamide
LDH	lactate dehydrogenase
AAT	aspartate aminotransferase

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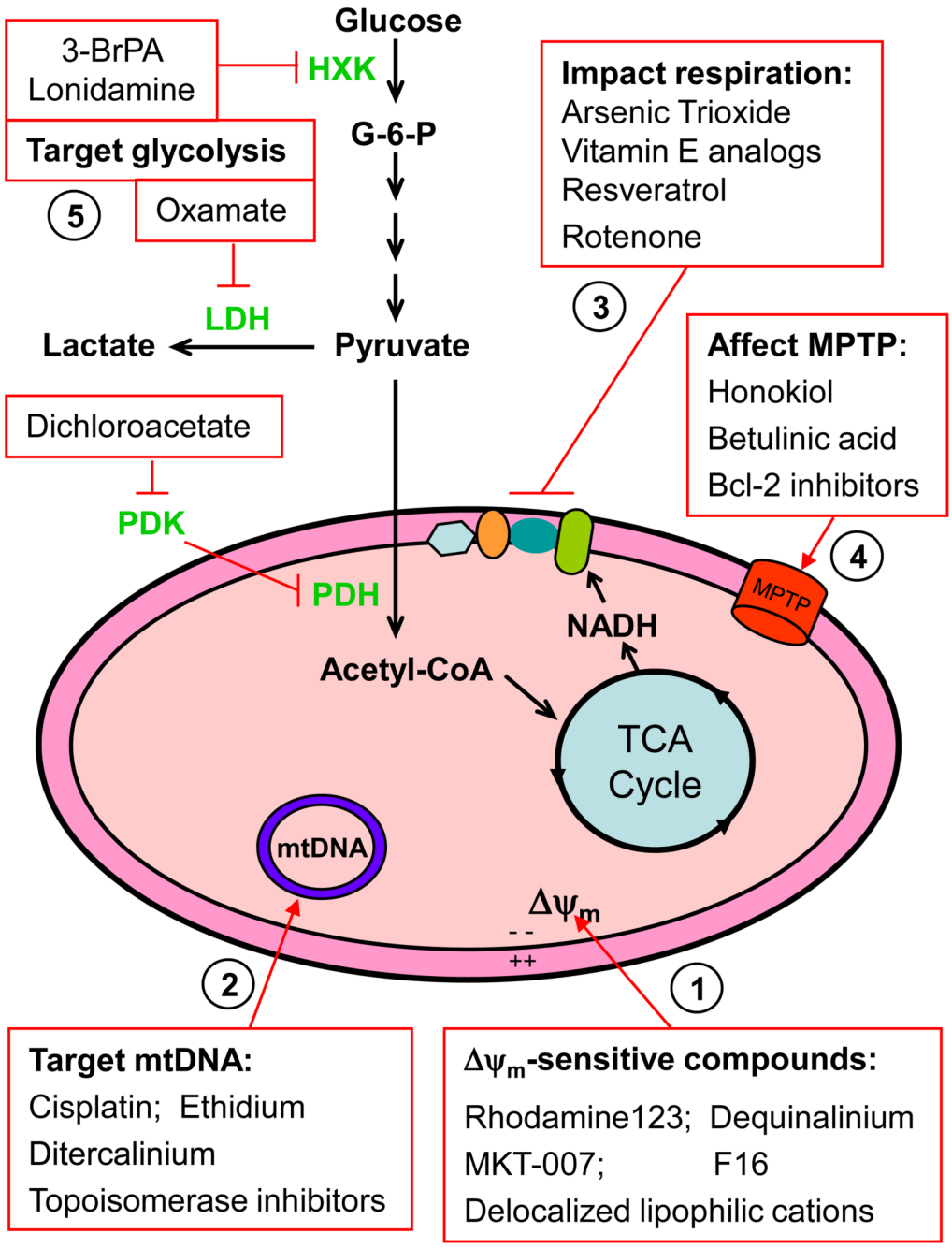

Figure 1.

Overview of possible sites and functions of mitochondria as potential targets for anticancer therapy. (1) Certain compound, often with positive charge, may preferentially accumulate in the mitochondria of cancer cells due to the elevated transmembrane potential (Δψm), with the inner surface of the inner mitochondrial membrane being highly negatively-charged. (2) Mitochondrial DNA, which encodes 13 key components of the respiratory chain complexes, are vulnerable to damage by certain DNA-interacting compounds due to the unique structure of mitochondrial DNA, lack of histone protection, and relatively weak DNA repair capacity in the mitochondria. (3) Inhibition of mitochondrial respiration through targeting the electron transport complexes has been shown to elevate ROS production, deplete ATP and induce

apoptosis. (4) Targeting the mitochondriapermeability transition pore (MPTP) may alter $\Delta\psi m$, induce change in membrane permeability, and result in a release of apoptotic factors. (5) Enzymes of the glycolytic pathways are often found elevated in cancer cells with mitochondrial dysfunction, likely being a mechanism to compensate energy supply and provide metabolic intermediates for cell growth and proliferation. Inhibition of glycolytic enzymes has been shown to preferentially kill cancer cells, especially those with significant mitochondrial dysfunction or under hypoxic conditions. 3BrPA, 3-bromopyruvate; HXK, hexokinase; LDH, lactate dehydrogenase; PDH, pyruvade dehydrogenase; PDK, pyruvate dehydrogenase kinase; $\Delta\psi m$, mitochondria transmembrane potential; mtDNA, mitochondrial DNA ($\Delta\psi m$). MPTP, mitochondrial permeability transition pore.

Table 1

Compounds that target mitochondria or impact the metabolic alterations associated with mitochondrial dysfunction in cancer cells.

Class	Compounds name	Status	Cancer type	Reference
Targeting mitochondria transmembrane potential				
	Rhodamine123	Phase I clinical trial	Prostate cancer	Jones et al., 2005
	MKT-077	Phase I clinical trial	Solid tumors	Britten et al., 2000 Propper et al.,1999
	F16	Pre-clinical testing	Breast cancer, fibrosarcoma, intestinal cancer	Fantin et al., 2002
	Dequalinium	Pre-clinical testing	Colon cancer, meloma, bladder cancer	Bleday et al., 1986; Helige et al., 1992; Weiss et al., 1987
Targeting cancer mitochondrial respiration				
	Arsenic trioxide	In clinical use and clinical trials in various cancer types.	APL, Liver cancer, melanoma, AML, glioma, CLL, lymphoma, breast cancer, non-small cell lung cancer, pancreatic cancer	Bael et al., 2008, Chang et al., 2009, Fox et al., 2008, Shen et al., 1997, Kindler et al.,2008, Niu et al.,1999, Tarhini et al.,2008
	Vitamin E analogue	Phase II study	Melanoma, prostate cancer, colorectal cancer, mesothelioma, breast cancer	Tsavachidou et al.,2009 Neuzil et al., 2007
	Resveratrol	Phase II clinical trials	Colorectal cancer, myeloma, follicular lymphoma	Jang et al., 1997; Goldberg et al.,2003; Walle et al., 2004; Meng et al., 2004; Boocock et al.,2007; Vitaglione et al.,2005; Levi et al., 2005
	Rotenone	Experimental tool		Chance et al., 1963
Targeting mitochondrial membrane permeability				
	Honokiol	Pre-clinical testing	Esophagus cancer	Chen et al., 2009a
	Betulinic acid	Pre-clinical testing	Brain tumor, colon cancer, melanoma	Fulda & Debatin, 2000; Fulda et al., 1999; Jung et al., 2007; Pisha et al., 1995
	Bcl-2 inhibitors (Gossypol)	Phase III clinical trial	Small cell lung cancer, prostate cancer, breast cancer, lymphoma, leukemia	Van et al., 2001

Class	Compounds name	Status	Cancer type	Reference
				O'Brien et al., 2005 Glenn et al., 2009
Targeting mtDNA				
	Cisplatin	In clinical use	Lung cancer, breast cancer, ovarian cancer, germ cell tumor, sarcoma	Sculier et al., 2009, Besse et al., 2009, Decatris et al., 2004, Murata et al., 1990, De et al.,1989
	Topoisomerase inhibitors (Etoposide)	In clinical use	Lymphoma, lung cancer, brain tumor, germ cell tumor	Fischer et al., 2008, Kondaqunta et al, 2006, Mascaux et al., 2000 Lawrence et al., 1993
	Ditercalinium	Pre-clinical testing	Fibrosarcoma, cervical cancer	Rodríguez et al., 2009, Okamaoto et al., 2003
	Vitamin K3	Pre-clinical testing	Pancreatic cancer	Akiyoshi et al., 2009 Marchionatti et al., 2009 Osada & Yoshida, 2009
Targeting metabolic alterations				
	3-Bromopyruvate	Pre-clinical testing	Hepatoma, colon cancer, lymphoma	Bustamante and Pedersen, 1977;
	Dichloroacetate	Phase II clinical trial	Brain tumor, some solid tumor, Non-small cell	Xu et al., 2005 Bonnet et al., 2007; McFate et al., 2008
	Lonidamine	Phase III clinical trial	lung cancer, breast cancer	De Marinis et al., 1999; Berruti et al., 2002
	Oxamate	Pre-clinical testing	Cervical cancer,	Goldberg et al., 1965; Hamilton et al., 1995