

Anticancer Drugs Targeting the Mitochondrial Electron Transport Chain

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Abstract

Significance: Mitochondria are emerging as highly intriguing organelles showing promise but that are yet to be fully exploited as targets for anticancer drugs. **Recent Advances:** A group of compounds that induce mitochondrial destabilization, thereby affecting the physiology of cancer cells, has been defined and termed ‘mitocans.’ Based on their mode of action of targeting in and around mitochondria, we have placed these agents into several groups including hexokinase inhibitors, compounds targeting Bcl-2 family proteins, thiol redox inhibitors, VDAC/ANT targeting drugs, electron transport chain-targeting drugs, lipophilic cations targeting the inner membrane, agents affecting the tricarboxylic acid cycle, drugs targeting mtDNA, and agents targeting other presently unknown sites. **Critical Issues:** Mitocans have a potential to prove highly efficient in suppressing various malignant diseases in a selective manner. They include compounds that are currently in clinical trial and offer substantial promise to become clinically applied drugs. Here we update and redefine the individual classes of mitocans, providing examples of the various members of these groups with a particular focus on agents targeting the electron transport chain, and indicate their potential application in clinical practice. **Future Directions:** Even though reactive oxygen species induction is important for the anticancer activity of many mitocans, the precise sequence of events preceding and following this pivotal event are not yet fully clarified, and warrant further investigation. This is imperative for effective deployment of these compounds in the clinic. *Antioxid. Redox Signal.* 15, 2951–2974.

Introduction

DESPITE CONSIDERABLE ADVANCES IN PREVENTION, diagnosis, and treatment, the incidence of cancer remains high. Cancer has surpassed the cardiovascular diseases as the leading cause of death in the US population under the age of 85 (96,200), and it has the dubious privilege of being the leading cause of death in the developed world overall. Also in the developing world, the incidence of cancer is dramatically increasing as a result of growing population and lifestyle changes (95). The difficulties associated with cancer treatment can be ascribed to a number of reasons, including the extreme heterogeneity of mutations even within one cancer type (100, 164). This heterogeneity makes it unlikely that cancer could be controlled by targeting a single gene or signaling pathway (34). We can therefore either choose to interfere with a specific pathway by tailor-made therapeutics based on individual histological or genetic analysis of a patient, or search for therapeutics directed at processes invariantly occurring in most cancers irrespective of the underlying genetic defect, yet absent or sufficiently different in normal somatic cells. This includes such diverse phenomena as angiogenesis and mitochondria-controlled metabolic and apoptosis pathways.

With respect to mitochondria and cancer, initial observations were made at the beginning of the third decade of the twentieth century by Otto Warburg, who noticed that cancer cells consume more glucose and produce more lactate than normal cells (a comprehensive review on the life and work of Otto Warburg in the context of modern knowledge can be found in Ref. 108). Confusingly, this was later misinterpreted by Warburg and others, contending that all cancer cells must have damaged respiration, when in fact the changes in most types of cancer may be more accurately explained in terms of regulation of both glycolysis and respiration (206). It should be noted, however, that in a certain (probably minor) subset of cancers, this envisaged damage to mitochondria or respiration actually occurs in a form of relatively rare mutations in nuclear genes encoding essential components of oxidative phosphorylation, such as complex II (CII) in paraganglioma and pheochromocytoma (83), or other metabolic pathways, exemplified by the enzyme isocitrate dehydrogenase in gliomas (46). In addition, mutations directly in mitochondrial DNA occur in up to 60% of cancers (116). Even though most of these mitochondrial mutations are silent, about one quarter of them have a potential to alter mitochondrial function, which may contribute to the phenomenon described by Warburg.

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Mitochondria are central to the phenotype characteristic of cancer cells, as mitochondria from cancer cells increase the glycolytic rate in the cytosol of nonmalignant cells, whereas mitochondria from nonmalignant cells decrease the rate of glycolysis in the cytosol of malignant cells (28). It therefore appears that cancer cell mitochondria are different from the mitochondria in normal cells. The characteristic alterations of cancer cell mitochondria include the association of upregulated hexokinase II with the porin-like voltage-dependent anionic channel (VDAC) in the mitochondrial outer membrane (MOM) (130), attenuated entry of pyruvate into the tricarboxylic acid cycle (TCA) in response to hypoxia-inducible factor 1 (HIF1), and Myc upregulation and corresponding increase in glutamine metabolism (45, 108, 206), downregulation of complex V (CV) function (43), elevated mitochondrial membrane potential (11, 49, 84, 138, 190), and increased propensity to generate reactive oxygen species (ROS) (196).

Considering the mitochondrial characteristics listed above that are typical of many different cancer types, the possibility of selective targeting of cancer cell mitochondria represents an enticing option for anticancer therapy (79, 170). Mitochondria, responsible not only for the maintenance and regulation of cellular metabolism but also for the execution of apoptosis (109), may be effectively coerced by pharmacological interventions into expediting the demise of cancer cells using an array of compounds we proposed to name 'mitocans' (a word derived from 'mitochondria' and 'cancer') (149, 153). Mitocans induce cancer cell death by directly targeting various sites in and around the mitochondrion, thereby bypassing the frequent defects in apoptotic machinery upstream of mitochondria. In the present review, we will provide an overview of the mitocans (some of them are well known whereas others are quite novel) and divide them into classes based on their mitochondrial target. We will highlight the role of mitocans targeting the electron transport chain (ETC), which comprise a number of intriguing compounds often highly selective for cancer cells.

Mitocan Classes

In this section we divide the mitocans into eight distinct classes of compounds with defined modes of action, stemming from their molecular targets around or inside the mitochondria (149, 153). The individual classes of mitocans are listed in Table 1, graphically depicted in Figure 1, and are numbered based on the location of their respective targets from outside of MOM to inside the mitochondrial matrix. Each of the classes is represented by a number of compounds, some of which are covered in the next paragraphs.

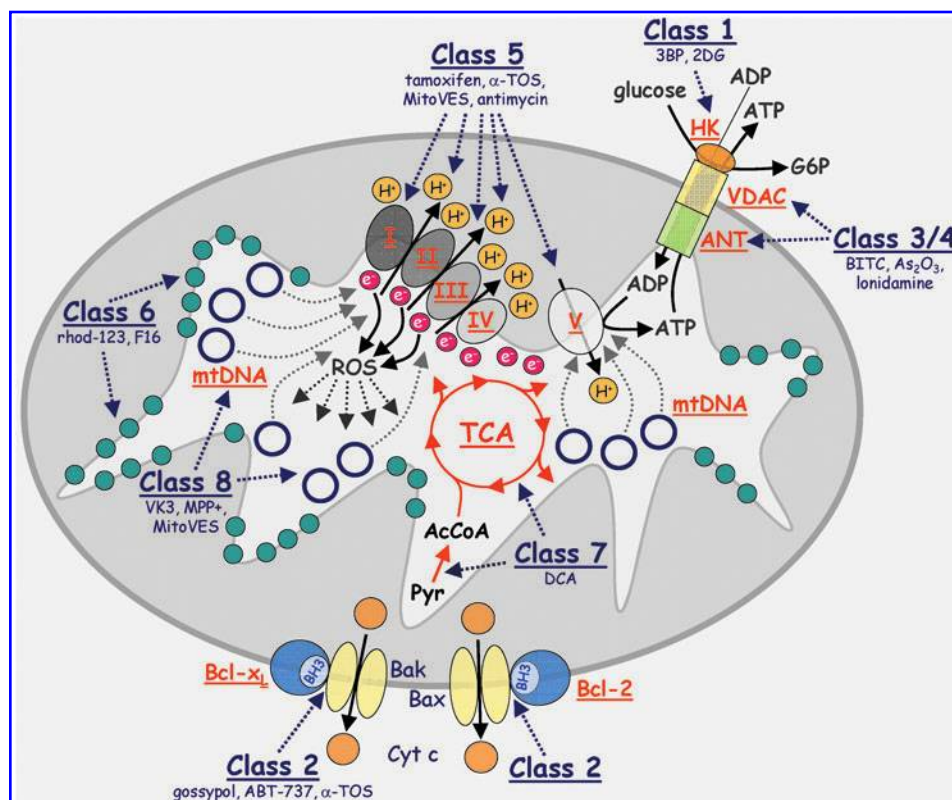
Class 1: Hexokinase inhibitors

Hexokinase (HK) is an enzyme, whose main role is to phosphorylate glucose, converting it to glucose-6-phosphate (G6P), which is a substrate for metabolic pathways ultimately coupled with ATP generation. HK has a very important function in cancer. Besides converting glucose to G6P, which can then enter the metabolic machinery to, ultimately, yield ATP, HK is associated with the cytosolic side of VDAC, a transmembrane protein in the MOM. The isotype HK-II is over-expressed in cancer cells in response to hypoxia, being transcriptionally regulated by HIF1. A channel is formed by VDAC and the adenine nucleotide transporter (ANT) extending across the MOM and the mitochondrial inner membrane (MIM), respectively, interconnecting the mitochondrial matrix to the cytosol. The function of the channel is to transport outside mitochondria the ATP made by the electron transport chain (ETC) of the MIM. When expressed at higher levels, as in cancer cells, HK-II binds both ATP and glucose, resulting in the production of G6P. A direct correlation has been established between the growth of carcinomas and the levels of HK activity (28). According to Koobs (107), mitochondrial-bound HK limits respiration when tumor cells are utilizing glucose (known as the Crabtree effect), even though large amounts of ADP continue to be produced. The continuous phosphorylation of glucose by

TABLE 1. CLASSIFICATION OF MITOCANS

| Class number | Type | Examples | References |
|--------------|--|---|------------------------------------|
| 1 | Hexokinase inhibitors | 3-Bromopyruvate 2-Deoxyglucose | (36, 106, 130) (13, 184) |
| 2 | Compounds targeting Bcl-2 family proteins | Gossypol ABT-737 Antimycin A | (102) (205) (201) |
| 3 | Thiol redox inhibitors | α -Tocopheryl succinate Isothiocyanates | (181) (198, 217) |
| 4 | VDAC/ANT targeting drugs | Arsenic trioxide Lonidamine, arsenites, steroid analogues like CD437 | (134, 166) (12) |
| 5 | See Table 2 for detail | | |
| 6 | Lipophilic cations targeting inner membrane | Rhodamine-123 F16 (KLAKKLAK) ₂ peptide | (15, 98, 114) (66) (64) |
| 7 | Drugs targeting the tricarboxylic acid cycle | DCA | (22) |
| 8 | Drugs targeting mtDNA | Vitamin K3, fialuridine, 1-methyl-4-phenylpyridinium, MitoVES | (123, 177) (137, 203) |
| 9 | Drugs targeting other (unknown) sites | Betulinic acid | Neuzil et al., unpublished (73) |

FIG. 1. Schematic illustration of the molecular targets of individual classes of mitocans. The classes of mitocans comprise the following, as enumerated from the outside of the mitochondria towards the matrix. Class 1: hexokinase inhibitors; Class 2: BH3 mimetics and related agents that impair the function of the anti-apoptotic Bcl-2 family proteins; Class 3: thiol redox inhibitors; Class 4: agents targeting VDAC and ANT; Class 5: compounds targeting the mitochondrial electron transport chain; Class 6: hydrophobic cations targeting the MIM; Class 7: compounds that affect the TCA; Class 8: agents that interfere with mtDNA; and Class 9, compounds acting on mitochondria whose molecular target is currently unknown (not shown). (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).



ATP (proceeding by the mitochondrial-bound HK) reduces the level of phosphate available for oxidative phosphorylation, and thereby prevents attaining maximal rates for state 3 respiration (8). Hence, HKII via its mitochondrial localization also helps to stabilize mitochondria, suppressing apoptotic death of cancer cells and promoting their survival (130).

Several hexokinase inhibitors have been found to inhibit cancer growth and of these, considerable focus has been on 2-deoxyglucose (2DG) and 3-bromopyruvate (3BP). The basis for the action of 2DG is to inhibit HK activity and hence, glycolysis, with the result that the binding of HK to VDAC is prevented. By this mechanism, 2DG promotes the susceptibility of malignant cells to other forms of treatment (184). This finding, as well as recent reports that 2DG promotes cancer cell apoptosis when used in combination with another mitocan, the anti-diabetic drug, metformin, provides the basis for testing these compounds in the clinical setting (13). 3BP is an alkylating agent that inhibits both HK activity and the mitochondrial complex II (CII) and consequently is included in the mitocan class 2 and 5 (Fig. 1). Recent data indicate that 3BP acts by binding covalently to HKII, causing its dissociation from VDAC (36). 3BP causes cancer cell death due to the rapid depletion of ATP and suppresses tumor growth considerably in animal models. For these reasons, 3BP is another candidate for cancer clinical trials (106, 130).

Class 2: Compounds targeting Bcl-2 family proteins

Bcl-2 family proteins comprise a class of polypeptides whose state of relative balance in levels of the family members decides the cell's 'fate'. When the levels of expression of the pro-apoptotic members are greater, then it will result in the demise

of the cell, whereas greater levels of expression of the anti-apoptotic Bcl-2 family proteins will provide a pro-survival 'environment'. Although recent findings revealed novel functions for the Bcl-2 family members, including a role in the biogenesis of mitochondria (189), we will only briefly discuss these proteins as targets for class 2 mitocans, resulting in promoting apoptosis of cancer cells. The basis for the action of this mitocan class stems from the finding that the anti-apoptotic and pro-apoptotic Bcl-2 family proteins interact via their Bcl-2 homology-3 (BH3) domains, thereby preventing the BH3 domains from forming large channels or pores in the MOM, a prerequisite for apoptotic signaling downstream of mitochondria (221). Since the MOM channel is made of oligomers of the pro-apoptotic Bax or Bak protein BH3 domains increased expression of the anti-apoptotic BH3 interacting proteins Bcl-2, Bcl-x_L, or Mcl-1 will protect cancer cells from apoptosis. Moreover, the anti-apoptotic Bcl-2 family proteins are often overexpressed in cancer cells (122). Therefore, small molecules or BH3 mimetics, targeting the interaction between the anti- and pro-apoptotic Bcl-2 protein members is of clinical importance (102, 224).

The BH3 mimetics include the natural polyphenolic compound gossypol. This agent has been shown to interact with BH3 binding domains, thereby interfering with the interaction between Bcl-2, Bcl-x_L, or Mcl-1 and the pro-apoptotic proteins, Bax or Bad. The result is the oligomerization of Bax or Bad to form channels and activation of the post-mitochondrial apoptotic signaling (160). Gossypol has served as a structure for developing more efficient BH3 mimetics, such as the highly intriguing compound, ABT-737 (Abbott Laboratories, ref. 205). ABT-737, as well as an oral version, ABT-263 (199), are now being tested in clinical trials. The apoptogenic compound α -tocopheryl succinate (α -TOS) has been reported to interact with

the BH3 binding domain of Bcl-2 and Bcl-x_L, which suppressed their binding interaction with the pro-apoptotic protein Bak, arresting proliferation of prostate cancer cells and inducing their death by apoptosis (181). This activity of α -TOS is interesting in that it complements a second apoptogenic activity due to its ability to also interact directly with the ubiquinone sites of the mitochondrial CII (see below for details). In addition, the mitochondrial complex III (CIII) Q_i site inhibitor, antimycin A, also acts as a BH3 mimetic (201), suggesting that on a more general level, compounds that interact with ubiquinone binding sites in the mitochondrial electron transport chain may have a tendency to be BH3 mimetics as well, consistent with the possibility that the BH3 binding site in Bcl2 family members might also be a ubiquinone binding site (153).

Class 3 and 4: Thiol redox inhibitors and VDAC/ANT targeting drugs

The redox environment of cancer cells is distinctly different from that of normal cells in that cancer cells show much higher intrinsic levels of ROS and also, as a result, appear more vulnerable to agents that induce further elevations in oxidative stress, since their antioxidant capacity is relatively inferior (93, 193). Therefore, compounds that oxidize thiol group and/or deplete the mitochondrial glutathione pool will cause substantial apoptosis of cancer cells (72, 196). Agents like arsenic trioxide (134, 166) or isothiocyanates, represented by phenylethyl isothiocyanate (PEITC) (198, 217) have been shown to possess relative selectivity in killing cancer cells by upsetting the normal homeostasis in the cellular redox environment. Intriguingly, PEITC has been reported to efficiently kill resistant leukemia cells (197).

The permeability transition pore complex (PTPC) forms as a super channel comprising the VDAC/ANT system of proteins embedded in the MOM and MIM, respectively, interconnecting the mitochondrial matrix with the cytosol, and serves as a mode of transport for a variety of solutes and small molecules including ATP and ADP (228). Deregulation of the VDAC/ANT complex results in apoptosis induction in cancer cells. Compounds that modulate the PTPC include lonidamine, arsenites, and steroid analogues (represented by CD437) (12). Interestingly, an arsenite analogue 4-(N-(S-glutathionylacetyl)amino) phenylarsineoxide (GSAO) was shown to inhibit the function of ANT by crosslinking its cysteine residues. This was followed by generation of oxidative stress and induction of apoptosis, which was selective for proliferating, angiogenic endothelial cells while being nontoxic to growth-arrested endothelial cells (54). These results indicate that GSAO can selectively kill endothelial cells in the context of a growing tumor, acting in an anti-angiogenic manner. Similar findings were reported for the mitocan, α -TOS (see more on its molecular mechanism below).

Class 5: Electron transport chain targeting drugs

Members of this class are discussed in more detail in a separate section below.

Class 6: Lipophilic cations targeting the inner mitochondrial membrane

The molecular target of this group of compounds is given by the relatively high transmembrane potential that exists across the MIM ($\Delta\psi_{m,i}$). In fact, it has been documented that cancer

cells have a considerably higher $\Delta\psi_{m,i}$ than that in nonmalignant cells due to altered mitochondrial bioenergetics (138). This feature will dictate the intracellular targeting of lipophilic cations which, as a result of the increased $\Delta\psi_{m,i}$ in cancer cells, will make these mitocans relatively more selective for cancer cells, since the Nernst law defines that with each increase of $\Delta\psi_{m,i}$ by -60 mV, a corresponding 10-fold increase occurs in the accumulation of cationic compounds in the MIM (139, 207).

A prime example of this mitocan class targeting the inner membrane is rhodamine-123, which was reported to accumulate in mitochondria of src-transformed cells as early as in the 1980s (98), showing selectivity for cancer cells (114). Following from this, the selective toxicity of a number of delocalized lipophilic cationic agents, including the peptide (KLAKKLAK)₂ towards cancer cells was found (64). A compound termed F16, with a delocalized positive charge, was identified by high-throughput screening and was found to be selective and effective against breast carcinomas with high levels of HER2 expression (66).

Class 7: Drugs targeting the tricarboxylic acid cycle

The TCA cycle, also referred to as the citric acid cycle or Krebs cycle, is a source of electrons that are fed into the ETC that are used to drive the electrochemical proton gradient required for the generation of ATP. The TCA cycle is based on the addition of acetyl-CoA (formed in the mitochondrial matrix by conversion of pyruvate, catalyzed by pyruvate dehydrogenase) to oxaloacetate to form citrate. Citric acid is then, in a series of reactions, converted to oxaloacetate, which then again adds a molecule of acetyl-CoA. During this process, electrons are released to drive the proton gradient that is coupled to the generation of ATP. An interesting step in the TCA cycle involves succinyl dehydrogenase (SDH), also known as the mitochondrial respiratory complex II, which converts succinate to fumarate, releasing two electrons that are used to reduce ubiquinone (UbQ) to ubiquinol (UbQH₂), which is then carried to CIII of the ETC.

A number of compounds exist that target the TCA cycle as well as the reaction converting pyruvate to acetyl-CoA, a prerequisite for pyruvate to enter the mitochondria and the TCA cycle. The enzyme pyruvate dehydrogenase, catalyzing the reaction, is negatively regulated by phosphorylation via the pyruvate dehydrogenase kinase (PDK), which is often upregulated in cancer by HIF1 and Myc transcription factors (45). This skews the balance towards the predominantly glycolytic phenotype typical of cancer cells. Dichloroacetate (DCA), a relatively basic compound, is selective for killing cancer cells by suppressing the activity of PDK (22), thereby promoting the activity of its target, pyruvate dehydrogenase. In this way, DCA increases the supply of pyruvate into the TCA cycle and causes a shift from anaerobic glycolysis to oxidative glycolytic metabolism accompanied by dissipation of $\Delta\psi_{m,i}$, generation of ROS, and activation of the K⁺ channel, events that typify cancer cells (22). DCA is already in clinical use to treat patients with mitochondrial deficiencies, and therefore, its development as an anticancer drug should be less complicated than with a completely novel agent.

Class 8: Drugs targeting mtDNA

Mitochondria are unique organelles because they carry their own genetic information encoded on a small circular

genome, referred to as mitochondrial DNA (mtDNA). The mammalian mitochondrial genome has a size of over 16 kB, and encodes 13 subunits of the mitochondrial complexes I, III, IV, and V, 24 tRNAs, 12S, and 16S rRNA, and also contains a region called the D-loop sequence, which is important in the regulation of mtDNA replication (5).

To date, several compounds have been reported that interfere with the function and stability of mtDNA and other drugs that affect the activity of the mitochondrial DNA polymerase- γ . For example, vitamin K3 (menadione) targets mtDNA by inhibiting the activity of DNA polymerase γ that is specific for replication of mtDNA, with ensuing induction of apoptosis (177). Similar effects were reported for fialuridine which induces mitochondrial structural defects (123). The Parkinsonian toxin 1-methyl-4-phenylpyridinium causes a reduction in the copy number of mtDNA by destabilizing the structure of the mitochondrial D-loop (137,203).

We have been studying vitamin E analogues as anticancer drugs, epitomized by the redox-silent α -TOS (see Chapters 3.2 and 4 for details on the apoptogenic signaling induced by α -TOS and similar agents). To enhance the toxicity of this drug, we modified it by adding a triphenylphosphonium (TPP⁺) group onto it, targeting the drug to the mitochondrial inner membrane (MIM) (57, 58), as has been done previously for a variety of antioxidants (103, 145). This mitochondrially targeted vitamin E succinate (MitoVES) was found to be superior to the untargeted α -TOS in its apoptogenic activity. Interestingly, we found that MitoVES modulates mtDNA and more specifically, at subapoptotic doses, suppressed the D-loop derived transcript levels in a range of cancer cells, a phenomenon that was not observed for α -TOS. This was accompanied by inhibition of cancer cell proliferation (Neuzil et al., unpublished). It is not clear at this stage, whether modulation of mtDNA by MitoVES is a direct effect on the mitochondrial genome or whether it is mediated by ROS generated in the

MitoVES-treated cells (see Chapter 4 for details). Nevertheless, MitoVES provides an intriguing possibility for interfering with tumour progression by means of suppressing the proliferation of cancer cells without necessarily inducing apoptosis.

Class 9: Drugs targeting other sites

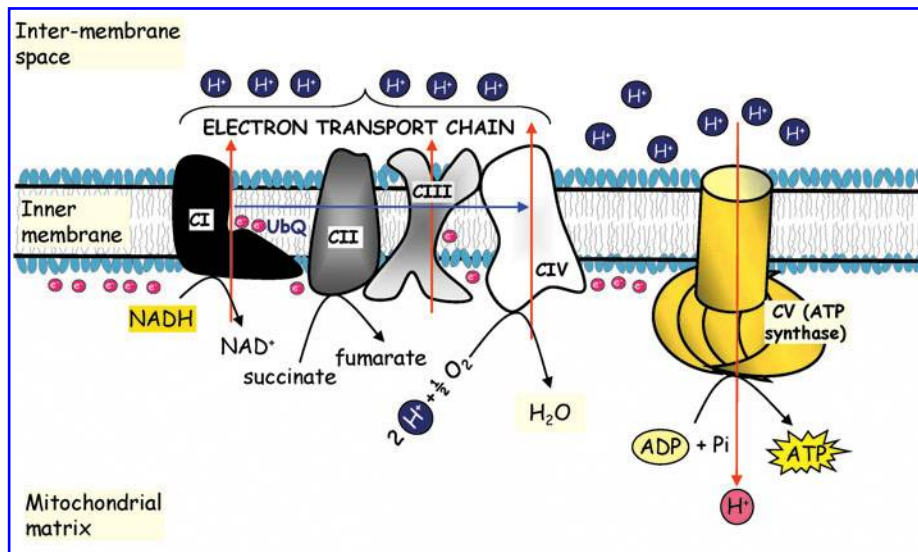
This group comprises a number of agents that affect mitochondria but their targets have not yet been fully elucidated. While effects on specific components of mitochondria or mitochondrial function have been reported for many such compounds, in these cases the molecular target has not yet been defined. A prime example of such compounds as is betulinic acid (73) of potential clinical relevance.

Anticancer Drugs That Target the Electron Transport Chain

Mitocans comprise a large group of different compounds, many of which were discussed above and in recent reviews (72, 75, 80, 169, 207). The following sections focus in more detail on the class 5 of mitocans, which target the mitochondrial ETC, the central system of mitochondrial energy production and the process of oxidative phosphorylation (176, 178).

The production of energy is achieved by transporting electrons in a coordinated manner from NADH or FADH₂ (generated from substrates in the TCA cycle) to the final acceptor, molecular oxygen, to produce water (Fig. 2). The four protein complexes of the mitochondrial ETC are embedded in the mitochondrial inner membrane, and, by passing electrons from complexes I and II via complex III to complex IV (abbreviated as CI etc.) with the help of electron carriers, UbQ and cytochrome c, generate energy that is maintained as an electrochemical proton gradient across the mitochondrial

FIG. 2. The structure of the electron transport chain (ETC), the target of Class 5 of mitocans. The ETC, localized on the interface of the MIM and the matrix, comprises complex I (CI; NADH:UbQ oxidoreductase), complex III (CIII; cytochrome bc₁ complex or ubiquinone-cytochrome c oxidoreductase), and complex IV (CIV; cytochrome c oxidase) that facilitate movement of protons across the MIM, from the matrix to the IMS. Electrons are transferred to CIV to give rise to molecular water and the proton extrusion provides an electrochemical potential gradient formed across the MIM with negative potential on the matrix face. This proton gradient powers the generation of ATP via complex V (CV; the F₀F₁ATP synthase). Complex II (CII; succinate dehydrogenase, SDH), converts succinate to fumarate as a part of the TCA cycle, and the liberated electrons are then fed into the ETC, whereby CII provides a link between the TCA cycle and the ETC. The subunits ND1, ND2, ND3, ND4, ND4L, ND5, and ND6 of CI, CYTB of CIII, COX1, COX2, and COX3 of CIV, and ATP6 and ATP8 of CV are encoded by the mtDNA, whereas the other subunits of these complexes plus all complexes of CII are encoded by the nuclear DNA. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).



inner membrane (MIM). CV, the F_1F_0 -ATPase, then uses the energy from the proton gradient to generate ATP from ADP and inorganic phosphate, which provides the cell with its fundamental energy substrate. ETC, due to the large electron flow, is the major source of mitochondrial ROS, with CI and CIII identified as prime superoxide-generating sites (1, 144). In addition, recent evidence also points to a role CII in ROS formation (120, 121). The superoxide by-product is not harmful when ROS production is controlled within the constraints of the cellular redox system, as the cell must maintain moderate ROS levels necessary to sustain the cellular signaling processes. However, deregulation leading to permanently higher levels of ROS may predispose, over time, to carcinogenesis (70). By contrast, an acute and substantial increase in ROS levels may have a much more immediate effect and commit the cell to undergo apoptosis (76, 128).

Cancer cells often show higher levels of oxidative stress and higher saturation of their antioxidant defenses, and consequently may be more sensitive to apoptosis induction by compounds disrupting the cellular redox balance (196). In addition, the ETC itself plays a role in apoptosis, because defects in the ETC are associated with abnormalities in apoptosis induction (112, 120). Furthermore, inhibition of ETC complexes results in increased ROS production, such that targeting the ETC complexes with compounds from the class 5 of mitocans provides a possible strategy for eliminating cancer cells with relatively high specificity. The next section provides an overview of the apoptosis-inducing class 5 mitocans (Table 2) targeted to the individual macromolecular complexes of the mitochondrial ETC and their anticancer potential. The list is not exhaustive, and further research will very likely identify additional candidates.

Complex I

The first and largest complex of the ETC, NADH:UbQ oxidoreductase or CI, functions by transferring electrons from NADH derived from the TCA cycle to UbQ, which passes them on to CIII. CI contributes to the maintenance of the proton gradient on the MIM, pumping four protons into the intermembrane space for each NADH oxidized. In most mammalian cells, CI comprises 45 subunits, including seven that are coded by the mitochondrial genome, and has a total molecular mass of about 1,000 kDa (178). The crystal structure of CI provided insight into the mechanism of proton pumping and electron transfer revealing that the electrons are removed from NADH by a flavin group, relayed to a series of iron-sulfur clusters, and finally donated to UbQ bound within CI (17, 62).

CI is proposed as one of the sites for superoxide formation within the ETC. It is not known precisely how the superoxide is formed, but the flavin group, the N2 iron sulfur cluster and the UbQ binding sites have each been suggested to participate in the process (17, 68, 86, 110, 111, 113). The basal level of superoxide production from CI is dramatically increased either due to retrograde electron transport or inhibition of CI. With regard to inhibition, more than 60 classes of compounds are known to inhibit the function of CI (50). Most of these inhibitors are specific for overlapping binding sites within the UbQ-binding region (159, 195), but despite this, substantial differences were seen with respect to the ability of individual compounds to induce superoxide production (67, 113). Some well-known CI inhibitors are considered neurotoxic and are

often used as pesticides, and as such, have been implicated in the development of neurological disorders such as Parkinson's disease, inducing similar symptoms in experimental animals (18, 53, 60, 162). Recent observation suggests that the neurotoxicity of rotenone and-methyl-4-phenylpyridinium (MPP⁺) is unrelated to the inhibition of CI, and instead results from their ability to destabilize microtubules (37). The equally potent CI inhibitor piericidin, which does not disrupt microtubules, is not neurotoxic. For this reason, rotenone, even though it efficiently induces apoptosis in cancer cells (124, 166, 214), is not considered for cancer treatment and piericidin could be a better candidate.

In the context of anticancer applications, a well-studied analogue of rotenone is deguelin, an inhibitor of the Akt and nuclear factor- κ B (NF- κ B) signaling pathways. Deguelin shows chemopreventive anti-tumor activity in experimental cancer and xenograph models without major toxic effects and also inhibits angiogenesis (39, 51, 117, 147, 156, 202). This anti-tumor activity is at least in part related to its interaction with heat shock protein HSP90, but direct inhibition of CI also plays a role, because cells with defective respiration are insensitive to deguelin-induced apoptosis (87, 104, 157). The lower affinity for CI (60 times lower for deguelin compared to rotenone) and the lack of the microtubule-related side effects may possibly contribute to the more favorable toxicity profile of deguelin (30,50).

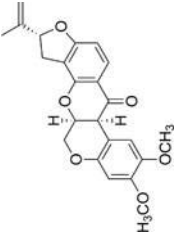
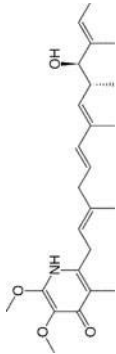
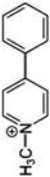
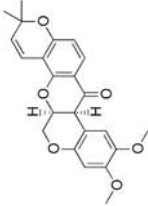

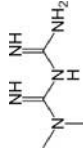
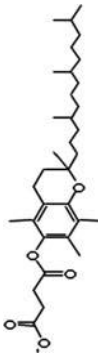
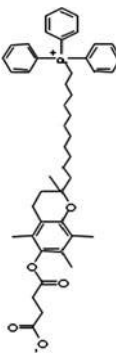
Interestingly, the anti-breast cancer drug, tamoxifen, routinely used in the clinic as an antagonist of the estrogen receptor in the treatment of hormone-dependent breast cancer was also reported to inhibit CI at the flavin site. This inhibition was accompanied by increased cellular hydrogen peroxide production, which was synergistically enhanced by estradiol (142). This observation could potentially extend the use of tamoxifen to other cancer types besides hormone-dependent breast cancers, and its prior history as a drug already clinically approved may make its application to other cancers more straightforward. Indeed, some anti-proliferative effects of tamoxifen against cells lacking the estrogen receptor, as well as against prostate cancer have been observed and the combination of tamoxifen with estradiol can induce apoptosis of tamoxifen-resistant breast cancer (14, 41, 161). Targeting the ETC may therefore be an important additional component of tamoxifen's anticancer activity, despite some doubt remaining as to whether CI is the only target of tamoxifen in the ETC (146).

Finally, the biguanidine, metformin, an AMP-activated protein kinase (AMPK) activator used in the clinic to treat type-2 diabetes also inhibits CI and blocks mitochondrial respiration, readily inducing apoptosis in p53 deficient cancer cells, and prevents the growth of p53-deficient xenographs (29). It also displays anti-tumor effects *in vivo* in pancreatic cancer and kills breast cancer cells as well as breast cancer stem-like cells; patients given metformin show a reduced incidence of cancer and increased effectiveness of chemotherapy to treat their tumors (reviewed in Ref. 38). Thus, metformin may be a promising option for cancer treatment when used either alone or in combination with other anti-cancer drugs (13).

Complex II

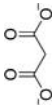
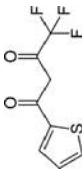
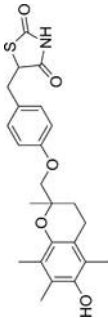
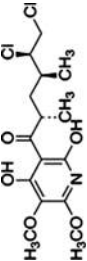
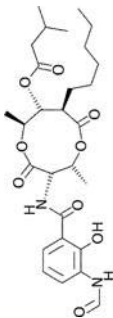
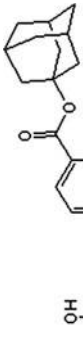
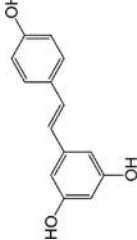
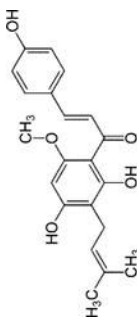
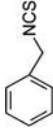
CII or succinate dehydrogenase (SDH), is the smallest of the respiratory complexes, and in mammals, all four of its

TABLE 2. CLASS 5 MITOCANS: COMPOUNDS TARGETING THE ELECTRON TRANSPORT CHAIN

| Complex | Targeting Compounds | Structure | Status |
|---|--|---|--|
| Complex I NADH:UbQ oxidoreductase | Rotenone |  | Laboratory use, standard CI inhibitor, neurotoxic, not considered for cancer treatment |
| | Piericidin |  | Effective CI inhibitor, no apparent neurotoxicity, not explored in cancer |
| | 1-Methyl-4-phenylpyridinium (MPP ⁺) |  | Neurotoxic, not considered for cancer treatment |
| | Deguelin |  | Preclinical <i>in vivo</i> cancer models, shows selectivity for cancer cells |
| Complex II Succinate dehydrogenase | Tamoxifen |  | Standard treatment in pre-menopausal hormone-positive breast cancer |
| | Metformin |  | Standard treatment in type-2 diabetes, inhibits AMPK and CI, >20 clinical trials for cancer (phase I, II, and III) |
| | α -Tocopheryl succinate (α -TOS) |  | Experimental drug, preclinical <i>in vivo</i> cancer models, selectivity for cancer cells. |
| Complex II Succinate dehydrogenase | Mitochondrially targeted vitamin E succinate (MitoVES) |  | Mitochondrially targeted experimental drug, increased efficiency in preclinical <i>in vivo</i> cancer models, selectivity for cancer cells |
| | 3-Bromopyruvate (3BP) | BrCH ₂ -CH ₂ -COOH | Inhibits hexokinase and CII, preclinical <i>in vivo</i> cancer models |

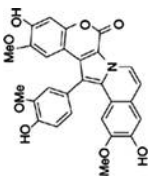
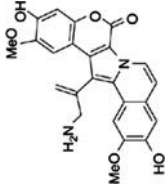
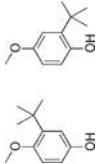
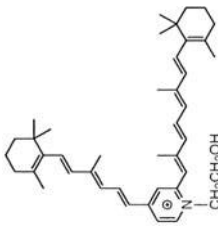
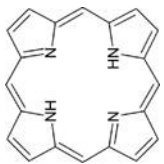
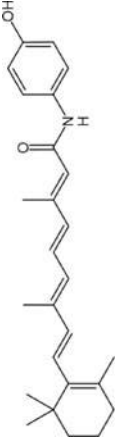
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TABLE 2. (CONTINUED)

| Complex | Targeting Compounds | Structure | Status |
|--|-------------------------|---|--|
| | Malonate |  | Experimental tool, neurotoxic, not considered for cancer treatment |
| | Nitropropionic acid | <chem>O=[N+]([O-])CC(=O)O</chem> | Experimental tool, neurotoxic, not considered for cancer treatment |
| | Thenoyltrifluoroacetone |  | Experimental tool, toxic, not considered for cancer treatment |
| | Troglitazone |  | Effective in experimental cancer models. Anti-diabetic drug, withdrawn due to liver toxicity |
| | Atpenin A5 |  | UbQ site inhibitor, unexplored in cancer |
| Complex III ubiquinone-cytochrome c oxidoreductase | Antimycin A |  | Experimental tool, standard CIII inhibitor |
| | Adaphostin |  | Effective against primary leukemia samples and in preclinical <i>in vivo</i> cancer models |
| | Resveratrol |  | Experimental cancer models, 9 cancer-related clinical trials (phase I and II) |
| | Xanthohumol |  | Experimental <i>in vitro</i> and <i>in vivo</i> cancer models |
| | Benzyl isothiocyanate |  | Preclinical <i>in vivo</i> cancer models |

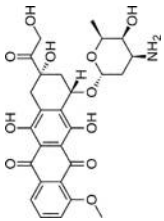
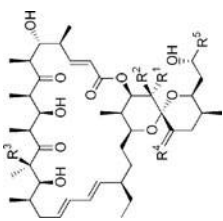
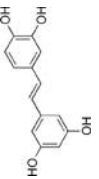
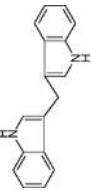
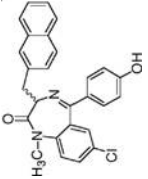
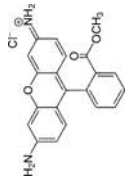
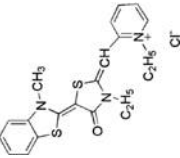
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TABLE 2. (CONTINUED)

| Complex | Targeting Compounds | Structure | Status |
|------------------------------------|---|---|---|
| | Lamellarin D |  | Experimental <i>in vitro</i> models, transformed cells more sensitive |
| | PM031379 |  | Experimental <i>in vitro</i> models, transformed cells more sensitive |
| Complex IV cytochrome c oxidase | Nitric oxide | $\cdot \text{N}=\text{O}$ | Physiological regulator |
| | 3-tert-Butyl-4-hydroxyanisole |  | Not explored in cancer |
| | N-Retinyl-N-retinylidene ethanolamine |  | Associated with macular degeneration, not explored in cancer |
| | Porphyrin photosensitizers (e.g. Porphimer sodium - Photofrin®) | Generic structure:  | Photofrin approved for esophageal cancer and non-small cell lung carcinoma, more than 20 clinical trials (phase I, II and III) in other types of cancer |
| | N-(4-Hydroxyphenyl) retinamide (fenretinid) |  | More than 30 clinical trials in cancer underway or completed (phase I, II and III) |

(continued)

TABLE 2. (CONTINUED)

| Complex | Targeting Compounds | Structure | Status |
|--|------------------------------------|---|--|
| | Doxorubicin |  | Widely used chemotherapeutic. CIV inhibition resulting in side-effect |
| Complex V ATP synthase, F₁F₀-ATPase | Oligomycin |  | Standard CV inhibitor, experimental tool. |
| | Piceatannol |  | Experimental <i>in vitro</i> cancer models, <i>in vivo</i> data contradictory |
| | 3,30-Diindolylmethane (DIM) |  | Preclinical <i>in vitro</i> cancer models |
| | Bz-423 |  | 8 clinical trials for cancer (phase I, II, and III), ongoing or completed Experimental <i>in vitro</i> models |
| | Rhodamine 123 |  | Clinical trials (phase I) in the past, no activity at present |
| | MK-077 |  | Clinical trials ended (phase I), hypomagnesaemia and renal toxicity |

Compounds in clinical use or undergoing clinical trials are in bold.

subunits are encoded by nuclear genes (178). Unlike Complex I, III, and IV, CII does not directly participate in the maintenance of the proton gradient. It is also unique in that it forms a direct link between the TCA cycle, where it catalyzes the oxidation of succinate to fumarate, and the mitochondrial ETC. CII transfers two electrons removed from succinate to oxidized UbQ to form reduced UbQH₂, which then transports the electrons onto CIII, before UbQ is recycled. In this way, CII can feed electrons into the UbQ pool, contributing to the electron flow that exists between CI and CIII, thereby indirectly helping to maintain mitochondrial membrane potential by proton pumping from respiratory complexes, CIII and CIV.

Unlike CI, the complete crystal structures of both prokaryotic and eukaryotic CII have been solved, providing insight into its architecture and the association of the four subunits, SDHA, SDHB, SDHC, and SDHD, helping to explain its mechanism of action (191, 219). The SDHA subunit, exposed to the mitochondrial matrix, comprises the active site of the SDH enzyme for converting succinate to fumarate. Electrons generated during this process are channeled via the iron-sulfur (Fe:S) clusters of the SDHB subunit to the UbQ-binding sites within subunits SDHC and SDHD embedded in the MIM. Of the two UbQ-binding sites suggested by the crystallographic studies, the proximal one (Q_P; closer to the matrix) is probably fully functional, whereas the distal site (Q_D) may be a nonfunctional 'pseudo-site' (129). Although CII has often not been considered a major source of mitochondrial superoxide generation, blocking the UbQ-binding sites with specific inhibitors nevertheless induces substantial ROS generation (see below).

One compound that triggers high levels of ROS generation from CII is the emerging anticancer drug, α -TOS (56, 57, 59), which belongs to a group of 'redox-silent' analogues of vitamin E (α -tocopherol). α -TOS has shown significant anticancer potential in various preclinical cancer models, including the difficult-to-treat mesotheliomas and HER2-positive breast carcinomas, without adverse effects or toxicity (154, 155, 187, 208). The UbQ-binding sites on CII appear necessary for the mechanisms of α -TOS anticancer activity, involving superoxide production, increased calcium levels, and the induction of apoptosis and its anticancer activity can be inhibited by pre-treating with antioxidants, supporting α -TOS working as a pro-oxidant (59, 78). α -TOS treatment of cancer cells sets in motion a number of intracellular processes, but the precise sequence and mutual relationship of each step have yet to be fully elucidated.

α -TOS treatment of cancer cells results in upregulation of the pro-apoptotic protein NOXA, in a p53-independent manner, an event that is regulated by the Mst1-FoxO1 axis (204) (Fig. 3). NOXA induction suppresses the anti-apoptotic function of Mcl-1, thereby promoting Bak/Bax polymerization and mitochondrial permeabilization (167, 211). At the same time, α -TOS promotes mitochondrial apoptosis by acting as a BH3 mimetic (181). These processes are depicted in Figure 4, which also indicates that the ROS generated in cells from targeting of CII by α -TOS may also cause dimerization of Bax and its ensuing mitochondrial translocation (44). Furthermore, α -TOS activates sphingomyelinase, resulting in the formation of the pro-apoptogenic product, ceramide (211). Ceramide production is an early event detectable within the first 15–20 min following α -TOS addition, well before the other drug-induced effects are observed. Another pro-apoptotic effect of α -TOS and the related compound, α -tocopheryloxybutyric acid (2) is to inhibit the Akt pro-survival signaling pathway. The mechanism of Akt inhi-

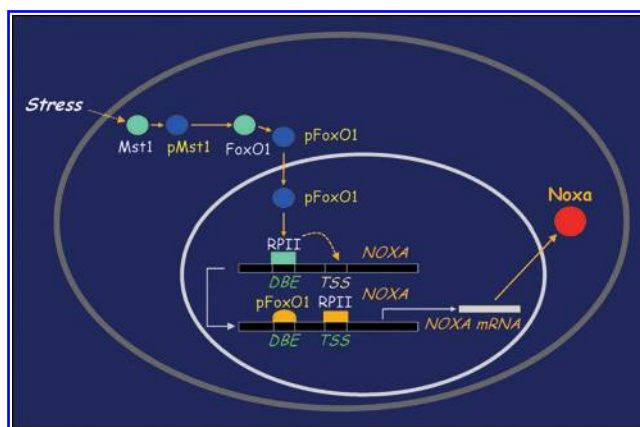


FIG. 3. Transcriptional regulation in apoptosis induced by VE analogues. Stress, presented by ROS and induced by agents such as the vitamin E analogue, α -TOS causes phosphorylation of Mst1/Hippo kinase. Consequently, the activated pMst1 phosphorylates the cytosolic transcription factor FoxO1, which then translocates into the nucleus. In resting cells, the DAF-16 binding element (DBE) of the NOXA promoter is occupied by RNA polymerase II (RPII). Under stressful conditions, pFoxO1 binds to the DBE due to its increased affinity for this site. As a consequence, RPII translocates to the transition start site (TSS), resulting in transcription of the NOXA gene. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

bition has not yet been fully clarified, but downregulation of Ras signaling and inhibition/downregulation of epidermal growth factor receptors have been implicated (55, 182). Finally, α -TOS can also inhibit cell cycle progression by reducing expression of the transcription factor E2F1 (4).

α -TOS has other indirect anticancer effects by attenuating angiogenesis as a result of the direct elimination of proliferating endothelial cells and by disrupting autocrine as well as paracrine signaling via the FGF2 pathway (151, 187). In this way, α -TOS eliminates tumor cells not only directly by inducing apoptosis but also indirectly by inhibiting angiogenesis. Furthermore, the relative specificity of α -TOS for targeting cancer cells is enhanced by the higher esterase activity in normal cells cleaving it to form vitamin E (α -tocopherol), which has antioxidant properties. This has been clearly shown in rat hepatocytes, where α -TOS protects against oxidative stress (150, 152, 225).

The knowledge that α -TOS targeted CII's UbQ-binding sites led us to design a more specific mitochondrially targeted analogue, MitoVES. The structure of this compound (Fig. 5A) causes it to compartmentalize to mitochondria (Fig. 5B), particularly to the surface of the MIM facing the matrix (57, 58). Consequently, cells treated with MitoVES respond rapidly by generating ROS within several minutes (Fig. 5C) which translates into much greater apoptogenic activity in cancer cells and superior anticancer efficacy in animal models (Fig. 6). MitoVES features physicochemical features endowing it with intriguing properties, as depicted in Figure 7. Thus, MitoVES selectively targets the Q_P site of CII such that the IC₅₀ value for electron transfer from CII to CIII was found to be 1.5 μ M, whereas the IC₅₀ value for conversion of succinate to fumarate was much greater at 70 μ M.

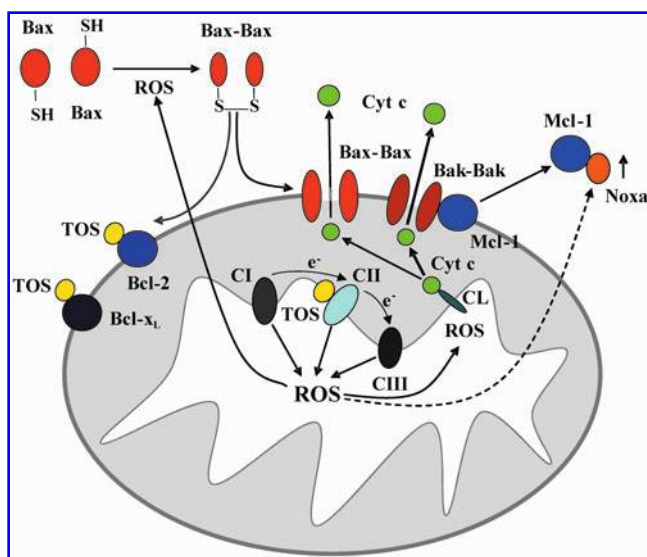


FIG. 4. Model of formation of the MOM channel during apoptosis induced by ROS. A number of anticancer agents induce apoptosis by generating ROS. These signaling molecules then give rise to a channel in the MOM, a requirement for the activation of the apoptosis signaling cascade downstream of mitochondria. There are two possibilities for how the channel can be formed, one being transcriptionally independent, the other dependent on transcription. These two possibilities are illustrated in this cartoon for the mitocan, α -TOS, a VE analogue that has the propensity to interact with the BH3 domains of the anti-apoptotic members of the Bcl-2 family members, such as Bcl-2 or Bcl- x_L . Once these domains become saturated, α -TOS is now free to diffuse across the MOM to CII in the MIM, where it jeopardizes the function of UbQ binding to CII, generating ROS, which then initiates the process of apoptosis by at least two different pathways. The transcription-independent (or direct) mechanism likely involves generation of a Bax-dependent channel in the MOM, based on the dimerization of Bax monomers in the cytosol due to formation of disulfide bridges between two monomeric Bax polypeptides. The dimeric Bax molecule changes its conformation, exposing the mitochondria-docking terminus, which results in the insertion of the dimers into the MOM, followed by Bax oligomerization and formation of the channel. The ROS generated from CII also activates transcriptional mechanisms yielding increased levels of BH3-only proteins like Noxa which divert the anti-apoptotic Bcl-3 family protein, Mcl-1, away from the pro-apoptotic protein, Bak, liberating Bak to form MOM channels. ROS also activate the oxidase activity of cytochrome c (Cyt c), resulting in peroxidation and altered conformation of the MIM phospholipid, cardiolipin (CL). The oxidized CL has a decreased affinity for Cyt c, which is released from the membrane anchoring, free to move into the cytosol via the Bax and/or Bak channel to activate the apoptotic cascade downstream of mitochondria. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Thenoyltrifluoroacetone (TTFA), the archetypal inhibitor of the UbQ binding sites of CII, also induces an increase in oxidative stress and calcium levels (133). However, in contrast to α -TOS, its practical application as an anticancer agent is restricted, as it is also very toxic to noncancerous cells, such as hepatocytes (226). Interestingly, the anti-diabetic and anti-inflammatory drug, troglitazone, which contains the α -tocopheryl moiety similar to α -TOS, also interferes with CII

activity (186). The drug was registered, but was subsequently withdrawn from the clinic due to liver toxicity (85). Another group of compounds targeting the UbQ site(s) of CII are atpenins, natural products isolated from bacteria, structurally similar to UbQ (136). The potential use of these agents as anticancer drugs has yet to be established.

Other compounds, including malonate and 3-nitropropionic acid (3NP), that specifically inhibit the succinyl dehydrogenase activity in the SDHA subunit of CII are also able to stimulate ROS production and induce cell death. Nevertheless, their use for cancer treatment may be problematic, because they exhibit neurotoxic properties, and in experimental animals they produce symptoms similar to Huntington disease, a severe human neurodegenerative disorder (23, 81, 92, 125). Hence, with regard to future anticancer drug development, the UbQ-binding sites in subunits SDHC and SDHD may prove to be more ideal targets than sites in the SDHA catalytic domain, given the associated problem of neurotoxicity. Inhibiting SDH activity also blocks the TCA cycle as well as the transfer of electrons to the ubiquinone pool and hence, the metabolic TCA cycle arrest could have much more serious consequences for the cell than the selective inhibition of electron transfer at the UbQ sites. In the latter case, the TCA cycle would not become fully inhibited, but the disruption of the electron flow at the UbQ sites would still allow for efficient superoxide formation.

The eligibility of CII as a target for anticancer drugs is not compromised by the occurrence of mutations in subunits SDHB, SDHC, and SDHD in cancer. These mutations are associated only with relatively infrequent and nonaggressive familial paragangliomas and pheochromocytomas, and in most common cancers such as breast cancer, only one in a million patients carries a mutation in CII (165, 179). Therefore, CII represents an invariant target in most cancers.

Complex III

The electron flows from CI and CII are integrated at CIII, also known as the cytochrome bc_1 complex or ubiquinone-cytochrome c oxidoreductase, which mediates transfer of electrons to another electron carrier, cytochrome c. CIII, similarly to CI, pumps protons across the MIM, contributing to the proton gradient that forms the mitochondrial membrane potential. Along with CI, CIII is considered to be a major source of superoxide radicals due to electron leakage, which is further increased either by the inhibition of the UbQ-binding sites or by an increase in the mitochondrial membrane potential (35, 61). The crystal structure of mammalian CIII containing 11 protein subunits (including one mitochondrially encoded) has been solved (94, 215) and the mechanisms for proton pumping and electron transfer have been determined. CIII contains two binding sites for UbQ involved in the 'Q cycle' in which two molecules of UbQH₂ are oxidized at the Q_o site per one molecule of UbQ reduced at the Q_i site, allowing CIII to transfer protons across the MIM (40). Specific inhibitors of the Q_i site such as antimycin A induce superoxide production at the Q_o site, but Q_o site inhibitors themselves do not have this effect (61). Antimycin A effectively eliminates cancer cells by apoptosis (214), although antimycin A is also a BH3 mimetic (201). Interference with the anti-apoptotic proteins of the Bcl-2 family could make antimycin A an interesting option for targeting cancers resistant to apoptosis due to upregulation of Bcl-2/Bcl- x_L .

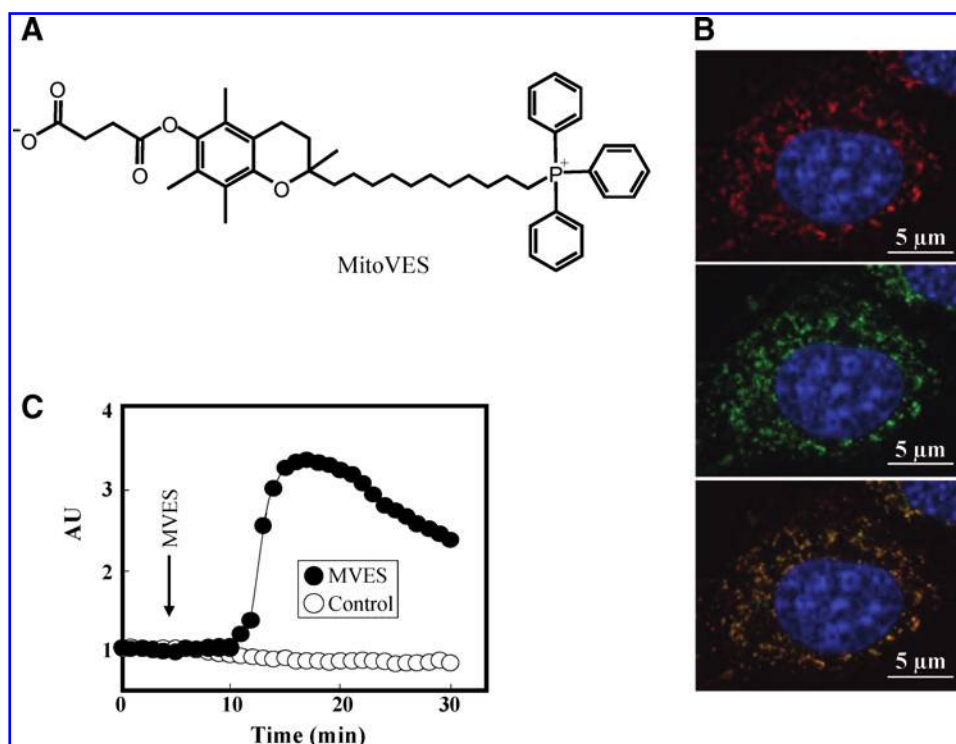


FIG. 5. Targeting of vitamin E succinate to MIM causes fast generation of ROS. (A) Structure of the 'full-length' mitochondrially targeted vitamin E succinate (MitoVES) with 11-C aliphatic chain spanning the tocopheryl head-group and the TPP⁺ moiety. **(B)** MitoVES associates primarily with mitochondria. This has been documented by confocal microscopy of mouse breast cancer cells NeuTL incubated with MitoTracker Red (*top image*) and FITC-modified MitoVES (*middle image*). The *bottom image* shows the overlay of the two upper images, indicating that the majority of MitoVES-dependent, green fluorescence overlays with red fluorescence, indicating mitochondria. **(C)** Human neuroblastoma cells, Tet21N, were transiently transfected with the *pHyPer-dMito* plasmid and their green fluorescence evaluated using time-lapse imaging confocal microscopy of live cells. The data document rapid generation of ROS (hydrogen peroxide) appearing after adding MitoVES. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

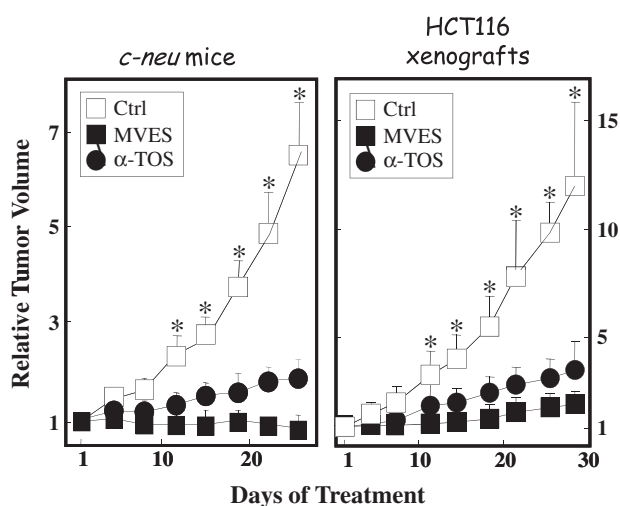


FIG. 6. Comparison of targeted and untargeted vitamin E analogs for their anti-tumor effects. The transgenic FVB/N202 *c-neu* mice with spontaneous formation of HER2-high breast carcinomas (*left*) and nude mice with human colorectal HCT116 cell line derived xenografts (*right*) were treated with α -TOS at 15 or MitoVES at 1–2 μ mol per animal per injection every 3–4 days. The tumors were regularly visualized and their volume quantified using an ultrasound imaging instrument (Vevo 770, VisualSonics) equipped with a 30- μ m resolution scan head.

Another compound, adaphostin, similarly to antimycin A, also targets the Q_i site of CIII to induce oxidative stress and apoptosis in cancer cells (115). Originally, adaphostin, a dihydroquinone derivative, was identified as an inhibitor of the Bcr-Abl kinase in chronic myeloid leukemia, but turned out to be also highly effective against Bcr-Abl-negative cancer cells as well as against cells with a mutated Bcr-Abl, resistant to the Bcr-Abl kinase inhibitor drug, imatinib mesylate. The action of adaphostin depends on stimulating superoxide production and appears to be relatively cancer cell-specific (31, 32, 47). Caspase-independent inhibition of the Raf1/MEK/ERK, Akt pathways and activation of the JNK and p38 MAP kinases have commonly been shown to occur during adaphostin-induced apoptosis (222) and the activation of p38 MAP kinase also results in cell cycle arrest in G1 (143).

Plant-derived polyphenols, represented by resveratrol (trans-3,4',5-trihydroxystilbene), are also efficient respiratory chain inhibitors of several ETC complexes, especially CIII, and induce mitochondria-mediated apoptosis (194, 229). The dichotomy of cytoprotective and cytotoxic effects associated with resveratrol (also linked with the beneficial health effects of red wine) may be concentration dependent and related to the widespread action on multiple cellular processes. In any case, the chemoprotective activities of resveratrol have been observed in various preclinical cancer models (7, 88, 229), and the anticancer potential of resveratrol is currently being investigated in several ongoing clinical trials, primarily in colorectal cancer (*e.g.*, NCT00256334, NCT00920803,

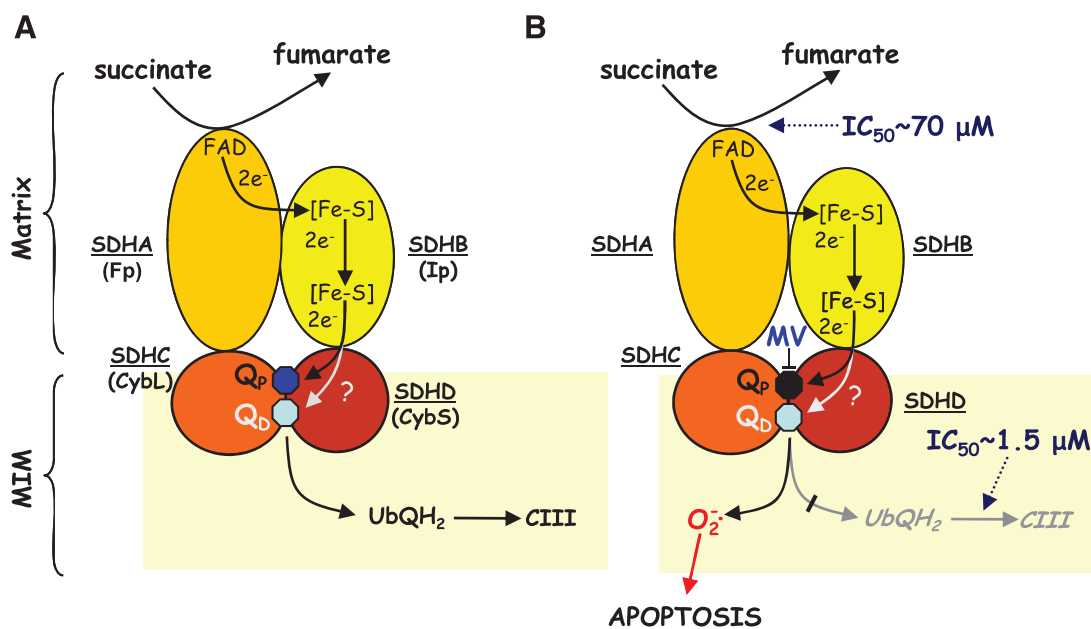


FIG. 7. Molecular mechanism for the generation of ROS by vitamin E analogues involving targeting of CII. (A) In the absence of a VE analogue, the electrons generated during conversion of succinate to fumarate by SDH at the SDHA (or Fp) subunit are relayed to the Fe-S clusters in the SDHB (or Ip) subunit, which direct the electrons to the Q_p (and/or, possibly, Q_d) site made up by residues from the SDHC (or CybL) and SDHD (or CybS) subunits. This results in a two-electron reduction of the oxidized form of UbQ to UbQH₂, which has low affinity for its binding site(s) in CII. UbQH₂ is then released from CII to bind to CIII, where it gives up the two electrons to CIII at the Q_i site to be transferred to UbQ at the Q_o site. Hence, the incoming UbQH₂ is reoxidized and released to return as UbQ to CII. (B) This 'electron shuttle' activity of UbQ becomes disrupted in the presence of agents such as the VE analogues, epitomized in this cartoon by MitoVES. In this case, agents like MitoVES interfere with the function of UbQ, most likely by displacing it. Therefore, the electrons generated during conversion of succinate to fumarate cannot be intercepted by their natural acceptor. As a consequence, they interact with molecular oxygen to yield superoxide, which then triggers a cascade of reactions culminating in the execution of apoptotic cell death. The IC₅₀ values for inhibition of the SDH activity and the CII→CIII electron transfer, i.e. ~70 and ~1.5 μM, respectively, determined using isolated beef heart mitochondria are also indicated. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

NCT00433576). Another representative from the polyphenol group is xanthohumol, derived from hops, which also effectively induces mitochondrial superoxide generation and apoptosis by CI and CIII inhibition associated with cytochrome c release from mitochondria (188). Xanthohumol shows other activities as well, including inhibiting the NFκB and Akt pathways, stimulating the apoptotic arm of the unfolded protein response, suppressing angiogenesis, and repressing tumor growth in experimental cancer models (3, 89, 127, 135, 141).

Isothiocyanates are natural compounds found in cruciferous vegetables such as broccoli, watercress, or cabbage, and like the polyphenols, they provide beneficial effects for preventing cancer. For example, benzyl isothiocyanate (BITC) inhibits CIII in cancer cells, initiating ROS-mediated apoptosis followed by translocation of Bax into the mitochondria in a JNK and p38 MAP kinase-dependent manner (148, 173, 216). These effects were specific for cancer cells with minimal effects on normal cells (216), a beneficial and advantageous property for any anticancer drug. BITC suppressed angiogenesis and xenograft tumor growth *in vivo* in experimental cancer models and was shown to be an efficient chemopreventative (209, 210, 218). Interestingly, phenethyl isothiocyanate (PEITC), a related compound, also selectively induced apoptosis in cancer cells by ROS accumulation, even though this drug mainly appeared to deplete the glutathione pool by inhibiting glutathione peroxidase (198). Given the similarity between both compounds, it is likely that PEITC will

also directly induce the production of ROS from the mitochondrial ETC (26).

Finally, CIII is also inhibited by lamellarin D, a hexacyclic pyrrol alkaloid derived from marine invertebrates and has been shown to act as a topoisomerase I inhibitor (48, 65). Mitochondrial respiration was disrupted at the level of CIII by lamellarin D treatment, which was accompanied by the loss of the transmembrane potential, calcium release, and induction of mitochondria-dependent apoptosis, all of which were relatively specific for cancer cells (9, 10, 105). Surprisingly, mitochondrial ROS generation was not stimulated by lamellarin D, suggesting a mechanism different from that of the CIII-targeting compounds discussed above. Interestingly, treating cancer cells with a modified lamellarin D derivative, PM031379, induced mitochondrial ROS generation and successfully induced apoptosis in a non-small cell lung carcinoma cell line, U1810, that was chemoresistant to the parental compound, lamellarin D (74). The difference between these two compounds was that whereas both induced the initial mitochondrial-specific apoptotic steps, only PM031379 induced the nuclear translocation of the apoptosis-inducing factor (AIF) in a ROS-dependent manner.

Complex IV

The final step in electron transport via the ETC is performed by CIV, also known as cytochrome c oxidase. This complex

removes electrons from cytochrome *c* and donates them to molecular oxygen, producing water while, at the same time, transporting protons across the MIM. Along with CI and CIII, CIV directly contributes to the maintenance of the proton gradient and the mitochondrial membrane potential. Of the 13 subunits that form CIV in mammals, three are encoded by the mitochondrial genome and 10 by the nuclear DNA (178). As the final element in the ETC, CIV has a physiologically important role in regulating ETC activity. For example, CIV is regulated by the HIF1 transcription factor in hypoxia, which results in an isoform switch increasing CIV activity (71). Furthermore, CIV is also regulated by nitric oxide, which competes for oxygen binding and inhibits mitochondrial respiration (140).

Surprisingly, there are not many anticancer agents that directly target CIV, in spite of the important role of this complex in regulating ETC activity. In fact, many of the compounds mentioned in connection with CIV actually modulate the expression of its various subunits at the level of mRNA expression and thereby indirectly modulate CIV mediated apoptosis. For example, N-(4-hydroxyphenyl) retinamide (4HPR), also known as fenretinide, downregulates CIV by destabilizing the mRNA transcript for subunit III of CIV, inducing ROS-mediated apoptosis in cancer cells (220). A number of clinical trials are currently underway to evaluate the merits of this compound for treating different types of cancer. Anticancer drugs from the anthracycline family, such as doxorubicin, also downregulate the expression of CIV subunits II and V at the level of mRNA. However, in this case, the CIV downregulation was associated with cardiotoxic side effects of these drugs and did not appear to relate to their anticancer activity (33). Interestingly, direct interaction with CIV and inhibition of its activity by doxorubicin and daunomycin has also been reported (163).

Induction of apoptosis in a leukemic cell line with the antioxidant and lipoxygenase inhibitor 3-tert-butyl-4-hydroxyanisole (BHA) was shown to also target and inhibit CIV directly at relatively high concentrations (185) but these effects could not be replicated with mitochondria derived from murine skeletal muscle (69, 158). Similarly, inhibition of CIV activity and apoptosis induction was also observed for the lipophilic cationic compound N-retinyl-N-retinylidene ethanolamine (A2E) in mammalian retinal pigment epithelia, as well as in other cells. However, A2E is associated with age-related macular degeneration and its potential use in cancer has not been explored (180, 192). With respect to CIV function in cancer cells, perhaps the most relevant compounds for current cancer therapy could be the porphyrin photosensitizers, such as Photofrin II, which in photodynamic therapy inhibit CIV, resulting in ROS production required for successful treatment (91). Similarly to the antioxidant MitoQ (103), the mitocan MitoVES (57, 58), polyphenols (19, 132), and manganese porphyrin superoxide dismutase mimics (6), photosensitizers were successfully delivered into mitochondria using cationic tags or peptide conjugates, resulting in increased phototoxicity (42, 118, 183).

Complex V

The proton gradient maintained by complexes I–IV drives the conversion of ATP by CV—the ATP synthase (F_1F_0 -AT-Pase), which attaches inorganic phosphate to ADP and

thereby supplies the cell with essential energy in a form utilized in many cellular processes. CV functions as a miniature molecular engine with two parts. Controlled dissipation of the proton gradient by the transmembrane F_0 portion provides torque that drives the rotational movement of the F_1 portion of the enzyme, resulting in the synthesis of three ATP molecules per one 360° turn of the enzyme (52, 101).

Inhibiting the F_0 portion of CV with oligomycin or its derivatives can induce apoptosis, but its selectivity for cancer-derived cell lines is limited, indicating that targeting CV in this manner may not be a very effective intervention for cancer (174, 175). Interestingly, oligomycin treatment can reduce the availability of ATP for caspase activation or changes in intracellular pH which will under certain conditions prevent apoptosis and/or switch to necrotic cell death. (63, 119, 126, 223).

CV is also a molecular target of resveratrol and related compounds such as piceatannol. Crystal structures of the F_1 subcomplex bound to resveratrol and piceatannol have been solved (77, 227) and this adds CV to the growing number of intracellular targets for resveratrol, providing the basis for the complexity of effects associated with this polyphenol. Resveratrol binding to CV may partly explain how this drug can promote the induction of apoptosis in cancer cells (194).

CV can also be inhibited by the metabolite 3,30-diindolylmethane (DIM) derived from the compound indole-3-carbinol (I3C) found in cruciferous vegetables. DIM stimulates ROS formation and induces apoptosis in a JNK and p38 kinase-dependent manner, as well as inhibiting the cell cycle by upregulating the p21 cell division kinase inhibitor (82). In addition, DIM modulates the aryl hydrocarbon receptor and displays anti-proliferative, pro-apoptotic, and anti-angiogenic activities in a number of cancer cell lines and experimental tumor models (172). Clinical testing of DIM verified its safety in human subjects, and it is used for treatment of recurrent respiratory papillomatosis, a benign noninvasive neoplasia caused by infection with the human papilloma virus (171, 212). Clinical trials in cancer patients are ongoing, including trial numbers NCT00212381 in cervical dysplasia and NCT00888654 in prostate cancer.

Bz-423, a 1,4-benzodiazepine derivative, is another inhibitor of CV activity which acts in a noncompetitive manner to induce superoxide production, albeit associated with less ATP depletion than for oligomycin (97). Bz-423 is effective against a number of cancer cell lines, inducing cell cycle arrest and apoptosis in a Bax- and Bak-dependent manner, associated with superoxide-dependent apoptosis signal-regulating kinase (ASK1) release from thioredoxin and JNK activation (20, 21). It is also currently being considered for the treatment of psoriasis. Last but not least, CV is targeted by lipophilic cationic dyes such as rhodamine 123 (27, 138) with advantages that they are more readily accumulated by cancer cell compared to normal cell mitochondria (49, 138, 190), which should provide greater anticancer specificity. Indeed, selective elimination of cancer cells and prevention of tumor growth has been observed for rhodamine 123 (15, 16, 90) which in phase I clinical trials of patients with hormone-refractory prostate cancer showed good retention in the prostatic tissue. However, rhodamine 123 did not show therapeutic benefits because its effects were not statistically significant at the maximum tolerated dose (99). A rhodamine analogue, MK-077, has entered into phase I clinical trials, but

its administration was associated with hypomagnesaemia and renal toxicity (25, 168).

Mechanism of Action and General Consideration for ETC Targeting Mitocans

Mitocans, compounds targeting cancer cell mitochondria and in particular, the mitochondrial ETC, constitute a promising group of cancer therapeutics. The advantage of the ETC in this context is that it is still fully or partially active in most tumors and that the mutation rate in the ETC complexes is relatively low. The only ETC complex that shows nuclear-coded mutations with links to cancer is CII, giving rise to relatively benign and/or infrequent neoplasias such as pheochromocytomas and paragangliomas (83). Certain subsets of cancers (about 15%) will also harbor nonsilent mitochondrial DNA mutations (116), and these mutations may compromise the induction of apoptosis due to nonfunctional ETC complexes that contain mitochondrially encoded subunits (112, 120). Even in this situation, the targeting of unaffected complexes (such as CII, with all subunits coded by the nucleus) may be plausible, as demonstrated by efficient killing of cells without functional CI by the CII inhibitor α -TOS (56, 59). It should be noted, however, that the efficiency of apoptosis induction by CII-interfering agents in cells with ETC compromised downstream of CII (*e.g.*, in CIII, CIV) or in CV has not yet been examined. It can be anticipated, though, that targeting CII will induce apoptosis in cancer cells regardless of the potential mutation in the downstream components of the ETC, since targeting CII causes generation of ROS that, in turn, induce apoptosis (56, 57, 59, 197, 204).

One of the pivotal events in apoptosis induction by ETC-targeting mitocans is the induction of ROS. This is particularly apparent for mitocans directed at CI–CIII, and is exemplified by numerous studies that show the inhibition of apoptosis induction by antioxidants. The mechanism of ROS generation (mostly in the form of superoxide) from the ETC complexes is highly complex and is not yet fully clarified for isolated mitochondria, and only vaguely inferred for mitochondria in cells (24). Similar lack of clarity persists concerning the exact sequence of events after the initiation of ROS formation. An attractive option is that ROS trigger general mechanisms common to many mitocans targeting the ETC complexes. One of these mechanisms could be direct opening of the permeability transition pore, followed by apoptosis (128). Besides this direct effect, ROS may also diffuse into the cytoplasm in the form of hydrogen peroxide and activate signaling processes via the ASK1- and or MST1/Hippo-controlled pathways. While the ASK1 pathway, activated in response to ROS by the release of the ASK1 kinase from thioredoxin, has been known for some time (131), ROS-mediated apoptosis induction via the Mst1/Hippo-FoxO1-NOXA signaling pathway has only recently been described for the CII inhibitor α -TOS (204). This pathway is conserved in higher organisms and is activated by the addition of hydrogen peroxide. It may thus represent, similarly to the ASK1 pathway, a general mechanism of cellular response to sudden increase of intracellular oxidative stress with Mst1/Hippo auto-activation in the presence of ROS. However, the involvement of this pathway in apoptosis induction has as yet not been replicated for other compounds targeting the ETC.

Despite the possible common mechanism, a number of mitocans discussed here also possess other activities unrelated to the ETC. For example, antimycin A and α -TOS function as BH3 'mimetics' (181, 201), deguelin inhibits HSP90 (104, 157), tamoxifen is an estrogen receptor modulator (213), metformin stimulates AMPK (29), and resveratrol exerts a variety of effects (88). Even though some of these activities may result in apoptosis induction, others may be harmful, as demonstrated for the CI inhibitors rotenone and MPP⁺, which also disrupt microtubules. This interference with microtubules results in undesirable neurotoxicity and Parkinson's-like syndromes (37). Targeting of mitocans directly to mitochondria using lipophilic cations or other methods, as recently demonstrated for vitamin E analogues (57, 58), polyphenols (19, 132), and porphyrins (42, 118, 183), may be a route to avoid some of the unwanted side-effects unrelated to mitochondrial activity of the compounds. This represents an important development in the field, a paradigm shift in cancer therapy.

Conclusions

Mitochondria represent, due to their distinctive nature in a number of cancers, intriguing therapeutic targets across the landscape of cancers, and mitocans may thus provide a valuable therapeutic option for the future. Although some mitocans, in particular those targeting CI or CII, exert undesirable neurotoxic side effects, a number of these compounds are selective for malignant cells. The selectivity can be further improved by mitochondrial targeting, as exemplified, for example, by MitoVES. Moreover, the availability of invariant targets combined with the preference most mitocans show for cancer cells provides an incentive for further development of these exciting compounds, which may become an essential component of our anticancer 'tool box' of the 21st century.

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References

1. Adam-Vizi V and Chinopoulos C. Bioenergetics and the formation of mitochondrial reactive oxygen species. *Trends Pharmacol Sci* 27: 639–645, 2006.
2. Akazawa A, Nishikawa K, Suzuki K, Asano R, Kumadaki I, Satoh H, Hagiwara K, Shin SJ, and Yano T. Induction of apoptosis in a human breast cancer cell overexpressing ErbB-2 receptor by alpha-tocopheryloxybutyric acid. *Jpn J Pharmacol* 89: 417–421, 2002.
3. Albini A, Dell'Eva R, Vene R, Ferrari N, Buhler DR, Noonan DM, and Fassina G. Mechanisms of the antiangiogenic activity by the hop flavonoid xanthohumol: NF-kappaB and Akt as targets. *FASEB J* 20: 527–529, 2006.
4. Alleva R, Benassi MS, Tomasetti M, Gellert N, Ponticelli F, Borghi B, Picci P, and Neuzil J. Alpha-tocopheryl succinate induces cytostasis and apoptosis in osteosarcoma cells: The role of E2F1. *Biochem Biophys Res Commun* 331: 1515–1521, 2005.
5. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger

- F, Schreier PH, Smith AJ, Staden R, and Young IG. Sequence and organization of the human mitochondrial genome. *Nature* 290: 457–465, 1981.
6. Asayama S, Kawamura E, Nagaoka S, and Kawakami H. Design of manganese porphyrin modified with mitochondrial signal peptide for a new antioxidant. *Mol Pharm* 3: 468–470, 2006.
 7. Athar M, Back JH, Tang X, Kim KH, Kopelovich L, Bickers DR, and Kim AL. Resveratrol: A review of preclinical studies for human cancer prevention. *Toxicol Appl Pharmacol* 224: 274–283, 2007.
 8. Baggetto LG and Testa-Parussini R. Role of acetoin on the regulation of intermediate metabolism of Ehrlich ascites tumor mitochondria: Its contribution to membrane cholesterol enrichment modifying passive proton permeability. *Arch Biochem Biophys* 283: 241–248, 1990.
 9. Ballot C, Kluza J, Lancel S, Martoriati A, Hassoun SM, Mortier L, Vienne JC, Briand G, Formstecher P, Bailly C, Nevieri R, and Marchetti P. Inhibition of mitochondrial respiration mediates apoptosis induced by the anti-tumoral alkaloid lamellarin D. *Apoptosis* 15: 769–781, 2010.
 10. Ballot C, Kluza J, Martoriati A, Nyman U, Formstecher P, Joseph B, Bailly C, and Marchetti P. Essential role of mitochondria in apoptosis of cancer cells induced by the marine alkaloid lamellarin D. *Mol Cancer Ther* 8: 3307–3317, 2009.
 11. Baracca A, Chiaradonna F, Sgarbi G, Solaini G, Alberghina L, and Lenaz G. Mitochondrial complex I decrease is responsible for bioenergetic dysfunction in K-ras transformed cells. *Biochim Biophys Acta* 1797: 314–323, 2010.
 12. Belzacq AS, El Hamel C, Vieira HL, Cohen I, Haouzi D, Metivier D, Marchetti P, Brenner C, and Kroemer G. Adenine nucleotide translocator mediates the mitochondrial membrane permeabilization induced by lonidamine, arsenite and CD437. *Oncogene* 20: 7579–7587, 2001.
 13. Ben Sahra I, Laurent K, Giuliano S, Larbret F, Ponzio G, Gounon P, Le Marchand-Brustel Y, Giorgetti-Peraldi S, Cormont M, Bertolotto C, Deckert M, Auberger P, Tanti JF, and Bost F. Targeting cancer cell metabolism: The combination of metformin and 2-deoxyglucose induces p53-dependent apoptosis in prostate cancer cells. *Cancer Res* 70: 2465–2475, 2010.
 14. Bergan RC, Reed E, Myers CE, Headlee D, Brawley O, Cho HK, Figg WD, Tompkins A, Linehan WM, Kohler D, Steinberg SM, and Blagosklonny MV. A Phase II study of high-dose tamoxifen in patients with hormone-refractory prostate cancer. *Clin Cancer Res* 5: 2366–2373, 1999.
 15. Bernal SD, Lampidis TJ, McIsaac RM, and Chen LB. Anticarcinoma activity *in vivo* of rhodamine 123, a mitochondrial-specific dye. *Science* 222: 169–172, 1983.
 16. Bernal SD, Lampidis TJ, Summerhayes IC, and Chen LB. Rhodamine-123 selectively reduces clonal growth of carcinoma cells *in vitro*. *Science* 218: 1117–1119, 1982.
 17. Berrisford JM and Sazanov LA. Structural basis for the mechanism of respiratory complex I. *J Biol Chem* 284: 29773–29783, 2009.
 18. Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, and Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci* 3: 1301–1306, 2000.
 19. Biasutto L, Mattarei A, Marotta E, Bradaschia A, Sassi N, Garbisa S, Zoratti M, and Paradisi C. Development of mitochondria-targeted derivatives of resveratrol. *Bioorg Med Chem Lett* 18: 5594–5597, 2008.
 20. Blatt NB, Boitano AE, Lyssiotis CA, Opipari AW Jr, and Glick GD. Bz-423 superoxide signals apoptosis via selective activation of JNK, Bak, and Bax. *Free Radic Biol Med* 45: 1232–1242, 2008.
 21. Boitano A, Ellman JA, Glick GD, and Opipari AW, Jr. The proapoptotic benzodiazepine Bz-423 affects the growth and survival of malignant B cells. *Cancer Res* 63: 6870–6876, 2003.
 22. Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, and Michelakis ED. A mitochondria-K⁺ channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. *Cancer Cell* 11: 37–51, 2007.
 23. Bonsi P, Cuomo D, Martella G, Sciamanna G, Tolu M, Calabresi P, Bernardi G, and Pisani A. Mitochondrial toxins in basal ganglia disorders: From animal models to therapeutic strategies. *Curr Neuroparmacol* 4: 69–75, 2006.
 24. Brand MD. The sites and topology of mitochondrial superoxide production. *Exp Gerontol* 45: 466–472, 2010.
 25. Britten CD, Rowinsky EK, Baker SD, Weiss GR, Smith L, Stephenson J, Rothenberg M, Smetzer L, Cramer J, Collins W, Von Hoff DD, and Eckhardt SG. A phase I and pharmacokinetic study of the mitochondrial-specific rhodacyanine dye analog MKT 077. *Clin Cancer Res* 6: 42–49, 2000.
 26. Brown KK, Cox AG, and Hampton MB. Mitochondrial respiratory chain involvement in peroxiredoxin 3 oxidation by phenethyl isothiocyanate and auranofin. *FEBS Lett* 584: 1257–1262, 2010.
 27. Bullough DA, Ceccarelli EA, Roise D, and Allison WS. Inhibition of the bovine-heart mitochondrial F1-ATPase by cationic dyes and amphipathic peptides. *Biochim Biophys Acta* 975: 377–383, 1989.
 28. Bustamante E, Morris HP, and Pedersen PL. Energy metabolism of tumor cells. Requirement for a form of hexokinase with a propensity for mitochondrial binding. *J Biol Chem* 256: 8699–8704, 1981.
 29. Buzzai M, Jones RG, Amaravadi RK, Lum JJ, DeBerardinis RJ, Zhao F, Viollet B, and Thompson CB. Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth. *Cancer Res* 67: 6745–6752, 2007.
 30. Caboni P, Sherer TB, Zhang N, Taylor G, Na HM, Greenamyre JT, and Casida JE. Rotenone, deguelin, their metabolites, and the rat model of Parkinson's disease. *Chem Res Toxicol* 17: 1540–1548, 2004.
 31. Chandra J, Hackbarth J, Le S, Loegering D, Bone N, Bruzek LM, Narayanan VL, Adjei AA, Kay NE, Tefferi A, Karp JE, Sausville EA, and Kaufmann SH. Involvement of reactive oxygen species in adaphostin-induced cytotoxicity in human leukemia cells. *Blood* 102: 4512–4519, 2003.
 32. Chandra J, Tracy J, Loegering D, Flatten K, Verstovsek S, Beran M, Gorre M, Estrov Z, Donato N, Talpaz M, Sawyers C, Bhalla K, Karp J, Sausville E, and Kaufmann SH. Adaphostin-induced oxidative stress overcomes BCR/ABL mutation-dependent and -independent imatinib resistance. *Blood* 107: 2501–2506, 2006.
 33. Chandran K, Aggarwal D, Migrino RQ, Joseph J, McAllister D, Konorev EA, Antholine WE, Zielonka J, Srinivasan S, Avadhani NG, and Kalyanaraman B. Doxorubicin inactivates myocardial cytochrome c oxidase in rats: Cardioprotection by Mito-Q. *Biophys J* 96: 1388–1398, 2009.

34. Check Hayden E. Cancer complexity slows quest for cure. *Nature* 455: 148, 2008.
35. Chen Q, Vazquez EJ, Moghaddas S, Hoppel CL, and Lesnefsky EJ. Production of reactive oxygen species by mitochondria: Central role of complex III. *J Biol Chem* 278: 36027–36031, 2003.
36. Chen Z, Zhang H, Lu W, and Huang P. Role of mitochondria-associated hexokinase II in cancer cell death induced by 3-bromopyruvate. *Biochim Biophys Acta* 1787: 553–560, 2009.
37. Choi WS, Palmiter RD, and Xia Z. Loss of mitochondrial complex I activity potentiates dopamine neuron death induced by microtubule dysfunction in a Parkinson's disease model. *J Cell Biol* 192: 873–882, 2011.
38. Chong CR and Chabner BA. Mysterious metformin. *Oncologist* 14: 1178–1181, 2009.
39. Chun KH, Kosmeder JW, 2nd, Sun S, Pezzuto JM, Lotan R, Hong WK, and Lee HY. Effects of deguelin on the phosphatidylinositol 3-kinase/Akt pathway and apoptosis in premalignant human bronchial epithelial cells. *J Natl Cancer Inst* 95: 291–302, 2003.
40. Crofts AR. The cytochrome bc1 complex: Function in the context of structure. *Annu Rev Physiol* 66: 689–733, 2004.
41. Croxtall JD, Emmas C, White JO, Choudhary Q, and Flower RJ. Tamoxifen inhibits growth of oestrogen receptor-negative A549 cells. *Biochem Pharmacol* 47: 197–202, 1994.
42. Cuchelkar V, Kopeckova P, and Kopecek J. Novel HPMA copolymer-bound constructs for combined tumor and mitochondrial targeting. *Mol Pharm* 5: 776–786, 2008.
43. Cuezva JM, Sanchez-Arago M, Sala S, Blanco-Rivero A, and Ortega AD. A message emerging from development: The repression of mitochondrial beta-F1-ATPase expression in cancer. *J Bioenerg Biomembr* 39: 259–265, 2007.
44. D'Alessio M, De Nicola M, Coppola S, Gualandi G, Pugliese L, Cerella C, Cristofanon S, Civitareale P, Ciriolo MR, Bergamaschi A, Magrini A, and Ghibelli L. Oxidative Bax dimerization promotes its translocation to mitochondria independently of apoptosis. *FASEB J* 19: 1504–1506, 2005.
45. Dang CV, Kim JW, Gao P, and Yustein J. The interplay between MYC and HIF in cancer. *Nat Rev Cancer* 8: 51–56, 2008.
46. Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM, Fantin VR, Jang HG, Jin S, Keenan MC, Marks KM, Prins RM, Ward PS, Yen KE, Liao LM, Rabinowitz JD, Cantley LC, Thompson CB, Vander Heiden MG, and Su SM. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* 462: 739–744, 2009.
47. Dasmahapatra G, Nguyen TK, Dent P, and Grant S. Adaphostin and bortezomib induce oxidative injury and apoptosis in imatinib mesylate-resistant hematopoietic cells expressing mutant forms of Bcr/Abl. *Leuk Res* 30: 1263–1272, 2006.
48. Davis RA, Carroll AR, Pierens GK, and Quinn RJ. New lamellarin alkaloids from the Australian ascidian, *Didemnum chartaceum*. *J Nat Prod* 62: 419–424, 1999.
49. Davis S, Weiss MJ, Wong JR, Lampidis TJ, and Chen LB. Mitochondrial and plasma membrane potentials cause unusual accumulation and retention of rhodamine 123 by human breast adenocarcinoma-derived MCF-7 cells. *J Biol Chem* 260: 13844–13850, 1985.
50. Degli Esposti M. Inhibitors of NADH-ubiquinone reductase: An overview. *Biochim Biophys Acta* 1364: 222–235, 1998.
51. Dell'Eva R, Ambrosini C, Minghelli S, Noonan DM, Albini A, and Ferrari N. The Akt inhibitor deguelin, is an angiopreventive agent also acting on the NF-kappaB pathway. *Carcinogenesis* 28: 404–413, 2007.
52. Devenish RJ, Prescott M, and Rodgers AJ. The structure and function of mitochondrial F1F0-ATP synthases. *Int Rev Cell Mol Biol* 267: 1–58, 2008.
53. Di Monte DA. The environment and Parkinson's disease: Is the nigrostriatal system preferentially targeted by neurotoxins? *Lancet Neurol* 2: 531–538, 2003.
54. Don AS, Kisker O, Dilda P, Donoghue N, Zhao X, Decollogne S, Creighton B, Flynn E, Folkman J, and Hogg PJ. A peptide trivalent arsenical inhibits tumor angiogenesis by perturbing mitochondrial function in angiogenic endothelial cells. *Cancer Cell* 3: 497–509, 2003.
55. Donapathy S, Louis S, Horvath E, Kun J, Sebt SM, and Malafa MP. RRR-alpha-tocopherol succinate down-regulates oncogenic Ras signaling. *Mol Cancer Ther* 5: 309–316, 2006.
56. Dong LF, Freeman R, Liu J, Zabalova R, Marin-Hernandez A, Stantic M, Rohlena J, Valis K, Rodriguez-Enriquez S, Butcher B, Goodwin J, Brunk UT, Witting PK, Moreno-Sanchez R, Scheffler IE, Ralph SJ, and Neuzil J. Suppression of tumor growth *in vivo* by the mitocan alpha-tocopheryl succinate requires respiratory complex II. *Clin Cancer Res* 15: 1593–1600, 2009.
57. Dong LF, Jameson VJ, Tilly D, Cerny J, Mahdavian E, Marin-Hernandez A, Hernandez-Esquivel L, Rodriguez-Enriquez S, Stursa J, Witting PK, Stantic B, Rohlena J, Truksa J, Kluckova K, Dyason JC, Ledvina M, Salvatore BA, Moreno-Sanchez R, Coster MJ, Ralph SJ, Smith RA, and Neuzil J. Mitochondrial targeting of vitamin E succinate enhances its pro-apoptotic and anticancer activity via mitochondrial complex II. *J Biol Chem* 286: 3717–3728, 2011.
58. Dong LF, Jameson VJ, Tilly D, Prochazka L, Rohlena J, Valis K, Truksa J, Zabalova R, Mahdavian E, Kluckova K, Stantic M, Stursa J, Freeman R, Witting PK, Norberg E, Goodwin J, Salvatore BA, Novotna J, Turanek J, Ledvina M, Hozak P, Zhivotovsky B, Coster MJ, Ralph SJ, Smith RA, and Neuzil J. Mitochondrial targeting of alpha-tocopheryl succinate enhances its pro-apoptotic efficacy: A new paradigm of efficient cancer therapy. *Free Radic Biol Med* 50: 1546–1555, 2011.
59. Dong LF, Low P, Dyason JC, Wang XF, Prochazka L, Witting PK, Freeman R, Swettenham E, Valis K, Liu J, Zabalova R, Turanek J, Spitz DR, Domann FE, Scheffler IE, Ralph SJ, and Neuzil J. Alpha-tocopheryl succinate induces apoptosis by targeting ubiquinone-binding sites in mitochondrial respiratory complex II. *Oncogene* 27: 4324–4335, 2008.
60. Drechsel DA and Patel M. Role of reactive oxygen species in the neurotoxicity of environmental agents implicated in Parkinson's disease. *Free Radic Biol Med* 44: 1873–1886, 2008.
61. Drose S and Brandt U. The mechanism of mitochondrial superoxide production by the cytochrome bc1 complex. *J Biol Chem* 283: 21649–21654, 2008.
62. Efremov RG, Baradaran R, and Sazanov LA. The architecture of respiratory complex I. *Nature* 465: 441–445, 2010.
63. Eguchi Y, Shimizu S, and Tsujimoto Y. Intracellular ATP levels determine cell death fate by apoptosis or necrosis. *Cancer Res* 57: 1835–1840, 1997.
64. Ellerby HM, Arap W, Ellerby LM, Kain R, Andrusiak R, Rio GD, Krajewski S, Lombardo CR, Rao R, Ruoslahti E, Bredesen DE, and Pasqualini R. Anticancer activity of

- targeted pro-apoptotic peptides. *Nature Medicine* 5: 1032–1038, 1999.
65. Facompre M, Tardy C, Bal-Mahieu C, Colson P, Perez C, Manzanares I, Cuevas C, and Bailly C. Lamellarin D: A novel potent inhibitor of topoisomerase I. *Cancer Res* 63: 7392–7399, 2003.
 66. Fantin VR, Berardi MJ, Scorrano L, Korsmeyer SJ, and Leder P. A novel mitochondriotoxic small molecule that selectively inhibits tumor cell growth. *Cancer Cell* 2: 29–42, 2002.
 67. Fato R, Bergamini C, Bortolus M, Maniero AL, Leoni S, Ohnishi T, and Lenaz G. Differential effects of mitochondrial complex I inhibitors on production of reactive oxygen species. *Biochim Biophys Acta* 1787: 384–392, 2009.
 68. Fato R, Bergamini C, Leoni S, Strocchi P, and Lenaz G. Generation of reactive oxygen species by mitochondrial complex I: Implications in neurodegeneration. *Neurochem Res* 33: 2487–2501, 2008.
 69. Festjens N, Kalai M, Smet J, Meeus A, Van Coster R, Saelens X, and Vandenabeele P. Butylated hydroxyanisole is more than a reactive oxygen species scavenger. *Cell Death Differ* 13: 166–169, 2006.
 70. Fruehauf JP and Meyskens FL, Jr. Reactive oxygen species: A breath of life or death? *Clin Cancer Res* 13: 789–794, 2007.
 71. Fukuda R, Zhang H, Kim JW, Shimoda L, Dang CV, and Semenza GL. HIF-1 regulates cytochrome oxidase subunits to optimize efficiency of respiration in hypoxic cells. *Cell* 129: 111–122, 2007.
 72. Fulda S, Galluzzi L, and Kroemer G. Targeting mitochondria for cancer therapy. *Nat Rev Drug Discov* 9: 447–464, 2010.
 73. Fulda S, Scaffidi C, Susin SA, Krammer PH, Kroemer G, Peter ME, and Debatin KM. Activation of mitochondria and release of mitochondrial apoptogenic factors by betulinic acid. *J Biol Chem* 273: 33942–33948, 1998.
 74. Gallego MA, Ballot C, Kluz J, Hajji N, Martorati A, Castera L, Cuevas C, Formstecher P, Joseph B, Kroemer G, Bailly C, and Marchetti P. Overcoming chemoresistance of non-small cell lung carcinoma through restoration of an AIF-dependent apoptotic pathway. *Oncogene* 27: 1981–1992, 2008.
 75. Galluzzi L, Larochette N, Zamzami N, and Kroemer G. Mitochondria as therapeutic targets for cancer chemotherapy. *Oncogene* 25: 4812–4830, 2006.
 76. Garrido C, Galluzzi L, Brunet M, Puig PE, Didelot C, and Kroemer G. Mechanisms of cytochrome c release from mitochondria. *Cell Death Differ* 13: 1423–1433, 2006.
 77. Gledhill JR, Montgomery MG, Leslie AG, and Walker JE. Mechanism of inhibition of bovine F1-ATPase by resveratrol and related polyphenols. *Proc Natl Acad Sci USA* 104: 13632–13637, 2007.
 78. Gogvadze V, Norberg E, Orrenius S, and Zhivotovsky B. Involvement of Ca(2+) and ROS in alpha-tocopheryl succinate-induced mitochondrial permeabilization. *Int J Cancer* 127: 1823–1832, 2010.
 79. Gogvadze V, Orrenius S, and Zhivotovsky B. Mitochondria in cancer cells: What is so special about them? *Trends Cell Biol* 18: 165–173, 2008.
 80. Gogvadze V, Orrenius S, and Zhivotovsky B. Mitochondria as targets for cancer chemotherapy. *Semin Cancer Biol* 19: 57–66, 2009.
 81. Gomez-Lazaro M, Galindo MF, Melero-Fernandez de Mera RM, Fernandez-Gomez FJ, Concannon CG, Segura MF, Comella JX, Prehn JH, and Jordan J. Reactive oxygen species and p38 mitogen-activated protein kinase activate Bax to induce mitochondrial cytochrome c release and apoptosis in response to malonate. *Mol Pharmacol* 71: 736–743, 2007.
 82. Gong Y, Sohn H, Xue L, Firestone GL, and Bjeldanes LF. 3,3'-Diindolylmethane is a novel mitochondrial H(+)-ATP synthase inhibitor that can induce p21(Cip1/Waf1) expression by induction of oxidative stress in human breast cancer cells. *Cancer Res* 66: 4880–4887, 2006.
 83. Gottlieb E and Tomlinson IP. Mitochondrial tumour suppressors: A genetic and biochemical update. *Nat Rev Cancer* 5: 857–866, 2005.
 84. Gough DJ, Corlett A, Schlessinger K, Wegrzyn J, Larner AC, and Levy DE. Mitochondrial STAT3 supports Ras-dependent oncogenic transformation. *Science* 324: 1713–1716, 2009.
 85. Graham DJ, Green L, Senior JR, and Nourjah P. Troglitazone-induced liver failure: A case study. *Am J Med* 114: 299–306, 2003.
 86. Grivennikova VG and Vinogradov AD. Generation of superoxide by the mitochondrial Complex I. *Biochim Biophys Acta* 1757: 553–561, 2006.
 87. Hail N, Jr. and Lotan R. Apoptosis induction by the natural product cancer chemopreventive agent deguelin is mediated through the inhibition of mitochondrial bioenergetics. *Apoptosis* 9: 437–447, 2004.
 88. Harikumar KB and Aggarwal BB. Resveratrol: A multi-targeted agent for age-associated chronic diseases. *Cell Cycle* 7: 1020–1035, 2008.
 89. Harikumar KB, Kunnumakkara AB, Ahn KS, Anand P, Krishnan S, Guha S, and Aggarwal BB. Modification of the cysteine residues in IkappaBalpha kinase and NF-kappaB (p65) by xanthohumol leads to suppression of NF-kappaB-regulated gene products and potentiation of apoptosis in leukemia cells. *Blood* 113: 2003–2013, 2009.
 90. Herr HW, Huffman JL, Huryk R, Heston WD, Melamed MR, and Whitmore WF, Jr. Anticarcinoma activity of rhodamine 123 against a murine renal adenocarcinoma. *Cancer Res* 48: 2061–2063, 1988.
 91. Hilf R. Mitochondria are targets of photodynamic therapy. *J Bioenerg Biomembr* 39: 85–89, 2007.
 92. Huang LS, Sun G, Cobessi D, Wang AC, Shen JT, Tung EY, Anderson VE, and Berry EA. 3-nitropropionic acid is a suicide inhibitor of mitochondrial respiration that, upon oxidation by complex II, forms a covalent adduct with a catalytic base arginine in the active site of the enzyme. *J Biol Chem* 281: 5965–5972, 2006.
 93. Huang P, Feng L, Oldham EA, Keating MJ, and Plunkett W. Superoxide dismutase as a target for the selective killing of cancer cells. *Nature* 407: 390–395, 2000.
 94. Iwata S, Lee JW, Okada K, Lee JK, Iwata M, Rasmussen B, Link TA, Ramaswamy S, and Jap BK. Complete structure of the 11-subunit bovine mitochondrial cytochrome bc1 complex. *Science* 281: 64–71, 1998.
 95. Jemal A, Bray F, Center MM, Ferlay J, Ward E, and Forman D. Global cancer statistics. *CA: Cancer J Clin* 61: 69–90, 2011.
 96. Jemal A, Siegel R, Xu J, and Ward E. Cancer statistics, 2010. *CA Cancer J Clin* 60: 277–300, 2010.
 97. Johnson KM, Cleary J, Fierke CA, Opipari AW, Jr., and Glick GD. Mechanistic basis for therapeutic targeting of the mitochondrial F1F0-ATPase. *ACS Chem Biol* 1: 304–308, 2006.
 98. Johnson LV, Walsh ML, and Chen LB. Localization of mitochondria in living cells with rhodamine 123. *Proc Natl Acad Sci USA* 77: 990–994, 1980.

99. Jones LW, Narayan KS, Shapiro CE, and Sweatman TW. Rhodamine-123: Therapy for hormone refractory prostate cancer, a phase I clinical trial. *J Chemother* 17: 435–440, 2005.
100. Jones S, Zhang X, Parsons DW, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Kamiyama H, Jimeno A, Hong SM, Fu B, Lin MT, Calhoun ES, Kamiyama M, Walter K, Nikolskaya T, Nikolsky Y, Hartigan J, Smith DR, Hidalgo M, Leach SD, Klein AP, Jaffee EM, Goggins M, Maitra A, Iacobuzio-Donahue C, Eshleman JR, Kern SE, Hruban RH, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, and Kinzler KW. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. *Science* 321: 1801–1806, 2008.
101. Junge W, Sielaff H, and Engelbrecht S. Torque generation and elastic power transmission in the rotary F(O)F(1)-ATPase. *Nature* 459: 364–370, 2009.
102. Kang MH and Reynolds CP. Bcl-2 inhibitors: Targeting mitochondrial apoptotic pathways in cancer therapy. *Clin Cancer Res* 15: 1126–1132, 2009.
103. Kelso GF, Porteous CM, Coulter CV, Hughes G, Porteous WK, Ledgerwood EC, Smith RA, and Murphy MP. Selective targeting of a redox-active ubiquinone to mitochondria within cells: Antioxidant and antiapoptotic properties. *J Biol Chem* 276: 4588–4596, 2001.
104. Kim WY, Oh SH, Woo JK, Hong WK, and Lee HY. Targeting heat shock protein 90 overrides the resistance of lung cancer cells by blocking radiation-induced stabilization of hypoxia-inducible factor-1 α . *Cancer Res* 69: 1624–1632, 2009.
105. Kluza J, Gallego MA, Loyens A, Beauvillain JC, Sousa-Faro JM, Cuevas C, Marchetti P, and Bailly C. Cancer cell mitochondria are direct proapoptotic targets for the marine antitumor drug lamellarin D. *Cancer Res* 66: 3177–3187, 2006.
106. Ko YH, Smith BL, Wang Y, Pomper MG, Rini DA, Torbenson MS, Hullihen J, and Pedersen PL. Advanced cancers: Eradication in all cases using 3-bromopyruvate therapy to deplete ATP. *Biochem Biophys Res Comm* 324: 269–275, 2004.
107. Koobs DH. Phosphate mediation of the Crabtree and Pasteur effects. *Science* 178: 127–133, 1972.
108. Koppenol WH, Bounds PL, and Dang CV. Otto Warburg's contributions to current concepts of cancer metabolism. *Nat Rev Cancer* 11: 325–337, 2011.
109. Kroemer G, Galluzzi L, and Brenner C. Mitochondrial membrane permeabilization in cell death. *Physiol Rev* 87: 99–163, 2007.
110. Kudin AP, Bimpong-Buta NY, Vielhaber S, Elger CE, and Kunz WS. Characterization of superoxide-producing sites in isolated brain mitochondria. *J Biol Chem* 279: 4127–4135, 2004.
111. Kussmaul L and Hirst J. The mechanism of superoxide production by NADH:ubiquinone oxidoreductase (complex I) from bovine heart mitochondria. *Proc Natl Acad Sci USA* 103: 7607–7612, 2006.
112. Kwong JQ, Henning MS, Starkov AA, and Manfredi G. The mitochondrial respiratory chain is a modulator of apoptosis. *J Cell Biol* 179: 1163–1177, 2007.
113. Lambert AJ and Brand MD. Inhibitors of the quinone-binding site allow rapid superoxide production from mitochondrial NADH:ubiquinone oxidoreductase (complex I). *J Biol Chem* 279: 39414–39420, 2004.
114. Lampidis TJ, Bernal SD, Summerhayes IC, and Chen LB. Selective toxicity of rhodamine 123 in carcinoma cells *in vitro*. *Cancer Res* 43: 716–720, 1983.
115. Le SB, Hailer MK, Buhrow S, Wang Q, Flatten K, Peditakis P, Bible KC, Lewis LD, Sausville EA, Pang YP, Ames MM, Lemasters JJ, Holmuhamedov EL, and Kaufmann SH. Inhibition of mitochondrial respiration as a source of adaphostin-induced reactive oxygen species and cytotoxicity. *J Biol Chem* 282: 8860–8872, 2007.
116. Lee HC, Chang CM, and Chi CW. Somatic mutations of mitochondrial DNA in aging and cancer progression. *Ageing Res Rev* 9 Suppl 1: S47–58, 2010.
117. Lee HY, Oh SH, Woo JK, Kim WY, Van Pelt CS, Price RE, Cody D, Tran H, Pezzuto JM, Moriarty RM, and Hong WK. Chemopreventive effects of deguelin, a novel Akt inhibitor, on tobacco-induced lung tumorigenesis. *J Natl Cancer Inst* 97: 1695–1699, 2005.
118. Lei W, Xie J, Hou Y, Jiang G, Zhang H, Wang P, Wang X, and Zhang B. Mitochondria-targeting properties and photodynamic activities of porphyrin derivatives bearing cationic pendant. *J Photochem Photobiol B* 98: 167–171, 2010.
119. Leist M, Single B, Castoldi AF, Kuhnle S, and Nicotera P. Intracellular adenosine triphosphate (ATP) concentration: A switch in the decision between apoptosis and necrosis. *J Exp Med* 185: 1481–1486, 1997.
120. Lemarie A and Grimm S. Mitochondrial respiratory chain complexes: Apoptosis sensors mutated in cancer? *Oncogene*, 2011.
121. Lemarie A, Huc L, Pazarentzos E, Mahul-Mellier AL, and Grimm S. Specific disintegration of complex II succinate:ubiquinone oxidoreductase links pH changes to oxidative stress for apoptosis induction. *Cell Death Differ* 18: 338–349, 2011.
122. Lessene G, Czabotar PE, and Colman PM. BCL-2 family antagonists for cancer therapy. *Nat Rev Drug Discov* 7: 989–1000, 2008.
123. Lewis W, Levine ES, Griniuvienė B, Tankersley KO, Colacino JM, Sommadossi JP, Watanabe KA, and Perrino FW. Fialuridine and its metabolites inhibit DNA polymerase gamma at sites of multiple adjacent analog incorporation, decrease mtDNA abundance, and cause mitochondrial structural defects in cultured hepatoblasts. *Proc Natl Acad Sci USA* 93: 3592–3597, 1996.
124. Li N, Ragheb K, Lawler G, Sturgis J, Rajwa B, Melendez JA, and Robinson JP. Mitochondrial complex I inhibitor rotenone induces apoptosis through enhancing mitochondrial reactive oxygen species production. *J Biol Chem* 278: 8516–8525, 2003.
125. Liot G, Bossy B, Lubitz S, Kushnareva Y, Sejbuk N, and Bossy-Wetzel E. Complex II inhibition by 3-NP causes mitochondrial fragmentation and neuronal cell death via an NMDA- and ROS-dependent pathway. *Cell Death Differ* 16: 899–909, 2009.
126. Liu X, Luo X, Shi Y, Zhu GD, Penning T, Giranda VL, and Luo Y. Poly (ADP-ribose) polymerase activity regulates apoptosis in HeLa cells after alkylating DNA damage. *Cancer Biol Ther* 7: 934–941, 2008.
127. Lust S, Vanhoecke B, M VANG, Boelens J, H VANM, Kaileh M, Vanden Berghe W, Haegeman G, Philippe J, Bracke M, and Offner F. Xanthohumol activates the proapoptotic arm of the unfolded protein response in chronic lymphocytic leukemia. *Anticancer Res* 29: 3797–3805, 2009.
128. Madesh M and Hajnoczky G. VDAC-dependent permeabilization of the outer mitochondrial membrane by

- superoxide induces rapid and massive cytochrome c release. *J Cell Biol* 155: 1003–1015, 2001.
129. Maklashina E and Cecchini G. The quinone-binding and catalytic site of complex II. *Biochim Biophys Acta* 1797: 3047–3056, 2010.
130. Mathupala SP, Ko YH, and Pedersen PL. Hexokinase II: Cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. *Oncogene* 25: 4777–4786, 2006.
131. Matsuzawa A and Ichijo H. Redox control of cell fate by MAP kinase: Physiological roles of ASK1-MAP kinase pathway in stress signaling. *Biochim Biophys Acta* 1780: 1325–1336, 2008.
132. Mattarei A, Biasutto L, Marotta E, De Marchi U, Sassi N, Garbisa S, Zoratti M, and Paradisi C. A mitochondriotropic derivative of quercetin: A strategy to increase the effectiveness of polyphenols. *Chembiochem* 9: 2633–2642, 2008.
133. Mehta A and Shaha C. Apoptotic death in *Leishmania donovani* promastigotes in response to respiratory chain inhibition: Complex II inhibition results in increased pentamidine cytotoxicity. *J Biol Chem* 279: 11798–11813, 2004.
134. Miller WH, Jr. Molecular targets of arsenic trioxide in malignant cells. *Oncologist* 7: 14–19, 2002.
135. Miranda CL, Stevens JF, Helmrich A, Henderson MC, Rodriguez RJ, Yang YH, Deinzer ML, Barnes DW, and Buhler DR. Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (*Humulus lupulus*) in human cancer cell lines. *Food Chem Toxicol* 37: 271–285, 1999.
136. Miyadera H, Shiomi K, Ui H, Yamaguchi Y, Masuma R, Tomoda H, Miyoshi H, Osanai A, Kita K, and Omura S. Atpenins, potent and specific inhibitors of mitochondrial complex II (succinate-ubiquinone oxidoreductase). *Proc Natl Acad Sci USA* 100: 473–477, 2003.
137. Miyako K, Kai Y, Irie T, Takeshige K, and Kang D. The content of intracellular mitochondrial DNA is decreased by 1-methyl-4-phenylpyridinium ion (MPP+). *J Biol Chem* 272: 9605–9608, 1997.
138. Modica-Napolitano JS and Aprille JR. Basis for the selective cytotoxicity of rhodamine 123. *Cancer Res* 47: 4361–4365, 1987.
139. Modica-Napolitano JS and Aprille JR. Delocalized lipophilic cations selectively target the mitochondria of carcinoma cells. *Adv Drug Deliv Rev* 49: 63–70, 2001.
140. Moncada S and Erusalimsky JD. Does nitric oxide modulate mitochondrial energy generation and apoptosis? *Nat Rev Mol Cell Biol* 3: 214–220, 2002.
141. Monteiro R, Calhau C, Silva AO, Pinheiro-Silva S, Guerreiro S, Gartner F, Azevedo I, and Soares R. Xanthohumol inhibits inflammatory factor production and angiogenesis in breast cancer xenografts. *J Cell Biochem* 104: 1699–1707, 2008.
142. Moreira PI, Custodio J, Moreno A, Oliveira CR, and Santos MS. Tamoxifen and estradiol interact with the flavin mononucleotide site of complex I leading to mitochondrial failure. *J Biol Chem* 281: 10143–10152, 2006.
143. Mukhopadhyay I, Sausville EA, Doroshow JH, and Roy KK. Molecular mechanism of adaphostin-mediated G1 arrest in prostate cancer (PC-3) cells: Signaling events mediated by hepatocyte growth factor receptor, c-Met, and p38 MAPK pathways. *J Biol Chem* 281: 37330–37344, 2006.
144. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J* 417: 1–13, 2009.
145. Murphy MP and Smith RA. Targeting antioxidants to mitochondria by conjugation to lipophilic cations. *Ann Rev Pharmacol Toxicol* 47: 629–656, 2007.
146. Nadanaciva S, Bernal A, Aggeler R, Capaldi R, and Will Y. Target identification of drug induced mitochondrial toxicity using immunocapture based OXPHOS activity assays. *Toxicol In Vitro* 21: 902–911, 2007.
147. Nair AS, Shishodia S, Ahn KS, Kunnumakkara AB, Sethi G, and Aggarwal BB. Deguelin, an Akt inhibitor, suppresses I κ B α kinase activation leading to suppression of NF- κ B-regulated gene expression, potentiation of apoptosis, and inhibition of cellular invasion. *J Immunol* 177: 5612–5622, 2006.
148. Nakamura Y, Kawakami M, Yoshihiro A, Miyoshi N, Ohigashi H, Kawai K, Osawa T, and Uchida K. Involvement of the mitochondrial death pathway in chemopreventive benzyl isothiocyanate-induced apoptosis. *J Biol Chem* 277: 8492–8499, 2002.
149. Neuzil J, Dong LF, Ramanathapuram L, Hahn T, Chladova M, Wang XF, Zabalova R, Prochazka L, Gold M, Freeman R, Turanek J, Akporiaye ET, Dyason JC, and Ralph SJ. Vitamin E analogues as a novel group of mitocans: Anticancer agents that act by targeting mitochondria. *Mol Aspects Med* 28: 607–645, 2007.
150. Neuzil J and Massa H. Hepatic processing determines dual activity of alpha-tocopheryl succinate: A novel paradigm for a shift in biological activity due to pro-vitamin-to-vitamin conversion. *Biochem Biophys Res Commun* 327: 1024–1027, 2005.
151. Neuzil J, Swettenham E, Wang XF, Dong LF, and Stapelberg M. alpha-Tocopheryl succinate inhibits angiogenesis by disrupting paracrine FGF2 signaling. *FEBS Lett* 581: 4611–4615, 2007.
152. Neuzil J, Tomasetti M, Mellick AS, Alleve R, Salvatore BA, Birringer M, and Fariss MW. Vitamin E analogues: A new class of inducers of apoptosis with selective anticancer effects. *Curr Cancer Drug Targets* 4: 355–372, 2004.
153. Neuzil J, Tomasetti M, Zhao Y, Dong LF, Birringer M, Wang XF, Low P, Wu K, Salvatore BA, and Ralph SJ. Vitamin E analogs, a novel group of "mitocans," as anticancer agents: The importance of being redox-silent. *Mol Pharmacol* 71: 1185–1199, 2007.
154. Neuzil J, Weber T, Gellert N, and Weber C. Selective cancer cell killing by alpha-tocopheryl succinate. *Br J Cancer* 84: 87–89, 2001.
155. Neuzil J, Weber T, Schroder A, Lu M, Ostermann G, Gellert N, Mayne GC, Olejnicka B, Negre-Salvayre A, Sticha M, Coffey RJ, and Weber C. Induction of cancer cell apoptosis by alpha-tocopheryl succinate: Molecular pathways and structural requirements. *FASEB J* 15: 403–415, 2001.
156. Oh SH, Woo JK, Jin Q, Kang HJ, Jeong JW, Kim KW, Hong WK, and Lee HY. Identification of novel antiangiogenic anticancer activities of deguelin targeting hypoxia-inducible factor-1 alpha. *Int J Cancer* 122: 5–14, 2008.
157. Oh SH, Woo JK, Yazici YD, Myers JN, Kim WY, Jin Q, Hong SS, Park HJ, Suh YG, Kim KW, Hong WK, and Lee HY. Structural basis for depletion of heat shock protein 90 client proteins by deguelin. *J Natl Cancer Inst* 99: 949–961, 2007.
158. Okubo T, Yokoyama Y, Kano K, and Kano I. Molecular mechanism of cell death induced by the antioxidant tert-butylhydroxyanisole in human monocytic leukemia U937 cells. *Biol Pharm Bull* 27: 295–302, 2004.
159. Okun JG, Lummen P, and Brandt U. Three classes of inhibitors share a common binding domain in mitochondrial complex I (NADH:ubiquinone oxidoreductase). *J Biol Chem* 274: 2625–2630, 1999.

160. Oliver CL, Miranda MB, Shangary S, Land S, Wang S, and Johnson DE. (-)-Gossypol acts directly on the mitochondria to overcome Bcl-2- and Bcl-X(L)-mediated apoptosis resistance. *Mol Cancer Ther* 4: 23–31, 2005.
161. Osipo C, Gajdos C, Cheng D, and Jordan VC. Reversal of tamoxifen resistant breast cancer by low dose estrogen therapy. *J Steroid Biochem Mol Biol* 93: 249–256, 2005.
162. Pan-Montojo F, Anichtchik O, Denning Y, Knels L, Pursche S, Jung R, Jackson S, Gille G, Spillantini MG, Reichmann H, and Funk RH. Progression of Parkinson's disease pathology is reproduced by intragastric administration of rotenone in mice. *PLoS One* 5: e8762, 2010.
163. Papadopoulos LC and Tsiftoglou AS. Mitochondrial cytochrome c oxidase as a target site for daunomycin in K-562 cells and heart tissue. *Cancer Res* 53: 1072–1078, 1993.
164. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA, Jr., Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, and Kinzler KW. An integrated genomic analysis of human glioblastoma multiforme. *Science* 321: 1807–1812, 2008.
165. Peczkowska M, Cascon A, Prejbisz A, Kubaszek A, Cwikla BJ, Furmanek M, Erlic Z, Eng C, Januszewicz A, and Neumann HP. Extra-adrenal and adrenal pheochromocytomas associated with a germline SDHC mutation. *Nat Clin Pract Endocrinol Metab* 4: 111–115, 2008.
166. Pelicano H, Feng L, Zhou Y, Carew JS, Hileman EO, Plunkett W, Keating MJ, and Huang P. 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* 278: 37832–37839, 2003.
167. Prochazka L, Dong LF, Valis K, Freeman R, Ralph SJ, Turanek J, and Neuzil J. alpha-Tocopheryl succinate causes mitochondrial permeabilization by preferential formation of Bak channels. *Apoptosis* 15: 782–794, 2010.
168. Propper DJ, Braybrooke JP, Taylor DJ, Lodi R, Styles P, Cramer JA, Collins WC, Levitt NC, Talbot DC, Ganesan TS, and Harris AL. Phase I trial of the selective mitochondrial toxin MKT077 in chemo-resistant solid tumours. *Ann Oncol* 10: 923–927, 1999.
169. Ralph SJ and Neuzil J. Mitochondria as targets for cancer therapy. *Mol Nutr Food Res* 53: 9–28, 2009.
170. Ralph SJ, Rodriguez-Enriquez S, Neuzil J, and Moreno-Sanchez R. Bioenergetic pathways in tumor mitochondria as targets for cancer therapy and the importance of the ROS-induced apoptotic trigger. *Mol Aspects Med* 31: 29–59, 2010.
171. Reed GA, Sunega JM, Sullivan DK, Gray JC, Mayo MS, Crowell JA, and Hurwitz A. Single-dose pharmacokinetics and tolerability of absorption-enhanced 3,3'-diindolylmethane in healthy subjects. *Cancer Epidemiol Biomarkers Prev* 17: 2619–2624, 2008.
172. Safe S, Papineni S, and Chintharlapalli S. Cancer chemotherapy with indole-3-carbinol, bis(3'-indolyl)methane and synthetic analogs. *Cancer Lett* 269: 326–338, 2008.
173. Sahu RP, Zhang R, Batra S, Shi Y, and Srivastava SK. Benzyl isothiocyanate-mediated generation of reactive oxygen species causes cell cycle arrest and induces apoptosis via activation of MAPK in human pancreatic cancer cells. *Carcinogenesis* 30: 1744–1753, 2009.
174. Salomon AR, Voehringer DW, Herzenberg LA, and Khosla C. Understanding and exploiting the mechanistic basis for selectivity of polyketide inhibitors of F(0)F(1)-ATPase. *Proc Natl Acad Sci USA* 97: 14766–14771, 2000.
175. Salomon AR, Voehringer DW, Herzenberg LA, and Khosla C. Apoptolodin, a selective cytotoxic agent, is an inhibitor of F0F1-ATPase. *Chem Biol* 8: 71–80, 2001.
176. Saraste M. Oxidative phosphorylation at the fin de siecle. *Science* 283: 1488–1493, 1999.
177. Sasaki R, Suzuki Y, Yonezawa Y, Ota Y, Okamoto Y, Demizu Y, Huang P, Yoshida H, Sugimura K, and Mizushima Y. DNA polymerase gamma inhibition by vitamin K3 induces mitochondria-mediated cytotoxicity in human cancer cells. *Cancer Sci* 99: 1040–1048, 2008.
178. Scheffler I. *Mitochondria, Second Edition* Hoboken, New Jersey, USA: John Wiley & Sons, Inc.; 2008.
179. Schiavi F, Boedeker CC, Bausch B, Peczkowska M, Gomez CF, Strassburg T, Pawlu C, Buchta M, Salzmann M, Hoffmann MM, Berlis A, Brink I, Cybulla M, Muresan M, Walter MA, Forrer F, Valimaki M, Kaweck A, Sztukowski Z, Schipper J, Walz MK, Pigny P, Bauters C, Willet-Brozick JE, Baysal BE, Januszewicz A, Eng C, Opocher G, and Neumann HP. Predictors and prevalence of paraganglioma syndrome associated with mutations of the SDHC gene. *JAMA* 294: 2057–2063, 2005.
180. Shaban H, Gazzotti P, and Richter C. Cytochrome c oxidase inhibition by N-retinyl-N-retinylidene ethanolamine, a compound suspected to cause age-related macula degeneration. *Arch Biochem Biophys* 394: 111–116, 2001.
181. Shiau CW, Huang JW, Wang DS, Weng JR, Yang CC, Lin CH, Li C, and Chen CS. alpha-Tocopheryl succinate induces apoptosis in prostate cancer cells in part through inhibition of Bcl-xL/Bcl-2 function. *J Biol Chem* 281: 11819–11825, 2006.
182. Shun MC, Yu W, Park SK, Sanders BG, and Kline K. Downregulation of epidermal growth factor receptor expression contributes to alpha-TEA's proapoptotic effects in human ovarian cancer cell lines. *J Oncol* 2010: 824571, 2010.
183. Sibrian-Vazquez M, Nesterova IV, Jensen TJ, and Vicente MG. Mitochondria targeting by guanidine- and biguanidine-porphyrin photosensitizers. *Bioconjug Chem* 19: 705–713, 2008.
184. Simons AL, Ahmad IM, Mattson DM, Dornfeld KJ, and Spitz DR. 2-Deoxy-D-glucose combined with cisplatin enhances cytotoxicity via metabolic oxidative stress in human head and neck cancer cells. *Cancer Res* 67: 3364–3370, 2007.
185. Slapke J, Schewe T, Hummel S, Winkler J, and Kopf M. Lung strips from guinea pigs as test system for lipoxigenase inhibitors. Inhibition of arachidonic acid-induced contractions by 3-t-butyl-4-hydroxyanisole and nordihydroguaiaretic acid. *Biomed Biochim Acta* 42: 1309–1318, 1983.
186. Soller M, Drose S, Brandt U, Brune B, and von Knethen A. Mechanism of thiazolidinedione-dependent cell death in Jurkat T cells. *Mol Pharmacol* 71: 1535–1544, 2007.
187. Stapelberg M, Gellert N, Swettenham E, Tomasetti M, Witting PK, Procopio A, and Neuzil J. Alpha-tocopheryl succinate inhibits malignant mesothelioma by disrupting the fibroblast growth factor autocrine loop: Mechanism and the role of oxidative stress. *J Biol Chem* 280: 25369–25376, 2005.
188. Strathmann J, Klimo K, Sauer SW, Okun JG, Prehn JH, and Gerhauser C. Xanthohumol-induced transient superoxide anion radical formation triggers cancer cells into apoptosis via a mitochondria-mediated mechanism. *FASEB J* 24: 2938–2950, 2010.

189. Suen DF, Norris KL, and Youle RJ. Mitochondrial dynamics and apoptosis. *Genes Dev* 22: 1577–1590, 2008.
190. Summerhayes IC, Lampidis TJ, Bernal SD, Nadakavukaren JJ, Nadakavukaren KK, Shepherd EL, and Chen LB. Unusual retention of rhodamine 123 by mitochondria in muscle and carcinoma cells. *Proc Natl Acad Sci USA* 79: 5292–5296, 1982.
191. Sun F, Huo X, Zhai Y, Wang A, Xu J, Su D, Bartlam M, and Rao Z. Crystal structure of mitochondrial respiratory membrane protein complex II. *Cell* 121: 1043–1057, 2005.
192. Suter M, Reme C, Grimm C, Wenzel A, Jaattela M, Esser P, Kociok N, Leist M, and Richter C. Age-related macular degeneration. The lipofusion component N-retinyl-N-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells. *J Biol Chem* 275: 39625–39630, 2000.
193. Szatrowski TP and Nathan CF. Production of large amounts of hydrogen peroxide by human tumor cells. *Cancer Res* 51: 794–798, 1991.
194. Tinhofer I, Bernhard D, Senfter M, Anether G, Loeffler M, Kroemer G, Kofler R, Csordas A, and Greil R. Resveratrol, a tumor-suppressive compound from grapes, induces apoptosis via a novel mitochondrial pathway controlled by Bcl-2. *FASEB J* 15: 1613–1615, 2001.
195. Tocilescu MA, Fendel U, Zwicker K, Kerscher S, and Brandt U. Exploring the ubiquinone binding cavity of respiratory complex I. *J Biol Chem* 282: 29514–29520, 2007.
196. Trachootham D, Alexandre J, and Huang P. Targeting cancer cells by ROS-mediated mechanisms: A radical therapeutic approach? *Nat Rev Drug Discov* 8: 579–591, 2009.
197. Trachootham D, Zhang H, Zhang W, Feng L, Du M, Zhou Y, Chen Z, Pelicano H, Plunkett W, Wierda WG, Keating MJ, and Huang P. Effective elimination of fludarabine-resistant CLL cells by PEITC through a redox-mediated mechanism. *Blood* 112: 1912–1922, 2008.
198. Trachootham D, Zhou Y, Zhang H, Demizu Y, Chen Z, Pelicano H, Chiao PJ, Achanta G, Arlinghaus RB, Liu J, and Huang P. Selective killing of oncogenically transformed cells through a ROS-mediated mechanism by beta-phenylethyl isothiocyanate. *Cancer Cell* 10: 241–252, 2006.
199. Tse C, Shoemaker AR, Adickes J, Anderson MG, Chen J, Jin S, Johnson EF, Marsh KC, Mitten MJ, Nimmer P, Roberts L, Tahir SK, Xiao Y, Yang X, Zhang H, Fesik S, Rosenberg SH, and Elmore SW. ABT-263: A potent and orally bioavailable Bcl-2 family inhibitor. *Cancer Res* 68: 3421–3428, 2008.
200. Twombly R. Cancer surpasses heart disease as leading cause of death for all but the very elderly. *J Natl Cancer Inst* 97: 330–331, 2005.
201. Tzung SP, Kim KM, Basanez G, Giedt CD, Simon J, Zimmerberg J, Zhang KY, and Hockenbery DM. Antimycin A mimics a cell-death-inducing Bcl-2 homology domain 3. *Nat Cell Biol* 3: 183–191, 2001.
202. Udeani GO, Gerhauser C, Thomas CF, Moon RC, Kosmeder JW, Kinghorn AD, Moriarty RM, and Pezzuto JM. Cancer chemopreventive activity mediated by deguelin, a naturally occurring rotenoid. *Cancer Res* 57: 3424–3428, 1997.
203. Umeda S, Muta T, Ohsato T, Takamatsu C, Hamasaki N, and Kang D. The D-loop structure of human mtDNA is destabilized directly by 1-methyl-4-phenylpyridinium ion (MPP+), a parkinsonism-causing toxin. *Eur J Biochem* 267: 200–206, 2000.
204. Valis K, Prochazka L, Boura E, Chladova J, Obsil T, Rohlena J, Truksa J, Dong LF, Ralph SJ, and Neuzil J. Hippo/Mst1 stimulates transcription of the proapoptotic mediator NOXA in a FoxO1-dependent manner. *Cancer Res* 71: 946–954, 2011.
205. van Delft MF, Wei AH, Mason KD, Vandenberg CJ, Chen L, Czabotar PE, Willis SN, Scott CL, Day CL, Cory S, Adams JM, Roberts AW, and Huang DC. The BH3 mimetic ABT-737 targets selective Bcl-2 proteins and efficiently induces apoptosis via Bak/Bax if Mcl-1 is neutralized. *Cancer Cell* 10: 389–399, 2006.
206. Vander Heiden MG, Cantley LC, and Thompson CB. Understanding the Warburg effect: The metabolic requirements of cell proliferation. *Science* 324: 1029–1033, 2009.
207. Wang F, Ogasawara MA, and Huang P. Small mitochondria-targeting molecules as anticancer agents. *Mol Aspects Med* 31: 75–92, 2010.
208. Wang XF, Dong L, Zhao Y, Tomasetti M, Wu K, and Neuzil J. Vitamin E analogues as anticancer agents: Lessons from studies with alpha-tocopheryl succinate. *Mol Nutr Food Res* 50: 675–685, 2006.
209. Warin R, Chambers WH, Potter DM, and Singh SV. Prevention of mammary carcinogenesis in MMTV-neu mice by cruciferous vegetable constituent benzyl isothiocyanate. *Cancer Res* 69: 9473–9480, 2009.
210. Warin R, Xiao D, Arlotti JA, Bommarreddy A, and Singh SV. Inhibition of human breast cancer xenograft growth by cruciferous vegetable constituent benzyl isothiocyanate. *Mol Carcinog* 49: 500–507, 2010.
211. Weber T, Dalen H, Andera L, Negre-Salvayre A, Auge N, Sticha M, Lloret A, Terman A, Witting PK, Higuchi M, Plasilova M, Zivny J, Gellert N, Weber C, and Neuzil J. Mitochondria play a central role in apoptosis induced by alpha-tocopheryl succinate, an agent with antineoplastic activity: Comparison with receptor-mediated pro-apoptotic signaling. *Biochemistry* 42: 4277–4291, 2003.
212. Wiatrak BJ. Overview of recurrent respiratory papillomatosis. *Curr Opin Otolaryngol Head Neck Surg* 11: 433–441, 2003.
213. Wijayaratne AL, Nagel SC, Paige LA, Christensen DJ, Norris JD, Fowlkes DM, and McDonnell DP. Comparative analyses of mechanistic differences among antiestrogens. *Endocrinology* 140: 5828–5840, 1999.
214. Wolvetang EJ, Johnson KL, Krauer K, Ralph SJ, and Linnane AW. Mitochondrial respiratory chain inhibitors induce apoptosis. *FEBS Lett* 339: 40–44, 1994.
215. Xia D, Yu CA, Kim H, Xia JZ, Kachurin AM, Zhang L, Yu L, and Deisenhofer J. Crystal structure of the cytochrome bc1 complex from bovine heart mitochondria. *Science* 277: 60–66, 1997.
216. Xiao D, Powolny AA, and Singh SV. Benzyl isothiocyanate targets mitochondrial respiratory chain to trigger reactive oxygen species-dependent apoptosis in human breast cancer cells. *J Biol Chem* 283: 30151–30163, 2008.
217. Xu K and Thornalley PJ. Involvement of glutathione metabolism in the cytotoxicity of the phenethyl isothiocyanate and its cysteine conjugate to human leukaemia cells in vitro. *Biochem Pharmacol* 61: 165–177, 2001.
218. Yang YM, Conaway CC, Chiao JW, Wang CX, Amin S, Whysner J, Dai W, Reinhardt J, and Chung FL. Inhibition of benzo(a)pyrene-induced lung tumorigenesis in A/J mice by dietary N-acetylcysteine conjugates of benzyl and phenethyl isothiocyanates during the postinitiation phase is associated with activation of mitogen-activated protein kinases and p53 activity and induction of apoptosis. *Cancer Res* 62: 2–7, 2002.
219. Yankovskaya V, Horsefield R, Tornroth S, Luna-Chavez C, Miyoshi H, Leger C, Byrne B, Cecchini G, and Iwata S.

- Architecture of succinate dehydrogenase and reactive oxygen species generation. *Science* 299: 700–704, 2003.
220. You KR, Wen J, Lee ST, and Kim DG. Cytochrome c oxidase subunit III: A molecular marker for N-(4-hydroxyphenyl)retinamide-induced oxidative stress in hepatoma cells. *J Biol Chem* 277: 3870–3877, 2002.
 221. Youle RJ and Strasser A. The BCL-2 protein family: Opposing activities that mediate cell death. *Nat Rev Mol Cell Biol* 9: 47–59, 2008.
 222. Yu C, Rahmani M, Almenara J, Sausville EA, Dent P, and Grant S. Induction of apoptosis in human leukemia cells by the tyrosine kinase inhibitor adaphostin proceeds through a RAF-1/MEK/ERK- and AKT-dependent process. *Oncogene* 23: 1364–1376, 2004.
 223. Zamaraeva MV, Sabirov RZ, Maeno E, Ando-Akatsuka Y, Bessonova SV, and Okada Y. Cells die with increased cytosolic ATP during apoptosis: A bioluminescence study with intracellular luciferase. *Cell Death Differ* 12: 1390–1397, 2005.
 224. Zeitlin BD, Zeitlin JJ, and Nor JE. Expanding circle of inhibition: Small-molecule inhibitors of Bcl-2 as anticancer cell and antiangiogenic agents. *J Clin Oncol* 26: 4180–4188, 2008.
 225. Zhang JG, Nicholls-Grzemeski FA, Tirmenstein MA, and Fariss MW. Vitamin E succinate protects hepatocytes against the toxic effect of reactive oxygen species generated at mitochondrial complexes I and III by alkylating agents. *Chem Biol Interact* 138: 267–284, 2001.
 226. Zhang JG, Tirmenstein MA, Nicholls-Grzemeski FA, and Fariss MW. Mitochondrial electron transport inhibitors cause lipid peroxidation-dependent and -independent cell death: protective role of antioxidants. *Arch Biochem Biophys* 393: 87–96, 2001.
 227. Zheng J and Ramirez VD. Piceatannol, a stilbene phytochemical, inhibits mitochondrial F₀F₁-ATPase activity by targeting the F₁ complex. *Biochem Biophys Res Commun* 261: 499–503, 1999.
 228. Zhivotovsky B, Galluzzi L, Kepp O, and Kroemer G. Adenine nucleotide translocase: A component of the phylogenetically conserved cell death machinery. *Cell Death and Differentiation* 16: 1419–1425, 2009.
 229. Zini R, Morin C, Bertelli A, Bertelli AA, and Tillement JP. Effects of resveratrol on the rat brain respiratory chain. *Drugs Exp Clin Res* 25: 87–97, 1999.

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Abbreviations Used

2DG = 2-deoxyglucose
 3BP = 3-bromopyruvate
 3NP = nitropropionic acid
 4HPR = N-(4-hydroxyphenyl) retinamide
 α -TEA = α -tocopherylacetic acid ether
 α -TOS = α -tocopheryl succinate
 $\Delta\psi_{m,i}$ = mitochondrial inner trans-membrane potential
 A2E = N-retinyl-N-retinylidene ethanolamine
 AMPK = AMP-activated protein kinase
 ANT = adenine nucleotide transporter
 ASK1 = apoptosis signal-regulating kinase 1
 BH3 = benzyl isothiocyanate
 BHA = 3-tert-butyl-4-hydroxyanisole
 CI = complex I
 CII = complex II
 CIII = complex III
 CIV = complex IV
 CV = complex V
 DCA = dichloroacetate
 DIM = 3,30-diindolylmethane
 ETC = electron transport chain
 FGF2 = fibroblast growth factor 2
 G6P = glucose-6-phosphate
 GSAO = 4-(N-(S-glutathionylacetyl)amino) phenylarsineoxide
 HIF = hypoxia-inducible factor
 HK = hexokinase
 I3C = indole-3-carbinol
 IMS = intermembrane space
 MIM = mitochondrial inner membrane
 MitoQ = mitochondrially targetted ubiquinone
 MitoVES = mitochondrially targeted vitamin E succinate
 MOM = mitochondrial outer membrane
 MPP⁺ = 1-methyl-4-phenylpyridinium
 mtDNA = mitochondrial DNA
 NF- κ B = nuclear factor- κ B
 PD = pyruvate dehydrogenase kinase
 PEITC = phenethyl isothiocyanate
 PTPC = permeability transition pore complex
 Q_D = distal UbQ-binding site
 Q_P = proximal UbQ-binding site
 ROS = reactive oxygen species
 SDH = succinate dehydrogenase
 SDHA = succinate dehydrogenase subunit A
 SDHB = succinate dehydrogenase subunit B
 SDHC = succinate dehydrogenase subunit C
 SDHD = succinate dehydrogenase subunit D
 TCA = tricarboxylic acid cycle
 TPP⁺ = triphenylphosphonium
 TTFA = thenoyltrifluoroacetone
 UbQ = ubiquinone
 UbQH₂ = ubiquinol
 VDAC = voltage-dependent anionic channel
 VK3 = vitamin K3

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2. Jiri Neuzil, Jakub Rohlena, Lan-Feng Dong. 2011. K-Ras and mitochondria: Dangerous liaisons. *Cell Research* . [[CrossRef](#)]