Review

Cancer chemoprevention and mitochondria: Targeting apoptosis in transformed cells *via* the disruption of mitochondrial bioenergetics/redox state

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Cancer chemoprevention employs agents that block, hinder, or reverse tumorigenesis to prevent malignancy. Several putative cancer chemopreventive agents promote apoptosis in transformed cells initiated in animal carcinogenesis models or identified in human subjects, and/or in tumor cells cultured *in vitro*. Consequently, apoptosis induction is increasingly valued as a biologically significant anticancer mechanism in the arena of chemoprevention. *In vitro* studies suggest that the permeabilization of mitochondrial membranes is an important mechanistic determinant associated with the apoptosis induced by these agents. Mitochondrial membrane permeabilization (MMP) may occur *via* the control of proapoptotic Bcl-2 family members, and/or by the induction of the mitochondrial permeability transition. Both of these cell death-inducing regulatory mechanisms are ultimately responsive to the bioenergetic status/redox state of mitochondria. Interestingly, in addition to inducing MMP, various chemopreventive agents can directly modulate mitochondrial bioenergetics and/or redox tone in transformed cells. This review will examine prospective mechanisms associated with the disruption of mitochondrial function by chemopreventive agents that affect MMP and apoptosis. In doing so, we will construct a paradigm supporting the notion that the bioenergetic and/or redox characteristics of the mitochondria in transformed cells are important targets in the chemoprevention of cancer.

Keywords: Apoptosis / Bioenergetics / Cancer chemoprevention / Mitochondria / Reactive oxygen species

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1 Modulating tumorigenesis through chemoprevention

The rates of cell division and apoptosis typically govern the size of a normal cell population. These processes are tightly regulated by various checks and balances mechanisms that are activated by genetic information. Tumorigenesis can be viewed as a process that exhibits accelerated, albeit abnormal, cellular evolution where the genetic information controlling proliferation, differentiation, and apoptosis is transformed under environmental selective pressures [1]. Cumulative alterations in genetic information resulting from aberrant cell proliferation, microenvironmental changes, and inadequate DNA repair, serve to systematically dismantle safeguards that allow a transformed cell to differentiate, or be eliminated *via* apoptosis. Inherently, the accrual of defects in these signaling and apoptotic pathways in premalignant cells can contribute to the promotion and progression of the malignant phenotype, and ultimately resistance to therapy [2].

Observations in animal models suggest that tumor development follows three distinct phases, initiation, promotion, and progression. The initiation phase is a rapid (*i.e.*, taking

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Abbreviations: ANT, adenine nucleotide translocase; **CD437,** 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid; **DCF,** 2′,7′-dichlorofluorescein; **EGCG,** epigallocatechin-3-gallate; **ETC,** electron transport chain; **4HPR,** *N*-**(4-hydroxyphenyl)retinamide; mtDNA,** mitochondrial DNA; **MMP,** mitochondrial membrane permeabilization; **MPT,** mitochondrial permeability transition; **OXPHOS,** oxidative phosphorylation; **PTPC,** permeability transition pore complex; **ROS,** reactive oxygen species

place within hours to days), irreversible event that occurs when a normal cell is exposed to a carcinogen, which causes irreparable or misrepaired DNA damage, yet alone is not mutagenic. However, the resulting somatic mutation can be duplicated *via* mitosis to yield a clone of the mutated cell. The promotion phase, which is a protracted process that may require several years or decades to establish, denotes the sustained clonal expansion of a mutated cell to form an actively proliferating, multicellular premalignant lesion. The progression phase, like the initiation phase, is an irreversible process that occurs over a relatively short period (*i.e.*, usually less than 1 year). Progression of premalignant cells produces new clones with increased proliferative capacity, invasiveness, and metastatic potential [3].

Cancer continues to be one of the major causes of death worldwide and only modest progress has been made in reducing the morbidity and mortality of this dreadful disease. Extensive preclinical and clinical research has led to substantial progress in understanding the multi-step nature of the prolonged tumorigenesis process. This understanding has led to the conviction that most human malignancies should be fought on multiple fronts. Thus, in addition to cancer therapy, cancer prevention has become an important approach to control cancer [4, 5]. Common prevention strategies include avoiding exposure to known cancer-causing agents, enhancement of host defense mechanisms against cancer, life style modifications, and chemoprevention.

Given that the initiation and progression phases of tumorigenesis are relatively transient and irreversible events, conventional wisdom dictates that time as well as targets for cancer prevention can be attained by intervening in the protracted promotion phase rather than treating the isolated late event of malignant disease [4]. The phrase "cancer chemoprevention" was introduced by Sporn in 1976 when he referred to the prevention of the development of malignancy by vitamin A and its analogs, known collectively as retinoids. Sporn contended that the process of carcinogenesis had the potential to be controlled by physiological or pharmacological means during its preneoplastic stages, whereby the promotion of precancerous cells could be stabilized, arrested, or reversed [6]. Thus, cancer chemoprevention has emerged as an important means of modulating the process of carcinogenesis [7]. Approximately three decades of research suggest that this strategy is promising with respect to reducing the incidence of cancer in well-defined high-risk groups, as well as in the general population [5, 8].

By definition, chemoprevention is the use of chemical agents to slow the progression of, reverse, or inhibit carcinogenesis, thereby lowering the risk of developing invasive, or clinically significant disease [6]. Consequently, an effective chemopreventive agent should intervene early in the process of carcinogenesis to eliminate premalignant cells before they become malignant. Several thousand agents have been reported to have chemopreventive activity, and among them, more than 40 promising agents and agent combinations are being evaluated clinically for cancer chemoprevention. For example, retinoids (*e.g.*, all-*trans* retinoic acid, 9-*cis* retinoic acid, and *N*-(4-hydroxyphenyl)retinamide (4HPR)) have been effective in arresting or reversing premalignant lesions such as oral leukoplakia, uterine cervical dysplasia, and actinic keratoses [5, 7]. In randomized trials of patients with familial adenomatous polyposis, the nonsteroidal anti-inflammatory drugs (NSAIDs) sulindac and celecoxib inhibited the growth of adenomatous polyps and promoted their regression of [9, 10]. Furthermore, chemoprevention trials have shown that the antiestrogen tamoxifen can reduce the incidence of breast cancer [11], and the putative free radical scavenging green tea catechins (*e.g.*, epigallocatechin-3-gallate (EGCG)) can reduce the incidence of prostate cancer [12].

Many chemopreventive agents are believed to block or delay the promotion and/or progression of premalignant cells by modulating cell proliferation and/or differentiation [7, 13]. Since, these agents are supposed to encourage cytostasis in epithelial cells that have already sustained DNA damage, it has been suggested that these agents should be administered chronically to individuals with an increased risk of developing cancer. Of course, in this modality even minor adverse side effects would be unacceptable [13]. Indeed, several recent human cancer chemoprevention trials have reported the development of adverse side effects in the intervention cohorts. For example, chemoprevention trials employing NSAIDs like celecoxib [9] and rofecoxib [14] that purportedly target cyclooxygenase-2, or selective estrogen receptor antagonists like tamoxifen [15], have been rather disappointing because of the adverse cardiovascular events that have developed in subjects following the prolonged use of these agents.

Concerns like long-term toxicity and the possibility of developing chemoresistance are formidable obstacles that could limit the feasibility and success of a chronic application strategy in the chemoprevention for many cancers. An alternate approach involves the use of agents that can eliminate transformed cells in an expeditious manner through the induction of apoptosis, rather than merely slowing their proliferation and/or promoting some degree of differentiation. For example, the eradication of premalignant lesions, or possibly the prevention of secondary primary tumors, could be achieved with chemopreventive agents that have demonstrated the capacity to trigger apoptosis in tumor cells. By shifting the mechanism of chemoprevention from cytostasis or differentiation to apoptosis, chronic exposure to a particular chemopreventive agent would not be necessary, thereby limiting the risk of long-term toxicity and/or the development of chemoresistance. Novel approaches to drug delivery and staging could also facilitate chemopreventive agent-induced apoptosis in target cells and reduce possible short-term adverse side effects [4, 5]. An ever-

growing list of putative cancer chemopreventive agents have been shown to trigger apoptosis in tumor cells *in vivo* and/or *in vitro*, which would suggest that the commitment to cell death in these cells is an important mechanism associated with their expected cancer chemopreventive effects. Indeed, apoptosis induction is increasingly valued as a biologically significant anticancer mechanism in the arena of chemoprevention [4, 5]. Moreover, from a mechanistic perspective, a vast majority of these same agents appear to initiate apoptosis *via* the intrinsic or mitochondria-mediated pathway by encouraging mitochondrial membrane permeabilization (MMP) (reviewed in [4, 5]).

2 The intrinsic pathway of apoptosis

Apoptosis is the mechanism used by metazoans to regulate tissue homeostasis by eliminating redundant or potentially deleterious cells. The cellular machinery associated with apoptosis is highly conserved with many similarities existing between phylogenetically divergent species. Apoptosis is triggered by an initiation phase that is largely dependent on cell type and apoptotic stimuli (*e.g.*, oxidative stress, DNA damage, ion fluctuations, and cytokines). This is followed by an effector phase where distinct biochemical events systematically activate catabolic hydrolases (*i.e.*, proteases and nucleases). These enzymes participate in the degradation phase of apoptosis through the cleavage of proteins and DNA [2].

The recent advances in the elucidation of apoptosis pathways have come about through the characterization of the effector mechanisms. There are several components comprising the effector mechanisms of apoptosis, and two effector mechanisms associated with the activation of caspases (*i.e.*, cysteine proteases involved in apoptosis) and/or cellular degradation have been characterized extensively. These include the death receptor-mediated (*e.g.*, tumor necrosis factor receptor and Fas) effector mechanism and the mitochondrial-mediated effector mechanism [16]. These effector mechanisms are also referred to as the extrinsic and intrinsic pathways of apoptosis, respectively. These pathways can operate independently of each other to trigger the eradication of a cell. However, in certain cell systems, the activation of death receptors appears to require the downstream action of certain proapoptotic Bcl-2 family members (*i.e.*, Bax and Bid) and mitochondrial effectors to execute cell death [17]. Furthermore, growing evidence suggests that the ER, Golgi apparatus, and lysosomes can function as major points of integration for damage sensing in the cell, and these organelles can generate signaling intermediates that stimulate mitochondrial-mediated apoptosis [18].

The mitochondria in eukaryotic cells developed through the symbiotic relationship between an anaerobic archaebacterium (*i.e.*, the host) and an aerobic proteobacterium (*i.e.*, the endosymbiont). This union produced an ancestral eukaryotic cell that, besides being able to respire to generate ATP, was able to survive in an environment that had become enriched with oxygen [4, 19]. Most of the genetic information in the resident endosymbionts or protomitochondria was systematically transferred to the nuclear genome of eukaryotic cells during evolution. This process left the present-day mitochondria in human cells with a relatively small (*i.e.*, 16 569 bp) circular genome that encodes for proteins required in oxidative phosphorylation (OXPHOS) [20].

Like mitochondrial evolution, mitochondrial-mediated apoptosis also represents a phylogenetically ancient process. The endosymbiont hypothesis of apoptosis evolution proposes that certain proteins associated with the endosymbiont would have played a strategic role in the establishment of endosymbiosis. These proteins were compartmentalized by membranes along with the endosymbionts because they were indispensable for endosymbiont function and potentially toxic to the host [21]. Several apoptogenic mitochondrial proteins (*e.g.*, cytochrome *c*, endonuclease G, second mitochondrial activator of caspases (Smac), Omi/HtrA2, apoptosis-inducing factor (AIF), and its homolog AIF-homologous mitochondrion-associated inducer of death) have been identified that appear to meet these criteria, in principle. As eukaryotic cells evolved, aided by the bioenergetic windfall associated with endosymbiosis, they established nuclear control mechanisms (*e.g.*, p53 and Bcl-2 family members) to regulate both the release of apoptogenic mitochondrial proteins and ultimately cell elimination *via* an apoptotic mechanism [21]. Reactive oxygen species (ROS, *i.e.*, superoxide, hydrogen peroxide, and hydroxyl radicals) that are a byproduct of OXPHOS are arguably the archetypal signal transduction mediators in eukaryotic cells, and excessive ROS production is potentially deleterious to mitochondrial and other cellular functions. Therefore, it has been proposed that the mitochondria, through the self-directed induction of the mitochondrial permeability transition (MPT), can also regulate the release apoptogenic mitochondrial proteins and apoptosis [22], since the MPT can be triggered by excessive mitochondrial ROS generation and/or the disruption of mitochondrial redox homeostasis [22, 23]. Given that mitochondrial redox tone is essentially equivalent to cellular redox tone, this perhaps most ancient mechanism of mitochondrial-mediated apoptosis may serve a teleolonomic function in metazoans by deleting aberrant ROS-producing cells [4, 23, 24].

The MPT is a rate-limiting and self-amplifying process that is influenced by several mitochondrial proteins localized in the inner and outer mitochondrial membranes. Many of these proteins (*e.g.*, voltage-dependent anion channel, adenine nucleotide translocase (ANT), hexokinase, peripheral benzodiazepine receptor, and cyclophilin D) are believed to constitute the permeability transition pore com-

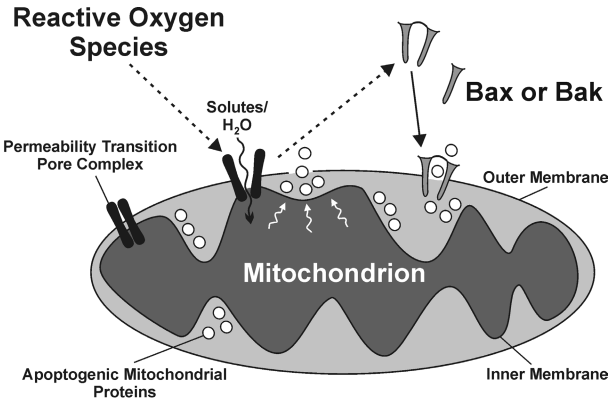

Figure 1. ROS regulation of mitochondria-mediated apoptosis. The MPT is regulated by several mitochondrial proteins believed to constitute the PTPC. Normally, the PTPC is in a closed or low conductance conformation. ROS can cause the proteins in the PTPC to adopt an open conformation, allowing water and solutes to infiltrate the mitochondrial matrix. This can trigger large amplitude swelling of the mitochondrial matrix, and permeabilization of the outer mitochondrial membrane due to physical disruption. Once the outer membrane fragments, apoptotic mitochondrial proteins are released to activate the degradation phase of apoptosis. ROS may also promote conformational changes in proapoptotic Bcl-2 family members (*e.g.*, Bax and Bak). In this scenario, these proteins ultimately form oligomers, which localize to the mitochondria and cause channel formation in the outer mitochondrial membrane. This activity can liberate apoptotic mitochondrial proteins to activate the degradation phase of apoptosis. Please refer to the text for additional details.

plex (PTPC) [25], although the components of this complex remain controversial. Normally, the proteins in the outer and inner mitochondrial membranes that constitute the PTPC are in close proximity to each other and in a closed or low conductance conformation. A host of factors, including pathologic stimuli (*e.g.*, ROS and Ca^{2+}) as well as various chemical agents, can alter the conformation of the PTPC. The PTPC is believed to be particularly vulnerable to ROS since it is regulated by proteins that contain thiols. This is especially true for mitochondrial proteins localized in the matrix, because of this compartment's relatively alkaline pH. Once the PTPC adopts an open conformation, water and solutes up to 1500 Da can infiltrate the mitochondrial matrix, which can cause colloidal osmotic swelling of the mitochondrion. If multiple PTPCs open concurrently and mitochondrial swelling is extensive, apoptogenic mitochondrial proteins are released to the cytoplasm *via* the physical rupture of the outer mitochondrial membrane (Fig. 1, reviewed in [4]). The MPT is a rate-limiting and self-amplifying process because the release of mitochondrial constituents (*e.g.*, Ca^{2+}) to the cytosol and the anomalous ROS production associated with mitochondrial disruption can trigger MPT in vicinal mitochondria [26]. Once released, the apoptogenic mitochondrial proteins can participate in the activation of apoptotic cellular degradation.

Bioenergetic catastrophe is one feature of the MPT that reinforces the ultimate demise of the dying cell. Besides the loss of apoptogenic mitochondrial proteins, many of which participate directly in the maintenance of mitochondrial homeostasis, the MPT typically results in the dissipation of the electrochemical gradient across the inner mitochondrial membrane known as the mitochondrial inner transmembrane potential ($\Delta\Psi_m$) and enhances ROS production *via* the disintegration of electron transport [27]. Together, these events progressively shut down mitochondrial OXPHOS. This may explain why inhibiting the degradation phase of apoptosis does not necessarily protect a cell form dying, possibly *via* a nonapoptotic mechanism, following the induction of the MPT [28].

In certain cell systems, the release of apoptogenic mitochondrial proteins like cytochrome *c* appears to act in an all-or-nothing fashion with respect to caspase activation and apoptosis induction [2]. Furthermore, in addition to MMP, a distinct reorganization of the mitochondrial cristae [29] and/or the oxidation of cardiolipin [30] may also be required to liberate sufficient cytochrome *c* to trigger protease activation and induce apoptosis. The regulation of MMP by the MPT or proapoptotic Bcl-2 family members (*i.e.*, Bax and Bak) constitutes two distinct processes that potentially have a common origin. We have previously discussed the role of ROS generation in the induction of the MPT. Similarly, the modulation of MMP by proapoptotic Bcl-2 family members has been linked to enhanced ROS generation and oxidative stress in several cell systems [31, 32]. Hence, during apoptosis induction by certain mechanisms, ROS generation may be rate limiting in the regulation of MMP by both the MPT and proapoptotic Bcl-2 family members. Furthermore, we would contend that the two principal players in MMP could cooperate in the induction of apoptosis during conditions of oxidative stress [33], since MMP and ROS can potentially participate in a feed-forward amplification loop where an increase in ROS causes MPT in a subset of mitochondria, which leads to additional ROS production and further modulation of MMP by redox-related conformational changes in Bax or Bak (Fig. 1) [34, 35].

3 Exploiting differences in mitochondrial bioenergetics and/or redox status in transformed cells as a method of cancer chemoprevention

While cancer is currently viewed as a disease that results from the aberrant expression of oncogenes that deregulate cellular proliferation, differentiation, and death, the relationship between these genes and the deregulation of energy production in transformed cells is only partially understood. In fact, most age-related diseases have a genetic component associated with their pathogenesis, which would suggest

that the genetic changes commonly associated with cancer (*e.g.*, the loss of p53 function, the deregulation of the phosphatidylinositol 3-kinase/Akt pathway, or the overexpression of H-ras) do not constitute an entirely accurate criteria for characterizing this disease. Given that cancer is a microenvironmental evolutionary process distinguished by the aberrant proliferation of transformed cells in defiance of normal homeostatic restraints [1], it is increasingly evident that the elucidation of the metabolic phenotype of tumor cells is equally as important as understanding the genetic changes these cells encounter during tumorigenesis given that in many instances these transformations are tightly coupled, and are probably required to maintain tumorigenicity [36].

Early in the 20th century, Warburg pioneered research on metabolic alterations in cancerous cells, and proposed a mechanism to explain how these cells evolved during carcinogenesis. In a series of controversial articles, Warburg hypothesized that a crucial event in malignancy involved the development of dysfunctional mitochondrial OXPHOS that resulted in a compensatory increase in glycolytic ATP production in tumor cells. Ultimately, malignant cells would satisfy their energy needs by producing most of their ATP *via* glycolysis [37]. Warburg further noted that the enhanced glucose consumption by tumor cells was accompanied by an increase in lactate production, which persisted even in the presence of oxygen (*i.e.*, the Warburg effect) suggesting that tumor cells acquire an imbalance or partial loss of mitochondrial electron transport and/or ATP synthesis during carcinogenesis [37, 38].

Considering that glycolysis is an extremely inefficient means of generating ATP compared to OXPHOS, this represented a unique metabolic phenotype in malignant cells that required them to maintain a high uptake and consumption of glucose to meet their ever-variable cellular energy expenditures [39]. In cells constituting benign tumors or premalignant lesions the rate of glycolysis is only a third of that exhibited by malignant tumors, and normal cells are believed to generate a 100-fold less ATP from glycolysis relative to OXPHOS [37, 40]. Normal cells typically have a fixed energy budget that is satisfied *via* the integration of glycolysis and OXPHOS [39].

Alterations in the metabolic phenotype of tumor cells are potentially associated with increased energy demand, ROS production, mutations and/or deletions in mitochondrial DNA (mtDNA), and microenvironmental changes (*e.g.*, hypoxia) *in vivo* [41, 42]. A prospective model depicting the changes in the metabolic phenotype of tumor cells during carcinogenesis is illustrated in Fig. 2. The model incorporates Warburg's characterization of the metabolic phenotype of malignant tumor cells with four assumptions. The first assumption holds that the abnormal proliferation associated with transformed cells requires more ATP than their normal counterparts to establish and maintain tumorigenicity. This notion is supported by numerous animal studies

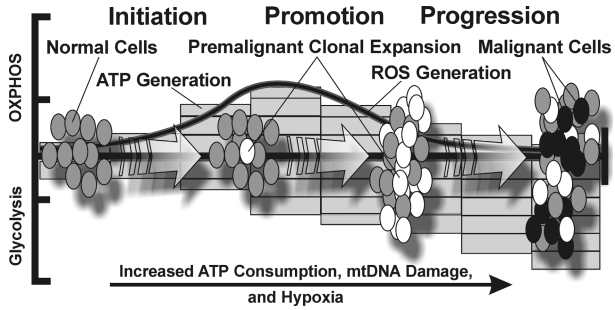

Figure 2. A hypothetical model depicting changes in the metabolic phenotype of transformed epithelial cells during carcinogenesis. Alterations in the metabolic phenotype of these transformed cells are potentially associated with increased energy demand, ROS production, mutations and/or deletions in mtDNA, and microenvironmental changes (*e.g.*, hypoxia) *in vivo*. The model integrates several assumptions. The first assumption holds that the abnormal proliferation associated with transformed cells requires more ATP than their normal counterparts to establish and maintain tumorigenicity. The second assumption holds that ROS production increases in cells during the initiation and promotion phases of tumorigenesis, while normal quiescent and/or differentiated epithelial cells are believed to have very low levels of ROS production and oxidative stress. The third assumption of the model allows for a decline in OXPHOS during the late promotion and progression stages of tumorigenesis due to alterations in mtDNA. For the fourth assumption, *in vivo* microenvironmental factors, specifically hypoxia, should promote the development of a glycolytic phenotype of tumor cells. Typically, under hypoxic conditions, OXPHOS would shut down since oxygen is a rate-limiting factor for respiration. Thus, hypoxia should enhance anaerobic glycolysis (*i.e.*, the Pasteur effect) in tumor cells to sustain ATP production and viability. In solid tumors, hypoxia during the late promotion and progression stages of tumorigenesis would also be expected to diminish ROS generation in the tumor cells. Please refer to the text for additional details. OXPHOS, ROS, and mtDNA, mtDNA.

that suggest caloric restriction can inhibit carcinogenesis as well as enhance mitochondrial-mediated apoptosis in tumor cells [4]. *In vitro* studies have revealed that proliferating thymocytes utilize three to five times more ATP than resting thymocytes [39, 43], and cell cycle progression can be blocked by repressing mitochondrial ATP production [44–46]. Furthermore, a recent study has shown that primary human fibroblasts serially transduced with oncogenes (*i.e.*, telomerase, simian virus large T antigen, simian virus small T antigen, and an oncogenic allele of H-ras) representing a progression of tumorigenic potential exhibited a progressive increase in ATP generation and oxygen consumption [36].

The increased ATP production, as well as an increase in the relative contribution to ATP production from glycolysis in cells with the highest tumorigenic potential, was attributed to the ability of the serially transduced cells to switch more readily to glycolytic (*i.e.*, anaerobic) ATP synthesis. Ramanathan *et al*. [36] also noted it was intriguing that the

cells with greater tumorigenic potential consumed more oxygen and yet exhibit diminished oxygen dependent (*i.e.*, aerobic) ATP synthesis. They speculated that such cells used the mitochondrial electron transport chain (ETC) for reasons other than the production of ATP. For example, for producing heat or ROS caused by leakage of protons across the inner mitochondrial membrane. They also suggested it was possible that the cells with greater tumorigenic potential used mitochondrial electron transport for pyrimidine synthesis rather than for ATP synthesis. This could facilitate DNA synthesis since the oxidation of dihydroorotate to orotate, which is required for *de novo* pyrimidine synthesis, is mediated by the ETC-dependent function of dihydroorotate dehydrogenase [47]. This would imply a possible coupling of nucleotide biosynthesis with the mitochondrial machinery to achieve the high rates of cell proliferation in the highly transformed state [36].

The second assumption holds that ROS production increases in cells during the initiation and promotion phases of tumorigenesis [48]. Normal quiescent and/or differentiated epithelial cells are believed to have very low levels of ROS production and oxidative stress [49]. However, moderate ROS production can trigger cell proliferation [48, 49], as well as the induction of an adaptive response like the induction of phase II ROS scavenging enzymes like those regulated by the nuclear factor erythroid 2-related factor 2 (*e.g.*, glutathione *S*-transferase and heme oxygenase-1), which may protect normal cells from ROS-induced damage [8]. Furthermore, a moderate increase in mitochondrial ROS production can promote an increase in OXPHOS that can act as a feed-forward mechanism for further increasing ROS production and mtDNA damage [50]. Several decades of research have pointed to excessive ROS generation in transformed cells, which are believed to be integral to the increased rate of proliferation, DNA damage, and oncogene activation in these cells [40, 51]. Moreover, many tumor cell types also constitutively overexpress antioxidant enzymes like those mentioned above [52, 53], suggesting that these cells are obligated to cope with higher ROS levels and/or oxidative stress relative to their normal counterparts [48].

The third assumption of the model allows for a decline in OXPHOS during the late promotion and progression stages of tumorigenesis due to alterations in mtDNA. Mutations and/or deletions in mtDNA have been identified in tumor cells originating from the bladder, breast, colon, head and neck, kidney, liver, skin, stomach, as well as leukemia and lymphoma [54, 55]. The mitochondrial genome encodes exclusively for 13 subunits of a small number of proteins in the inner mitochondrial membrane that participate in OXPHOS. Unlike nuclear DNA, mtDNA is far more susceptible to damage due to its relatively small size (*i.e.*, 16 569 bp), lack of protective histones, proximity to processes that generate ROS, and limited mtDNA repair mechanisms. Furthermore, mutations and/or deletions in mtDNA are more likely to occur in coding regions, since, unlike nuclear DNA, the mitochondrial genome lacks sizeable introns [4]. Given that cells typically contain many mitochondria with several copies of mtDNA *per* organelle, it is possible for wild type and mutant mtDNA to co-exist in a state called heteroplasmy. However, there is evidence suggesting that the development of heteroplasmy may well enhance mitochondrial ROS generation, promote further mtDNA damage, and accelerate the loss of OXPHOS [56, 57].

The fourth assumption considers the contribution of *in vivo* microenvironmental factors, specifically hypoxia, to the development of the glycolytic phenotype of tumor cells. Hypoxia is defined as a reduction in the normal oxygen tension of a tissue. This can be achieved by the uncontrolled proliferation of solid tumor cells, effectively causing them to outgrow their blood, and consequently oxygen, supply [58]. In fact, most of the cells comprising a tumor with a volume as small as 2 cm^3 probably subsist in a hypoxic environment [59]. Under hypoxic conditions, OXPHOS would shut down since oxygen is rate limiting for respiration [60]. Thus, hypoxia should enhance anaerobic glycolysis (*i.e.*, the Pasteur effect) in tumor cells to sustain ATP production and viability [41]. It is also important to note that during hypoxia the mitochondria would transform from ATP producers to robust ATP consumers [61]. Once OXPHOS is impeded or inactivated, the mitochondrial F1-ATPase would hydrolyze ATP to preserve proton motive force and $\Delta\Psi_m$ [61, 62]. In tumor cells this process is believed to be assisted through the enhanced expression of the type 2 isoform of the ANT (*i.e.*, the ANT2), which operates a reverse ATP/ADP exchange to import cytosolic ATP into the mitochondrial matrix [46, 63]. This reallocation of energy in cancerous cells for aberrant, but essential, ATP-requiring processes provides another possible example to support the first supposition that rapidly proliferating tumor cells require more ATP than their normal counterparts to establish and maintain tumorigenicity. The establishment of hypoxia in a tumor mass leads to a concomitant reduction in ROS production and oxidative stress in the cells constituting the tumor due to a loss or restriction of the normal oxygen tension in the tissue.

The decline of OXPHOS during carcinogenesis is often associated with a reduction in the abundance and/or mass of the mitochondria in tumor cells [64, 65]. Consequently, a reduction of apoptogenic mitochondrial proteins would also be expected to occur. Solid tumors typically respond to chemotherapy and radiation therapy by triggering mitochondrial-mediated apoptosis [66], and under certain circumstances death receptor-mediated apoptosis can be executed and/or amplified *via* the activity of mitochondrial effectors [67]. Thus, assuming that the participation of the mitochondria and apoptogenic mitochondrial proteins are to some extent required for apoptosis, the disintegration of the metabolic phenotype of tumor cells during the late promotion and progression phases of carcinogenesis inherently deacti-

vates mitochondrial-mediated apoptosis-inducing mechanisms. This is accomplished through the progressive decline in the number, constituents, and mass-effect of the mitochondria in tumor cells [64]. In essence, carcinogenesis allows tumor cells to divorce themselves from the symbiotic relationship that evolution has established with their mitochondria [4].

The notion that OXPHOS and mitochondrial-mediated apoptosis are intimately coupled is compelling [4]. The requisite loss of OXPHOS in tumor cells during late-stage carcinogenesis [38, 64] is equally compelling. Thus, we would contend that targeting mitochondrial OXPHOS and/or redox state to induce apoptosis in a cancer chemoprevention setting should prove to be a successful strategy to eradicate premalignant cells before they become malignant. In support of this contention, mechanistic studies have revealed that the proapoptotic effects of several putative chemopreventive agents (*e.g.*, 6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid (CD437) [68], 4HPR [69], capsaicin [70], resiniferatoxin [70], deguelin [71], rotenone [72], curcumin [73, 74], and α-tocopheryl succinate [75, 76]) are conspicuously diminished in tumor cells that have been depleted of mtDNA (*i.e.*, ρ^0 cells), which causes them to become functionally deficient in OXPHOS.

The inability of the ρ^0 mitochondria to conduct OXPHOS would appear to correspond to the metabolic alterations observed in various malignant tumor cells. Compared to their parental counterparts, ρ^0 tumor cells also produce less ROS signifying a reduction in oxidative stress in these cells [73, 77]. This would suggest that certain aspects of mitochondrial function are realistic targets for cancer chemoprevention [4]. Moreover, if increased OXPHOS and ROS are indeed coupled with the early promotion phase of tumorigenesis (Fig. 2) this would implicate a role for cellular redox tone in determining sensitivity to cytostasis or cell death triggered by chemopreventive agents that can enhance oxidative stress in transformed cells (Fig. 3). Fundamentally, it is hypothesized that transformed cells in the promotion stage of tumorigenesis are inherently obligated to vie with enhanced ROS production and/or oxidative stress, which should make these cells more likely to succumb to the effects of a chemopreventive agent that further escalates cellular ROS production [4, 48, 53, 78]. However, the window of opportunity for acquiring targets like OXPHOS and mitochondrial redox state probably closes as tumor cells progress to a more malignant phenotype.

4 Chemopreventive agents that potentially employ antimitochondrial mechanisms for preventing malignancy

In this section, we will discuss some of the possible mechanisms through which certain chemopreventive agents act to disrupt the mitochondrial function/redox status of transformed cells to trigger apoptosis.

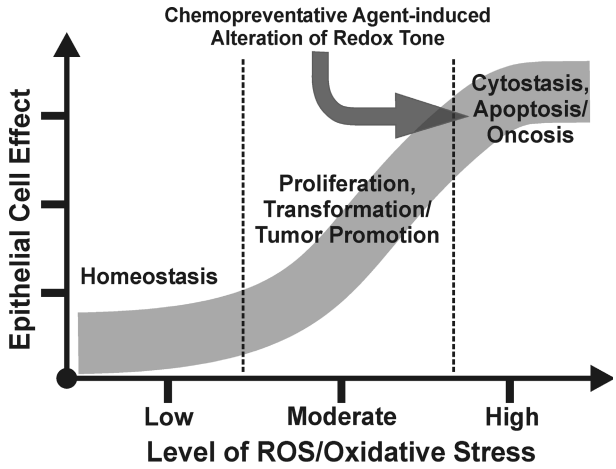

Figure 3. Exploiting the intrinsic oxidative stress of transformed cells in cancer chemoprevention. We speculate that many cancer chemopreventive agents exert their carcinostatic effects on transformed cells *via* the production of ROS, which ultimately disrupts the redox tone of these cells leading to cytostasis and/or cell death. Given that the promotion phase of tumorigenesis is associated with aberrant ROS production in transformed epithelial cells, it is reasonable to assume these cells would be obligated to vie with increased oxidative stress, and consequently should be more dependent on, relative to their normal counterparts, antioxidant enzymes, and other antioxidant defense mechanisms for their survival. These events should make transformed cells more likely to succumb to the cytotoxic effects of a chemopreventive agent that further escalates cellular ROS production [4, 48, 53, 78], perhaps through the obstruction of mitochondrial functions like OXPHOS. However, the window of opportunity for acquiring targets like OXPHOS and mitochondrial redox state probably closes as tumor cells progress to a more malignant phenotype.

4.1 The synthetic retinoids 4HPR and CD437

The 4HPR (also know as fenretinide, Fig. 4) is a synthetic analog of all-*trans* retinoic acid that was first produced by R. W. Johnson Pharmaceuticals in the late 1960s. The substitution of an amide-linked 4-hydroxyphenyl group for the carboxyl group of all-*trans* retinoic acid markedly reduced adverse side effects like liver toxicity. Furthermore, 4HPR lacks the ability to induce point mutations or chromosomal aberrations, and is therefore not genotoxic. These qualities suggested that 4HPR could be compatible for long-term use in a chemopreventive modality. Undeniably, in animal models, 4HPR has demonstrated chemopreventive efficacy against carcinogenesis of the breast, prostate, pancreas, and skin. Moreover, in a clinical setting, 4HPR slowed the progression of prostate cancer in men diagnosed with an early stage of the disease, protected against the development of ovarian cancer and a second breast malignancy in premeno-

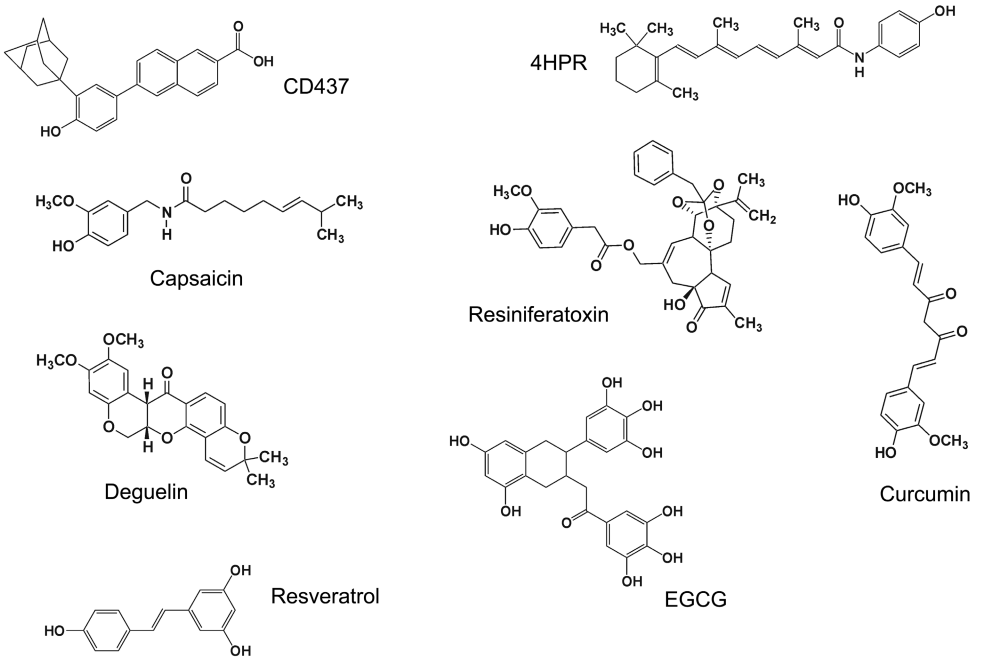

Figure 4. Chemical structures of the chemopreventive agents discussed in this review.

pausal women who had been treated to prevent the formation of a new tumor in the contralateral breast, and prevented relapse and the formation of secondary primary lesions in patients following the surgical removal of oral leukoplakia [33].

In some cell systems, during normal development and under pathological conditions, natural and synthetic retinoids reportedly induce apoptosis in a retinoid receptor-dependent manner. However, in most instances these same compounds fail to do so in the vast majority of the premalignant and malignant cells examined *in vitro* [79]. Numerous *in vitro* studies suggest that 4HPR's prospective anticancer mechanism is associated with the retinoid receptor-independent induction of apoptosis in transformed cells. In these cells, the process of apoptosis appears to require mitochondrial disruption resulting in MMP (reviewed in [33]). Perhaps the most common property of 4HPR-induced apoptosis is its inhibition by antioxidants (*e.g.*, vitamin C, vitamin E, *N*-acetylcysteine, butylated hydroxyanisole, and pyrrolidine dithiocarbamate), suggesting an essential role for ROS and oxidative stress in 4HPR's cytotoxicity. Furthermore, cells exposed to 4HPR reportedly undergo cardiolipin oxidation [80, 81], implying a mitochondrial susceptibility to cytochrome *c* release. The rapid production of ROS, particularly hydroperoxides, is commonly observed in a variety of tumor cells following short-term exposures to 4HPR *in vitro*. Indeed, within 15 min after exposure to 4HPR, the oxidation of 2′,7′-dichlorofluorescin to 2′,7′-dichlorofluorescein (DCF) increased in a linear fashion in these cells [33]. Once cellular esterases hydrolyze 2′,7′-dichlorofluorescin diacetate, 2′,7′-dichlorofluorescin can serve as a cytoplasmic peroxidase substrate, and its oxidization *via* the dismutation of hydroperoxides can serve as a surrogate indicator of oxidative stress in intact cell [82].

Several lines of evidence suggest that 4HPR-induced ROS generation arises from the mitochondria in cancer cells. There has been speculation that 4HPR can be converted to a radical species by the mitochondria [83], and classical inhibitors of mitochondrial electron transport (*e.g.*, rotenone, myxothiazol, and cyanide) diminish the hydroperoxide generation promoted by 4HPR in skin [69] and cervical [84] cancer cells. As mentioned earlier, ρ^0 cells are markedly resistant to 4HPR-induced apoptosis, as well as MPT induction and ROS generation, compared to their parental counterparts [69], and the apoptogenic effects of 4HPR are also markedly diminished in cells cultured under hypoxic conditions [85, 86]. Together, these observations signify that O_2 and/or OXPHOS are required for 4HPR's cytotoxicity [33].

Following a perturbational assessment of 4HPR-induced ROS production in skin cancer cells using various mitochondrial poisons and coenzyme Q analogs, we hypothesized that 4HPR was involved in an enzymatic, NADH-supported, redox metabolism *via* its interaction with Complex I and/or complex III of the mitochondrial ETC [69]. In this scenario, redox cycling by 4HPR would ultimately increase hydrogen peroxide production in the cytoplasm/mitochondrial intermembrane space. This hydrogen peroxide could diffuse into the mitochondrial matrix where it would presumably enhance mitochondrial oxidative stress. The

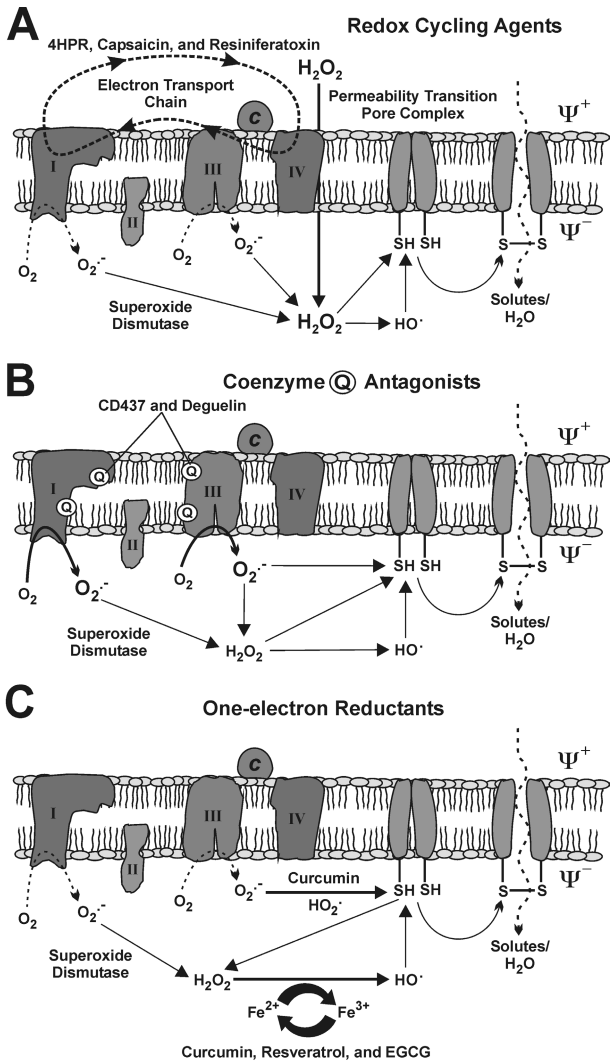

Figure 5. Potential mechanisms associated with the prooxidant activity of certain cancer chemopreventive agents. Please refer to the text for details. *c*, cytochrome *c*; Q, coenzyme Q; H_2O_2, hydrogen peroxide; $HO_2^{\bullet}$, hydroperoxyl radical; $HO^{\bullet}$, hydroxyl radical; and $O_2^{\bullet-}$, superoxide.

enhancement of hydroperoxide production by 4HPR could promote the MPT directly *via* the oxidation of thiols that regulate the proteins constituting the PTPC. This process could also conceivably occur indirectly by mediators like the highly reactive hydroxyl radical, perhaps generated by a Haber–Weiss reaction involving 4HPR-induced hydrogen peroxide and the OXPHOS byproduct superoxide (Fig. 5A). Given the sensitivity issues associated with ROS detection by fluorescent dyes like DCF in intact cells (*e.g.*, the potential crossreactivity with various ROS and ferrous (Fe^{2+}) iron, or reactive nitrogen species) [87], it is currently highly unlikely that the ROS directly involved with 4HPR's cytotoxic/apoptogenic effects can be pinpointed. It is also important to note that 4HPR reportedly caused ROS-dependent, antioxidant-inhibitable, conformational changes in both Bax and Bak, which lead to the exposure of their N-termini, and to the mitochondrial relocalization of Bax. This mechanism of MMP may act to augment direct ROS-induced mitochondrial disruption by 4HPR [35].

Our group has screened many retinoids in a search for more potent anticancer compounds with potentially fewer side effects than natural retinoic acids. In addition to 4HPR, the synthetic retinoid CD437 (also know as AHPN, Fig. 4) appears to be another top candidate in this regard [79]. Others have also found CD437, and related adamantyl-substituted retinoids, to be very active in biological systems relative to natural retinoic acids, and many other synthetic retinoids. Like 4HPR, the putative anticancer activity of CD437 appears to involve the retinoid receptor-independent induction of apoptosis in transformed cells [88, 89]. In addition, we have also observed selectivity in CD437-induced apoptosis in malignant epithelial cells relative to their normal counterparts [88, 90].

CD437 exposure promotes the rapid oxidation of dihydroethidium to ethidium, presumably *via* enhanced superoxide generation, in cultured myeloma [89] and skin cancer cells [68]. In these cells, the enhanced superoxide generation was believed to be predominately associated with the induction of the MPT. Interestingly, vitamin C inhibited CD437-induced apoptosis in skin cancer cells [68], and α-tocopherol acetate provided a similar protective effect in lymphocytic leukemia cells exposed to CD437 [91]. These findings suggested that ROS generation was intimately associated with triggering CD437-induced cell death in certain cell types. Moreover, vitamin C acid also diminished the CD437-mediated inhibition of oxygen consumption by skin cancer cells implying that the mitochondria were both the source as well as the target of oxidative stress in these cells [68].

Contrary to 4HPR, short-term exposures (*i.e.*, 1 to 2 h) of intact cells to CD437 does not promote a robust oxidation of 2′,7′-dichlorofluorescin in human skin cancer cells (unpublished results). This phenomenon has been observed with mitochondrial poisons (*i.e.*, rotenone, deguelin, antimycin A, 2-heptyl-4-hydroxyquinoline-*N*-oxide, myxothiazol, and stigmatellin) that presumably directly inhibit the ETC to produce ROS, largely those that can be detected by the oxidation of dihydroethidium [69, 71]. The ROS superoxide and hydroxyl radicals predominantly mediate the oxidation of dihydroethidium to ethidium [87], which can serve as an indicator of oxidative stress in intact cells [71, 87]. This reaction occurs with faster kinetics when hydroxyl radicals oxidize dihydroethidium, given the superior reactivity of hydroxyl radicals compared to superoxide [87]. Since, all of the aforementioned mitochondrial poisons function as antagonists of coenzyme Q in the ETC, it is possible that CD437 could also function as a coenzyme Q antagonist to enhance mitochondrial ROS production (Fig. 5B) [68]. This could amplify mitochondrial superoxide pro-

duction from the reactions of molecular O_2 with the reduced redox constituents of the ETC. This enhanced superoxide production could directly promote the MPT *via* PTPC protein thiol oxidation, or it could also do so indirectly through the production of hydrogen peroxide and/or hydroxyl radicals as described previously for 4HPR.

An elegant study by Marchetti *et al*. [89] was the first to illustrate that CD437-induced apoptosis required the rapid loss of $\Delta\Psi_m$ and MPT induction in myeloma cells. This study also illustrated that short-term exposure to CD437 could promote mitochondrial swelling, indicative of MPT, in isolated mouse liver mitochondria respiring on succinate. We have also observed mitochondrial swelling *in situ* in cultured skin cancer cells following a short-term exposure to CD437 [92]. In addition to antioxidants, CD437-induced MPT could also be modulated by various inhibitors of the PTPC (*i.e*., cyclosporin A, and trifluoperazine) [68, 89]. These later observations, and the results showing proteoliposomes containing purified mitochondrial ANT were permeabilized in the presence of CD437, suggested that this synthetic retinoid may induce the MPT *via* an interaction with the mitochondrial ANT [93]. If the obstruction of mitochondrial ANT were indeed a common trigger for CD437-induced MPT and apoptosis, we would hold that ρ^0 cells should be equally or more sensitive to these effects compared to their parental counterparts given the prospective role of the ANT in preserving the proton motive force and $\Delta\Psi_m$ in ρ^0 mitochondria [62]. The opposite was in fact the case [68], which could point to a possible cell type-specific effect of CD437 with respect to the ANT, MPT induction, and apoptosis.

4.2 The vanilloids capsaicin and resiniferatoxin

Chemical compounds that contain the vanillyl moiety (4-hydroxy-3-methoxybenzyl, *e.g*., capsaicin and resiniferatoxin, Fig. 4) are collectively classified as vanilloids. Vanilloid phytochemicals can be found in a variety of sources, some of which are routinely consumed by humans throughout the world. The dietary and/or medicinal use of vanilloids may be effective in inhibiting or reversing carcinogenesis, which has sparked a considerable interest in these compounds as potential cancer chemopreventive or therapeutic agents.

Recently, several vanilloids have demonstrated the ability to induce apoptosis in various cancer cell types. Vanilloids can interact with proteins and membranes to initiate pleiotropic effects, some of which are potentially cytotoxic. Certain vanilloids appear to interfere with enzymatic processes in the plasma membrane and/or the mitochondria by functioning as coenzyme Q antagonist (*e.g*., similar to CD437 and deguelin in Fig. 5B). For example, capsaicin reportedly blocks electron transfer at complex I of the mitochondrial ETC presumably by acting as a coenzyme Q antagonist (reviewed in [94]). This may account for the rapid depression of mitochondrial O_2 consumption by capsaicin and/or resiniferatoxin in cultured skin [70], lung [95], and pancreatic [96] carcinoma cells.

A structure-activity relationship exists among complex I inhibitors (*e.g*., capsaicin, rotenone, and stigmatellin) because these agents act at or close to the coenzyme Q binding site of the enzyme [97]. As mentioned previously, the interruption of electron flow at complex I, or complex III, can cause nonenzymatic ROS production due to the one-electron reduction of O_2 upstream of the inhibition site (Fig. 5B). Capsaicin, resiniferatoxin, and several synthetic vanilloids reportedly caused cyanide-sensitive ROS production in rat leukemia cells, suggesting that these agents were interacting at the mitochondrial ETC [98]. Well before the apparent manifestations of MPT induction (*i.e*., a marked dissipation of $\Delta\Psi_m$ and superoxide generation confirmed by the oxidation of dihydroethidium), short-term exposures (*i.e*., 30 min) to capsaicin or resiniferatoxin caused a three-fold increase in hydroperoxide production, confirmed by the oxidation of 2′,7′-dichlorofluorescin to DCF, in the cells from two human skin cancer cell lines [70]. The ρ^0 clones of these human skin cancer cells displayed only a slight, yet discernable and reproducible, increase in hydroperoxide generation following a similar exposure to capsaicin or resiniferatoxin. These results indicated that most of the initial hydroperoxide produced in the parental cells exposed to capsaicin or resiniferatoxin was of mitochondrial origin, and potentially associated with the inhibition of mitochondrial OXPHOS. Furthermore, the ρ^0 clones also exhibited clear resistance to capsaicin- and resiniferatoxin-induced MPT and apoptosis suggesting that oxidative stress and/or perturbations in mitochondrial OXPHOS were intimately associated with the vanilloid-induced apoptosis in the parental skin cancer cells [70].

We have reported previously that center *i* inhibitors of complex III of the mitochondrial ETC can conspicuously increase hydroperoxide generation by 4HPR in skin cancer cells, indicating that the activity of dehydrogenases in complex I may contribute to redox cycling by 4HPR [69]. Interestingly, in a recent study we also observed that both capsaicin and resiniferatoxin responded to co-treatment with the center *i* inhibitor 2-heptyl-4-hydroxyquinoline-*N*-oxide with a marked increase in the DCF fluorescence intensity when compared to the already three- to four-fold increases, relative to the controls, in the DCF fluorescence intensity triggered by these agents alone [73]. As mentioned previously, short-term exposure to the center *i* inhibitor 2-heptyl-4-hydroxyquinoline-*N*-oxide does not enhance DCF fluorescence in these cell, but a similar exposure promoted a five-fold increase in ethidium fluorescence intensity. This would suggest that DCF and ethidium potentially detect ROS generation in different compartments in these cells [73]. Together, these results would imply that, in addition to functioning possibly as coenzyme Q antagonists, capsaicin and resiniferatoxin could perhaps undergo redox cycling at

complex I of the mitochondrial ETC, much in the same way as hypothesized for 4HPR (Fig. 5A).

As shown in Fig. 4, 4HPR, capsaicin, and resiniferatoxin contain a hydroxyl derivative of an aromatic functional group in their chemical makeup. Phenolic compounds, by virtue of their ability to participate in electron transfer reactions [99], are thought to function as antioxidants in the intracellular milieu [3]. It is also possible they could become prooxidants in an oxidizing intracellular environment [99–102], which is characteristic of transformed cells [103, 104]. Furthermore, sustained redox cycling of phenolic compounds at the ETC could prolong the chemical half-life of reactive intermediates of coenzyme Q (*i.e.*, ubisemiquinone), thus allowing them to participate in ROS generation *via* the one-electron reduction of oxygen [4].

Further studies are warranted to determine whether the enhancement of ROS generation and/or the inhibition of mitochondrial OXPHOS are uniquely associated with the apoptogenic effects of capsaicin and resiniferatoxin in other cell types [94]. It would also be worthwhile to compare the activities of complex I and/or OXPHOS in skin cancer cells and in normal human epidermal keratinocytes, as well as the relative sensitivities of these cells to capsaicin- or resiniferatoxin-induced cytotoxicity [105].

4.3 The rotenoid deguelin

Deguelin (Fig. 4), rotenone, and related compounds are derived from *Derris* and *Lonchocarpus* species and collectively classified as rotenoids [71]. These agents constitute the active ingredients in the commercial botanical pesticide cubè resin [106]. The pesticidal action and toxicity of rotenoids result from the inhibition of enzyme-coupled oxidation of NADH, reduction of coenzyme Q, and proton translocation *via* complex I of the ETC [97] (Fig. 5B). Rotenoids may also prove to be useful in the prevention or treatment of cancer. For example, rotenone has been shown to decrease the incidence of chemically induced tongue carcinoma in rats [107], and spontaneous liver tumor formation in mice [108]. Furthermore, deguelin was effective in reducing the incidence of chemically induced skin tumors in mice [109], mammary tumors in rats [109], colonic aberrant crypt foci in mice [110], chemically induced lung tumor formation in mice [111], and preneoplastic lesion formation in mouse mammary gland in organotypic culture [112].

The mechanism through which rotenoids inhibit carcinogenesis has not been fully elucidated. However, it has been suggested that the suppression of ornithine decarboxylase, the first and rate-limiting enzyme in the polyamine biosynthesis pathway, may be involved [112]. The decrease in ornithine decarboxylase activity by deguelin is contingent on the inhibition of mitochondrial bioenergetics, which is achieved *via* its pesticidal action in disrupting enzyme-coupled processes in complex I to inhibit OXPHOS [113]. The modulation of ornithine decarboxylase activity by deguelin and other rotenoids in cultured cells is not a unique phenomenon given that structurally unrelated inhibitors of complex I can promote similar effects albeit with varying degrees of potency [114, 115].

Recent studies have reported that deguelin can trigger apoptosis in tumor cells *in vitro*. In skin cancer cells, deguelin-induced apoptosis appeared to be mediated by the ROS-induced cardiolipin oxidation and MPT induction. Moreover, deguelin rapidly (*i.e.*, within 30 min) and markedly (*i.e.*, by approximately 70%) inhibited the O_2 consumption by these cells. Since, the ρ^0 clones of the aforementioned cells were resistant to the deguelin-induced ROS production, MPT induction, and apoptosis it was assumed the primary mechanism associated with deguelin's apoptogenic effect centered on the inhibition of OXPHOS in the parental cells [71]. Similar observations have also been reported for apoptosis induction by rotenone [72].

In colon cancer cells, deguelin apparently caused the deregulation of the cell cycle checkpoint protein retinoblastoma to promote cell cycle arrest that was followed by apoptosis induction [116]. Deguelin has also been shown to decrease the activity of the phosphatidylinositol 3-kinase/Akt pathway in premalignant and malignant human bronchial epithelial cells, which was believed to trigger apoptosis in these cells [117]. In these cell systems, the deguelin-induced suppression of retinoblastoma [116] and Akt [117] appeared to be caused by the decreased phosphorylation of these signaling intermediates. This would imply that diminished bioenergetic activity was possibly associated with the events leading to apoptosis induction by deguelin in these cells. Unfortunately, most of the recent studies characterizing deguelin-induced apoptosis in transformed cells *in vitro* and/or *in vivo* have not examined any of the biochemical aspects of cell death (*e.g.*, ROS production, the dissipation of $\Delta\Psi_m$, and oxygen consumption) that could conceivably be linked to deguelin's direct effects on OXPHOS.

4.4 The polyphenols curcumin, EGCG, and resveratrol

Turmeric, which is derived from the dried rhizomes of *Curcuma longa*, is comprised predominantly of the polyphenolic constituent curcumin. Curcumin has been employed in traditional Asian Indian medicine for thousands of years to treat a range of diseases, and in contemporary medicine curcumin has emerged as an important tool for the chemoprevention of cancer. Curcumin possesses a variety of pharmacological qualities, including anti-inflammatory, antioxidant, and apoptogenic activities, which are well suited for efficacy in relation to cancer chemoprevention [118].

As it was proposed approximately 30 years ago [119], dietary polyphenolics like curcumin are believed to function systemically as antioxidants, and several recent comprehensive appraisals of curcumin's chemical biology sug-

gest that it is an effective scavenger of ROS [118, 120, 121]. Curcumin exposures in the micro- to millimolar range have been shown to scavenge ROS and reactive nitrogen species *in vitro* and *in vivo* [122]. The expression of phase II metabolic enzymes (*e.g.*, glutathione *S*-transferase and heme oxygenase-1) *via* the activation of the antioxidant response element is also enhanced by curcumin, which may also be associated with curcumin's observed antioxidant effects in normal cells [118, 120, 121]. Furthermore, curcumin was effective in preventing lipid peroxidation and protein oxidation caused by 2,2′-azobis(2-amidinopropane) hydrochloride or Fe^{2+}/ascorbate in isolated, deenergized mitochondria [123].

While contended to have antioxidant/protective properties in normal tissues and cells [118, 120, 121], low micromolar (*i.e.*, ≤50 μM) concentrations of curcumin have been shown to promote oxidative stress in transformed cells in culture [124–134]. These effects are associated with enhanced ROS production, the altering of cellular redox homeostasis (*e.g.*, the depletion of glutathione), and/or the obstruction of mitochondrial function (*e.g.*, dissipation of mitochondrial inner transmembrane potential $\Delta\Psi_m$). Moreover, curcumin has been reported to disrupt redox homeostasis and amplify oxidative stress [135, 136], promote the dissipation of $\Delta\Psi_m$ [136], enhance cytochrome *c* release [135], and/or encourage mitochondrial swelling [135, 136] in isolated, energized mitochondria.

As mentioned previously, curcumin has been shown to scavenge ROS and reactive nitrogen species *in vitro* and *in vivo* [122], and it is reportedly a "superb antioxidant" [137]. In biological systems, a typical antioxidant, whether it is enzymatic or nonenzymatic, should be able to reduce ROS so that they become less reactive than the parent species (*i.e.*, the conversion of superoxide to hydrogen peroxide by superoxide dismutase) [138]. This is typically accomplished by the two-electron reduction of O_2 and ROS (*e.g.*, the conversion of superoxide to hydrogen peroxide by superoxide dismutase, the conversion of hydrogen peroxide to water by peroxidases, or the conversion of O_2 to water by cytochrome *c* oxidase (complex IV)). If curcumin scavenges ROS, it must act as a reducing agent by donating a keto-enol and/or phenolic proton. These reactions are probably highly dependent on the concentration of curcumin, the agent it is reacting with, and the solvent for the reaction [139, 140]. Several reports suggest that curcumin can potentially increase oxidative stress by enhancing the reactivity of ROS like superoxide and hydrogen peroxide. There is evidence to suggest that curcumin may promote the one-electron reduction (*i.e.*, protonation) of superoxide to form the hydroperoxyl radical [139, 141], which is believed to be more reactive than superoxide in the intracellular milieu [142]. Furthermore, curcumin may also promote the reduction of transition metals such as iron (*i.e.*, the reduction of ferric (Fe^{3+}) iron to ferrous (Fe^{2+}) iron). The reduced transition metal can then catalyze the Fenton reaction, which converts hydrogen peroxide to the highly reactive hydroxyl radical [101, 135, 143–145] (Fig. 5C).

The enhancement of oxidative stress by curcumin in tumor cells ultimately results in mitochondrial-mediated apoptosis [124, 125, 127–134], which may be another mechanism associated with the anticancer activity of this polyphenolic compound [4, 5]. Indeed, the cancer chemopreventive properties of curcumin were correlated with enhanced levels of apoptosis induction in tumor cells from azoxymethane induced colon tumors in male Fisher 344 rats. Dietary administration of curcumin promoted a statistically significant increase in apoptotic tumor cells relative to the tumor cells derived from animals fed the control diet. The apoptogenic activity of curcumin was reportedly selective for the colon tumor cells, while vicinal normal enterocytes appeared to be unaffected [146]. Thus, the redox environment of tumor cells may make them more sensitive than their normal counterparts to curcumin-induced apoptosis [73].

In a recent study we conducted [73], low micromolar (*i.e.*, ≤40 μM) concentrations of curcumin were effective in promoting cell cycle arrest and/or apoptosis induction in human skin cancer cells. Apoptosis induction in these cells was associated with the dissipation of mitochondrial inner transmembrane potential and mitochondrial swelling, suggestive of the MPT, which appeared to be preceded by an increase in intracellular ROS production. A short-term (*i.e.*, 2 h) exposure of the skin cancer cells to curcumin promoted three- to four-fold increases in DCF and ethidium fluorescence intensity, respectively. The effects of the center *i* inhibitor of complex III, 2-heptyl-4-hydroxyquinoline-*N*-oxide, were examined on hydroperoxide production by 4HPR, capsaicin, resiniferatoxin, or curcumin. Co-treating the skin cancer cells with 2-heptyl-4-hydroxyquinoline-*N*-oxide caused a marked increase in the DCF fluorescence intensity of 4HPR, capsaicin, and resiniferatoxin when compared to the increase in DCF fluorescence intensity triggered by these agents alone. This was not the case for curcumin-induced hydroperoxide production. Moreover, curcumin did not diminish 4HPR-induced hydroperoxide generation in these cells, suggesting that it did not act as a ROS scavenger, at least in the DCF assay. These experiments suggested that the ROS generation triggered by curcumin in skin cancer cells was apparently derived from a process that was different from that which caused the ROS production following exposure to other phenolic compounds like 4HPR, capsaicin, and resiniferatoxin.

Pharmacologically lowering the constitutive mitochondrial ROS levels in skin cancer cells *via* a 6-d exposure to ng/mL concentrations of ethidium bromide, which is believed to selectively inhibit mtDNA polymerase γ, suppressed the cytotoxic effects of curcumin. Correspondingly, the ρ^0 counterparts of these cells were markedly resistant to ROS production, mitochondrial disruption, and apoptosis following curcumin exposure. These observations implied

that the diminution of mitochondrial ROS production protected cells against the cytotoxic effects of curcumin, and supported the notion that mitochondrial respiration and redox tone were pivotal determinants in apoptosis signaling by curcumin. This study was especially timely since an ever-growing body of evidence strongly suggests that the carcinostatic qualities of compounds like curcumin are probably not associated with systemic antioxidant effects [101, 147, 148].

EGCG (Fig. 4) is the principal polyphenolic component in green tea catechins. Capsules containing green tea catechins (predominately EGCG) caused no biologically significant adverse side effects when relatively high doses are administered orally to humans, and a recent chemoprevention pilot study showed that a short-term (*i.e.*, 12 month) administration of EGCG-containing capsules was safe and very effective in suppressing the progression of prostate premalignant lesions (*i.e.*, high-grade prostate intraepithelial neoplasia to prostate cancer [12]. In the aforementioned double-blind placebo-controlled study, 60 volunteers with high-grade prostate intraepithelial neoplasia were enrolled. Daily treatment consisted of three green tea catechin capsules, 200 mg each (a total of 600 mg/day), which were estimated to be approximately 52% EGCG by weight. These encapsulated doses of EGCG far exceed those that could be obtained by daily consumption of large quantities of green tea [149]. After 1 year, only one tumor was diagnosed among the 30 green tea catechin-treated men (an incidence of approximately 3%), while nine cancers were found among the 30 placebo-treated men (an incidence of approximately 30%). The total prostate-specific antigen did not change markedly between the two arms, but green tea catechins-treated men showed values constantly lower than the men did in the placebo group. The International Prostate Symptom Score and quality of life scores of green tea catechin-treated men with coexistent benign prostate hyperplasia improved, reaching statistical significance in the case of International Prostate Symptom Scores [12].

Several mechanisms have been postulated for the cancer chemopreventive properties of EGCG. These include: (i) protection against oxidative damage to DNA, proteins, and membrane lipids; (ii) prevention and/or detoxification of carcinogenic metabolites; (iii) the stimulation of the immune surveillance against tumor cells; and/or (iv) the induction of cell cycle arrest and/or apoptosis [3]. As mentioned previously, an ever-growing body of evidence strongly suggests that the carcinostatic qualities of polyphenolic compounds are not associated with systemic antioxidant effects [101, 147, 148, 150]. Furthermore, the aforementioned human trial suggested an intervention in the promotion phase of carcinogenesis, which would indicate that EGCG was normalizing prostatic tissue homeostasis *via* the eradication of transformed cells. Thus, the primary mechanism believed to be associated with EGCG's anticancer effects in premalignant and/or malignant cells involves apoptosis induction [151, 152]. This effect appears to be specific for transformed cells, while sparing their normal counterparts [153]. EGCG has also been shown to alter cellular redox homeostasis and/or disrupt mitochondrial function in tumor cells, which may be fundamental to its apoptogenic and anticancer activity [150, 154–162].

Like curcumin, polyphenolic compounds such as EGCG can participate in electron transfer reactions that ultimately cause them to function as prooxidants. For example, EGCG may reduce ferric (Fe^{3+}) iron to ferrous (Fe^{2+}) iron, which can catalyze Fenton reactions and the generation of the highly reactive hydroxyl radical from hydrogen peroxide [100, 153, 154, 163] (Fig. 5C). These reactions could disrupt mitochondria to induce the MPT. Indeed, EGCG has been show to promote ROS production, induce mitochondrial inner membrane depolarization suggestive of the MPT, and trigger caspase-dependent apoptosis in various tumor cell types [153–157, 160–162, 164].

Resveratrol (Fig. 4) is a polyphenol widely present in foods such as grapes, wine, and peanuts. Resveratrol has emerged as a potential antitumor agent because of its ability to inhibit various stages of carcinogenesis and eliminate preneoplastic cells *in vitro* and *in vivo* [165, 166]. While the protective properties of resveratrol in normal cells are potentially associated with its antioxidant activity and the ability to preserve mitochondrial function [167], the carcinostatic mechanism of resveratrol in transformed cells appears to involve apoptosis induction [168–178]. Recently, resveratrol has been shown to inhibit important mitochondrial enzymes including complex I [114] and the F0F1-ATPase [179, 180]. Moreover, in several transformed cell types, resveratrol can disrupt mitochondrial redox homeostasis [177] and induce mitochondrial-mediated apoptosis [100, 169–172, 174, 178] that appears to be sensitive to regulation by Bcl-2 family members [169, 171, 181]. It is anticipated that resveratrol could function as a prooxidant in a way similar to that described above for curcumin or EGCG (Fig. 5C). This activity may also produce reactive intermediates like the phenoxyl radical, which can promote indiscriminant cellular damage through the oxidization of proteins, lipids, and DNA [100, 102].

The disruption of mitochondrial bioenergetics and/or redox tone in transformed cells appears to be a common theme associated with the sensitivity to apoptosis induction by the chemopreventive compounds described in this review. Among all of these agents, EGCG and resveratrol appear to be the least toxic with respect to ROS production and apoptosis induction in cultured skin and/or prostate carcinoma cells. These agents typically required micromolar concentrations 10 to 20 times higher than 4HPR, CD437, capsaicin, resveratrol, deguelin, or curcumin to achieve similar cytotoxic effects (unpublished results). The studies of others cited here performed on various tumor cell types appear to confirm these observations. These *in vitro* concentration differences may ultimately reflect the potential

efficacy of these agents *in vivo* with respect to their anticipated carcinostatic effects.

5 Conclusions

Cancer is a complex disease that is manifested through the survival advantage inherent to tumor cells. Apoptosis can be subverted during tumorigenesis presumably through the systematic decay of regulatory control mechanisms. This ultimately results in the generation of a malignant phenotype and resistance to chemotherapy and radiation therapy. Therefore, if apoptosis can be considered a goal of cancer chemoprevention or therapy, it is imperative to advance the characterization of apoptosis mechanisms. As the understanding of these processes increases, so to will come the opportunity to employ agents that can selectively manipulate them. In this regard, we have provided a reasonable argument for the use of chemopreventive agents that potentially target mitochondrial bioenergetics/redox homeostasis in transformed cells to initiate mitochondrial-mediated apoptosis. This strategy may be an effective means of abating or controlling cancer. This idea is in its infancy, and its substantiation will require additional research efforts. Nevertheless, the data from recent *in vitro* studies summarized in this work would appear to support this line of reasoning.

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