Apoptosis as a Novel Target for Cancer Chemoprevention

Shi-Yong Sun, Numsen Hail, Jr., Reuben Lotan

Cancer chemopreventive agents are typically natural products or their synthetic analogs that inhibit the transformation of normal cells to premalignant cells or the progression of premalignant cells to malignant cells. These agents are believed to function by modulating processes associated with xenobiotic biotransformation, with the protection of cellular elements from oxidative damage, or with the promotion of a more differentiated phenotype in target cells. However, an increasing number of chemopreventive agents (e.g., certain retinoids, nonsteroidal anti-inflammatory drugs, polyphenols, and vanilloids) have been shown to stimulate apoptosis in premalignant and malignant cells *in vitro* or *in vivo*. Apoptosis is arguably the most potent defense against cancer because it is the mechanism used by metazoans to eliminate deleterious cells. Many chemopreventive agents appear to target signaling intermediates in apoptosis-inducing pathways. Inherently, the process of carcinogenesis selects against apoptosis to initiate, promote, and perpetuate the malignant phenotype. Thus, targeting apoptosis pathways in premalignant cells—in which these pathways are still relatively intact—may be an effective method of cancer prevention. In this review, we construct a paradigm supporting apoptosis as a novel target for cancer chemoprevention by highlighting recent studies of several chemopreventive agents that engage apoptosis pathways. [J Natl Cancer Inst 2004;96:662–72]

Cancer prevention has become an integral part of cancer control. Common prevention approaches include avoiding exposure to known cancer-causing agents, enhancing host defense mechanisms against cancer, modifying life styles, and chemoprevention. The National Cancer Institute has made cancer prevention research a priority in such diverse areas as early detection and screening, diet and nutrition, cessation of tobacco use, and chemoprevention. Cancer prevention programs are increasingly being added to the multidisciplinary, collaborative efforts at mainstream research institutions, and efforts have intensified to attract researchers to this field.

Carcinogenesis, Chemoprevention, and Apoptosis

Even with improvements in the early detection and treatment of cancer, overall mortality rates for most cancers of epithelial origin have not declined in the last 30 years *(1)*. Carcinomas account for more than 80% of human cancers, with skin, lung, colon, breast, prostate, and uterus being the most frequent sites. Carcinogenesis can be viewed as a process that involves accelerated, and abnormal, cellular changes in which the genes controlling proliferation, differentiation, and apoptosis are transformed under selective environmental pressures *(2)*. Tumor development follows three distinct phases: initiation, promotion, and progression *(3,4)*. The initiation phase is a rapid (within hours or days), irreversible event that occurs when a normal cell is exposed to a carcinogen that causes unrepairable or misrepaired DNA damage. DNA damage itself is not mutagenic unless the resulting somatic mutation is recapitulated via mitosis to yield a clone of the mutated cell. This promotion phase, a protracted process that may require several years or decades to establish, consists of the expansion of mutated cells to form an actively proliferating, multicellular premalignant lesion. During the progression phase, another irreversible event occurs over a relatively short period, perhaps less than 1 year, in which new clones with increased proliferative capacity, invasiveness, and metastatic potential are produced *(5)*. Because the initiation and progression phases are irreversible and relatively transient events, the promotion phase of carcinogenesis may provide the best targets for cancer prevention *(6,7)*.

The term "chemoprevention" was coined by Michael Sporn in 1976, when he referred to the prevention of the development of malignancy by vitamin A and its synthetic analogs, known collectively as retinoids. Sporn *(6)* contended that the process of carcinogenesis had the potential to be controlled physiologically or pharmacologically during its preneoplastic stages, in which he suggested that the promotion phase could be stabilized, arrested, or reversed *(6)*. Cancer chemoprevention has emerged as an important means of modulating the process of carcinogenesis *(1,8)*. Almost three decades of research indicate that this strategy is promising with respect to reducing the incidence of cancer in well-defined high-risk groups and in the general population *(8,9)*. Chemoprevention, by definition, is the use of agents to slow the progression of, reverse, or inhibit carcinogenesis, thereby lowering the risk of developing invasive or clinically significant disease *(1,6,8)*. Consequently, an effective chemopreventive agent should intervene early in the process of carcinogenesis to eliminate premalignant cells before they become malignant *(1,10–12)*.

Several thousand agents have been reported to have chemopreventive activity, and more than 40 promising agents and

Affiliations of the authors: Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, TX.

Correspondence to: Reuben Lotan, PhD, Department of Thoracic/Head and Neck Medical Oncology, Box 432, The University of Texas M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX 77030-4095 (e-mail: rlotan@mdanderson.org).

See "Note" following "References."

DOI: 10.1093/jnci/djh123

Journal of the National Cancer Institute, Vol. 96, No. 9, © Oxford University Press 2004, all rights reserved.

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

agent combinations are currently being evaluated clinically for cancer chemoprevention *(12)*. For example, natural and synthetic retinoids have been effective in arresting or reversing premalignant lesions, such as bronchial metaplasia, oral leukoplakia, uterine cervical dysplasia, and actinic keratoses *(1)*. In randomized trials of patients with familial adenomatous polyposis, the cyclooxygenase-2 inhibitors sulindac and celecoxib inhibited the growth of adenomatous polyps and promoted polyp regression *(13)*. Furthermore, chemoprevention trials have shown that the antiestrogen tamoxifen can reduce the incidence of breast cancer *(14)* and that the antioxidant vitamin E can reduce the incidence of prostate cancer *(8)*.

Many chemopreventive agents (e.g., retinoids and antiestrogens) are believed to block or delay the progression of transformed cells by modulating cell proliferation or differentiation *(1,10,12)*. Because these agents are thought to promote cytostatic effects, it has been suggested that they should be administered long-term to healthy individuals who have an increased cancer risk. Of course, in this modality, even minor adverse side effects would be unacceptable *(10)*. Long-term toxicity and the possibility of developing resistance to chemopreventive agents are formidable obstacles that could limit the feasibility and success of conventional chemoprevention for many cancers. An alternate chemopreventive approach entails the use of agents that quickly eliminate premalignant cells by inducing them to undergo apoptosis rather than merely slowing their proliferation and/or promoting some degree of differentiation. For example, premalignant lesions could be eradicated and secondary primary tumors prevented with chemopreventive agents that have the capacity to trigger apoptosis in transformed cells. By shifting the outcome 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 could also facilitate chemopreventive agent-induced apoptosis in target cells and reduce possible short-term adverse side effects.

Apoptosis is the mechanism used by metazoans to regulate tissue homeostasis through the elimination of redundant or potentially deleterious cells. Apoptosis induction is arguably the most potent defense against cancer. For example, immune system cells destroy cancerous cells *(15,16)*, and most chemotherapeutic agents inhibit tumor cell proliferation *(17,18)*, by inducing apoptosis. The cellular machinery associated with apoptosis is highly conserved, with many similarities existing between phylogenetically divergent species. This similarity may explain why mutations in genes that regulate apoptosis pathways (e.g., p53, Bcl-2 family members, and PTEN) are common in most human cancers, and it underscores the importance of apoptosis resistance in the process of carcinogenesis *(19–21)*. Furthermore, growing evidence suggests that certain chemopreventive agents can trigger apoptosis in transformed cells *in vivo* and *in vitro*, which appears to be associated with their effectiveness in modulating the process of carcinogenesis.

In Vivo Observations Supporting Apoptosis as a Target for Chemopreventive Agents

Animal studies have demonstrated that certain chemopreventive agents (Table 1) can induce apoptosis in tumor cells *in vivo*. In the TRAMP mouse model for prostate cancer *(22)*, treatment with oral infusions of a polyphenolic extract isolated from green tea, at doses equivalent to the human consumption of six cups of green tea per day, promoted a statistically significant inhibition of prostate cancer development and increased overall survival. The chemopreventive intervention in prostate carcinogenesis with the green tea extract also caused a statistically significant induction of apoptosis in prostate cancer cells. This activity reportedly reduced the dissemination of these cells, thereby inhibiting their progression and possible metastasis to distant organ sites *(22)*. Oral pretreatment of SKH-1 mice with lyophilized green tea solids for 2 weeks enhanced the ultraviolet (UV)-induced increases in the number of p53-positive cells, p21-positive cells, and apoptotic sunburn cells in the epidermis. Thus, green tea treatment stimulated early adaptive responses to UV irradiation in mouse epidermis by the induction of tumor suppressor genes (i.e., p53 and p21) and the enhancement of apoptosis *in vivo* (i.e., inducing apoptotic sunburn cells) *(23)*. Furthermore, oral administration of a polyphenolic black tea extract to mice with chemically induced skin tumors inhibited proliferation and enhanced apoptosis in transformed skin cells *(24)*.

In a mouse model for chemically induced liver carcinogenesis, dietary supplementation with epigallocatechin gallate (a polyphenol isolated from tea leaves) or caloric restriction diminished the frequency and expansion of hepatic lesions by stimu-

Table 1. Chemopreventive agents that induce apoptosis in carcinogenesis models or in human chemoprevention trials

Agent	Carcinogenesis model/human trial (reference)
Tea polyphenols	TRAMP mouse model for prostate cancer *(22)*, and UV- and chemically induced skin tumors in mice *(23,24)*
Epigallocatechin gallate	Chemically induced hepatic tumors in mice *(25)* and UV-induced skin tumors in mice *(26)*
S-adenosyl-L-methionine	1,2-Dimethylhydrazine/orotic acid-induced rat liver carcinogenesis *(27)*
Aspirin	Min/+ mouse model for colon carcinogenesis *(28)*
Perillyl alcohol	Azoxymethane (AOM)-induced rat colon carcinogenesis *(29)*
Sulindac	AOM-induced rat colon carcinogenesis *(30)*
Phenylethyl-3-methylcaffeate	AOM-induced rat colon carcinogenesis *(30)*
Curcumin	AOM-induced rat colon carcinogenesis *(30)*
α-Difluoromethylornithine	*N*-Nitrosomethylbenzylamine-induced esophageal cancer in Zn^{2+}-deficient rats *(32)*
Quercetin	AOM-induced mouse colon carcinogenesis *(31)*
Rutin	AOM-induced mouse colon carcinogenesis *(31)*
Capsaicin	4-Nitroquinoline 1-oxide (NQO)-induced tongue carcinogenesis in rats *(33)*
Rotenone	NQO-induced tongue carcinogenesis in rats *(33)*, and diethylnitosamine-induced hepatic lesions in mice *(34)*
Exisulind	Phase I trial in patients with familial adenomatous polyposis *(37)*
Mesalazine	Prospective pilot study in colorectal cancer patients *(35)*, and as a chemopreventive agent in patients with sporadic polyps of the large bowel *(36)*

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

lating the induction of apoptosis in transformed hepatocytes *(25)*. Topical applications of caffeine or epigallocatechin gallate inhibited carcinogenesis and selectively increased apoptosis in UVB-induced skin tumors in mice *(26)*. In a rat model for liver carcinogenesis *(27)*, *S*-adenosyl-L-methionine reduced the incidence of 1,2-dimethylhydrazine/orotic acid–induced hepatic tumors. The decrease in tumor incidence was accompanied by an increase in apoptotic cells in the residual hepatic lesions.

In the Min/+ mouse, an animal with a germline mutation in the adenomatous polyposis coli gene that is essential for normal intestinal cell growth and differentiation, aspirin decreased the spontaneous rate of colon tumor formation by 44% *(28)*. Aspirin also normalized enterocyte growth by increasing apoptosis and reducing the proliferation of preneoplastic intestinal mucosa cells *(28)*. During chemically induced colon carcinogenesis, male F344 rats fed a diet supplemented with perillyl alcohol (a monoterpene isolated from lavender) exhibited a statistically significant reduction in the incidence and multiplicity of invasive adenocarcinomas of the colon compared with animals fed the control (no intervention) diet. Histopathologic evaluation of the treated colons indicated that the chemopreventive activity of perillyl alcohol was mediated through the induction of apoptosis in tumor cells *(29)*. In a related study, the chemopreventive properties of sulindac, curcumin, and phenylethyl-3-methylcaffeate were associated with apoptosis induction in tumor cells from chemically induced colon tumors in male F344 rats. Dietary administration of sulindac, curcumin, or phenylethyl-3-methylcaffeate was associated with a statistically significant increase in the number of apoptotic tumor cells relative to that in animals fed the control diet *(30)*. The flavonoids quercetin and rutin were also examined *(31)* in the male F344 rat model for chemically induced colon tumors. Either agent given as a dietary supplement increased the frequency of apoptotic cells and caused a redistribution of these cells along the colon crypt axis in the focal area of dysplasia relative to that in animals fed the control diet.

α-Difluoromethylornithine (an ornithine decarboxylase inhibitor) was examined as a chemopreventive agent in *N*-nitrosomethylbenzylamine-induced esophageal cancer in Zn^{2+}-deficient rats *(32)*. Chronic exposure to α-difluoromethylornithine in the drinking water substantially reduced the incidence of esophageal tumors from 89% to 10%. Esophageal cells from α-difluoromethylornithine-treated animals, compared with esophageal cells from animals given drinking water alone, had increased induction of apoptosis, enhanced expression of the pro-apoptotic Bcl-2 family member Bax, and reduced expression of proliferating-cell nuclear antigen *(32)*. Dietary administration of the mitochondrial poisons rotenone or capsaicin during 4-nitroquinoline 1-oxide-induced tongue carcinogenesis in rats reduced tumor formation by triggering apoptosis in the transformed cells *(33)*, and dietary administration of rotenone inhibited diethylnitosamine-induced hepatic lesions in mice, again apparently by triggering apoptosis in the transformed hepatocytes *(34)*.

Human chemoprevention trials have also demonstrated an association between a clinical response and the induction of apoptosis in tumor cells *(35–37)*. In a phase I trial of the nonsteroidal anti-inflammatory drug exisulind as a chemopreventive agent in patients with familial adenomatous polyposis *(37)*, increased apoptosis in colon polyps was noted at the maximum tolerated dose. However, this dose appeared to have no statistically significant effect on cell proliferation in the colon polyps or in the frequency at which these lesions were detected. In a prospective pilot study examining the chemopreventive effects of the nonsteroidal anti-inflammatory drug mesalazine in patients with colorectal cancer *(35)*, induction of apoptosis was increased statistically significantly in tumor samples but was unchanged in normal intestinal mucosa. Cell proliferation in the malignant and normal tissue samples was hardly affected by mesalazine, indicating that this agent selectively induced apoptosis in tumor cells without affecting normal cell proliferation. The apparent selectivity in apoptosis induction *(35,37)* without the inhibition of cell proliferation in transformed intestinal mucosa cells may be dependent on the cell type and/or on the particular chemopreventive agent that was examined in these clinical studies.

Another study *(36)* examining the effects of mesalazine on apoptosis and proliferation in the uninvolved intestinal mucosa of patients with sporadic polyps of the large bowel reported a statistically significant increase in the frequency of apoptotic cells shortly (i.e., 1–3 days) after the initiation of treatment. The increase in apoptosis remained largely unchanged after longer exposures to mesalazine (up to 14 days). Cell proliferation in the intestinal mucosa was decreased more in the mesalazine treatment group than in the untreated groups.

Thus, these studies illustrate that certain chemopreventive agents can induce apoptosis in tumor cells in various animal models for carcinogenesis and in human chemoprevention trials. Moreover, the induction of apoptosis in tumor cells was apparently associated with the antitumor activity of the respective chemopreventive agents.

The design of clinical chemoprevention trials is evolving, but a few generalities can be defined for each phase *(38)*. A phase I study determines the dose-related safety of a prospective chemopreventive agent and assesses the pharmacokinetics and the toxicity of the agent. Initial doses and schedules in phase I trials are based on toxicity and efficacy data obtained from preclinical studies. Phase II trials use a randomized, blinded, placebo-controlled design to evaluate the dose–response relationship and common toxic side effects that are likely to be associated with long-term (i.e., 3 months or longer) administration of the chemopreventive agent. Several dose levels are evaluated and compared with the modulation of previously validated surrogate end point biomarkers (SEBs). SEBs are defined as measurable biologic processes or molecules that are closely linked to the progression pathway to invasive cancer and that undergo modulation in concert with neoplastic regression *(39)*. Phase II trials can be conducted with individuals who have premalignant lesions or with former cancer patients at risk of developing a second primary tumor. If safety and efficacy are judged satisfactory in these trials, the agent proceeds to evaluation in a randomized, prospective phase III clinical trial. The phase III trial is the ultimate test of a chemopreventive agent efficacy and is designed to measure the incidence of primary tumors and changes in SEBs in relation to dose and toxicity. The longer phase III trials require strict adherence to the chemoprevention protocol by patients and reproducibility in the chemopreventive agent's formulation.

Although large-scale randomized trials are the gold standard for testing the efficacy of cancer chemopreventive agents and, indeed, of anticancer agents that act at other stages in the cancer

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

process, these trials typically require large study populations, extensive resources, and many years for completion *(40)*. The use of valid SEBs for the intermediate effects of cancer chemoprevention (e.g., apoptosis induction or the loss of clonogenicity) would make it possible to design smaller, short-term prevention trials. SEBs can be assessed by molecular biology techniques, such as immunohistochemistry, applied to tissue specimens obtained by excision, punch, or core-needle biopsy examination. It is likely that a panel of SEBs, rather than a single SEB, will be more advantageous to monitor chemopreventive agent–induced apoptosis in patient samples. The most commonly used SEB for apoptosis induction in the chemoprevention clinical trials discussed thus far is DNA fragmentation. *In situ* staining for DNA fragmentation can be coupled with an indicator of cellular proliferation (e.g., proliferating-cell nuclear antigen, DNA ploidy, or Ki-67 antigen expression) to determine whether tissue homeostasis has been restored as a result of the chemopreventive intervention *(12,32)*. Other SEBs that can be used to detect chemopreventive agent–induced apoptosis in patient tissue samples include activation of caspases [i.e., cysteine proteases involved in apoptosis, such as the cleavage and activation of caspase-3 *(41)*], the cleavage of cytokeratin 18 by caspases *(42)*, and enhanced expression of Fas and/or Fas ligand *(43)*. Of course some of the major challenges associated with the use of SEBs in chemoprevention trials will be the standardization of the methods for measuring these intermediate biologic changes as well as the terminology used to describe them *(39)*.

Potential Mechanisms Associated with Chemopreventive Agent–Induced Apoptosis

Apoptosis is triggered by an initiation phase that is highly dependent on cell type and apoptotic stimuli (e.g., oxidative stress, DNA damage, ion fluctuations, and cytokines). In the subsequent effector phase, the cell undergoes distinct biochemical changes that result in the systematic activation of catabolic hydrolases (i.e., proteases and nucleases). These enzymes participate in the degradation phase of apoptosis through the cleavage of proteins and DNA *(15,44)*. Most of the recent advances in the elucidation of apoptosis pathways have come about through the characterization of the effector mechanisms. Effector mechanisms of apoptosis have several components, and two effector mechanisms associated with caspase activation have been characterized extensively—the extrinsic, or death receptor–mediated, effector mechanism and the intrinsic, or mitochondrial-mediated, effector mechanism *(45)*. In addition to mitochondria, other organelles, including the endoplasmic reticulum, Golgi apparatus, and lysosomes, may also have a role in damage sensing, proapoptotic signaling, and caspase activation *(46)*.

The extrinsic pathway of apoptosis is activated at the cell surface when a specific ligand binds to its corresponding cell surface death receptor (Fig 1, A). Death receptors (e.g., tumor necrosis factor receptor [TNFR], TNF-related apoptosis-inducing ligand [TRAIL] receptor, and Fas) belong to the TNFR superfamily (47). After ligand binding (e.g., TNF, TRAIL, and Fas ligand, respectively), death receptors cluster in the plasma membrane and promote the recruitment of adapter proteins *(47)*. Caspase 8 is an apical caspase in the death receptor pathway. The zymogen of caspase 8 can interact with the adapter proteins (e.g., FADD and RIP1) to generate the active form of caspase 8 *(47)*. After activation, caspase 8 can trigger the activation of

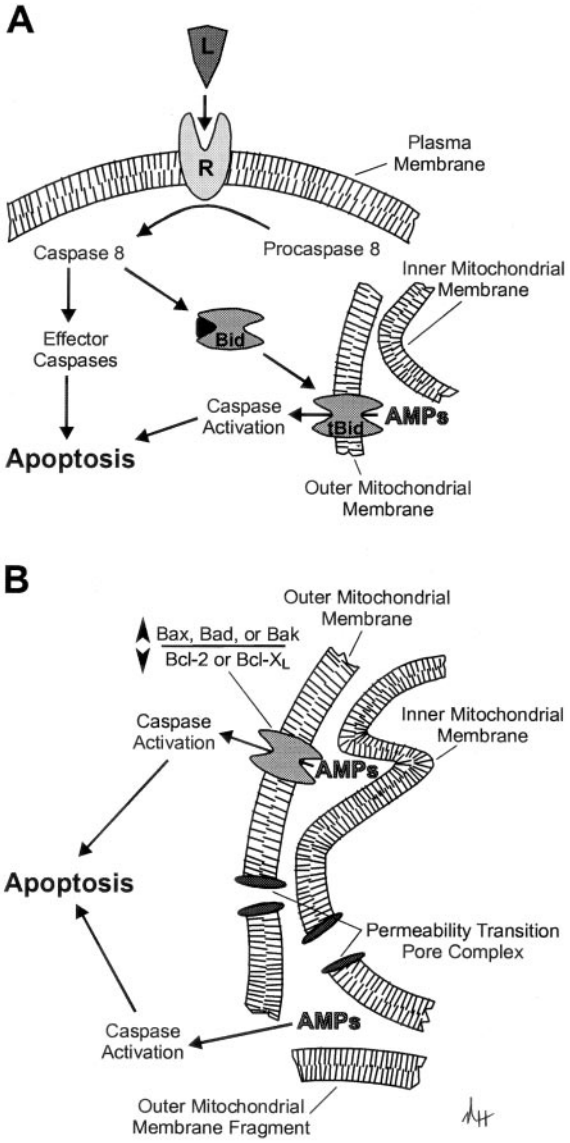

Fig. 1. Regulation of the extrinsic and intrinsic pathways of apoptosis. **A)** The extrinsic pathway is initiated when a death receptor ligand (L; i.e., the Fas ligand) binds to its death receptor (R; i.e., the Fas receptor). This interaction promotes the recruitment of adapter molecules (not shown) and results in the cleavage of procaspase 8 to yield active caspase 8. In certain cell systems, the activation of caspase 8 is sufficient to initiate the proteolytic cascade required for apoptosis. Caspase 8 can also cleave the proapoptotic Bcl-2 family member Bid, and then truncated Bid (tBid) can facilitate pore formation in the outer mitochondrial membrane to promote the release of proapoptotic mitochondrial proteins (AMPs). AMPs can trigger caspase activation and apoptosis. Cleavage of Bid by caspase 8 can serve as a link between the extrinsic and intrinsic pathways of apoptosis. **B)** The intrinsic pathway is regulated by the permeabilization of mitochondrial membranes. Antiapoptotic activity of certain Bcl-2 family members (e.g., Bcl-2 and Bcl-X_L) can be subverted by the induction of proapoptotic Bcl-2 family members (e.g., Bax, Bad, and Bak). In this scenario, the ratio of proapoptotic family members to antiapoptotic family member becomes greater, which causes pores to form in the outer mitochondrial membrane, liberating AMPs to activate caspases and induce apoptosis. The mitochondrial permeability transition is regulated at various levels by several mitochondrial proteins believed to constitute the permeability transition pore complex. Normally, the permeability transition pore complex is in a closed or low conductance conformation (not shown). Numerous pathologic stimuli, as well as various chemical agents, can cause the permeability transition pore complex to adopt an open conformation, resulting in large-amplitude swelling of the mitochondrial matrix and permeabilization of the outer mitochondrial membrane because of physical disruption. After the outer membrane fragments, AMPs are released to activate caspases and induce apoptosis.

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

downstream effector caspases such as caspase 3. In certain cell types, the activation of caspase 8 is sufficient to initiate the proteolytic cascade that results in apoptotic cellular degradation *(15,47)*.

In addition to activating effector caspases, caspase 8 also targets Bid, a proapoptotic member of the Bcl-2 family. In response to the binding of Fas ligand or TNF to its receptor, caspase 8 induces the cleavage of Bid to yield a truncated C-terminal fragment that translocates from the cytosol to the outer mitochondrial membrane. Oligomers of the C-terminal Bid fragment can trigger pore formation in the outer mitochondrial membrane, allowing apoptogenic mitochondrial proteins, such as cytochrome *c (48,49)* and endonuclease G *(50,51)*, to be released from the mitochondria (Fig. 1, A). Truncated Bid may also trigger conformational changes in Bax (another proapoptotic member of the Bcl-2 family) that allow it to localize in the outer mitochondrial membrane *(52)*, where Bax can associate with the voltage-dependent anion channel that is also located in the outer mitochondrial membrane. Complexes of Bax and the voltage-dependent anion channel promote pore formation in the outer mitochondrial membrane, allowing the release of apoptogenic mitochondrial proteins *(52)*. Regardless of the mechanism of caspase-8-induced permeabilization of the outer mitochondrial membrane, the presence of apoptogenic mitochondrial proteins in the cytosol can serve to amplify intracellular signals activated by death receptors to trigger apoptosis.

The intrinsic pathway of apoptosis relies solely on the permeabilization of mitochondrial membranes to release the apoptogenic mitochondrial proteins [e.g., cytochrome *c (53)*, endonuclease G *(50,51)*, Smac/DIABLO *(54)*, Omi/HtrA2 *(55)*, apoptosis-inducing factor (AIF) *(56)*, and its homolog AIF-homologous mitochondrion-associated inducer of death (AMID) *(57)*] required for caspase activation and apoptosis. As they do in the extrinsic pathway, Bcl-2 family members appear to play an important role in the regulation of the intrinsic pathway (Fig. 1, B). During conditions of cell stress, antiapoptotic Bcl-2 family members (e.g., Bcl-2 and Bcl-X_L) residing in the outer mitochondrial membrane can be destabilized by the induction of proapoptotic Bcl-2 family members (e.g., Bax, Bad, and Bak). In this scenario, the ratio of proapoptotic Bcl-2 family members to antiapoptotic Bcl-2 family members increases and, by mechanisms that are not completely understood, pores form in the outer mitochondrial membrane, liberating apoptogenic mitochondrial proteins to activate caspases and induce apoptosis *(58,59)*.

Another mechanism implicated in the permeabilization of mitochondrial membranes is the mitochondrial permeability transition. The mitochondrial permeability transition is a rate-limiting and self-amplifying process that is regulated at various levels by several mitochondrial proteins. Many of these proteins are believed to constitute the permeability transition pore complex *(17,60)* (Fig. 1, B). Normally, proteins in the outer and inner mitochondrial membranes that constitute the permeability transition pore complex are predictably in close proximity to each other and are in a closed or low-conductance conformation *(60)*. Numerous pathologic stimuli (e.g., reactive oxygen species and calcium) and various chemical agents can cause the permeability transition pore complex to adopt an open conformation *(17,60)*, which allows water and solutes can infiltrate the mitochondrial matrix *(61)*. This results in colloidal osmotic swelling of the mitochondrial matrix and permeabilization of the outer mitochondrial membrane, presumably resulting from physical rupture of the outer membrane *(62)*. After the outer membrane fragments, apoptogenic mitochondrial proteins are released to the cytoplasm, where they participate in the degradation phase of apoptosis.

A search of PubMed for studies published since 1994 revealed that several chemopreventive agents have the ability to induce apoptosis in a variety of premalignant and malignant cell types *in vitro* (Table 2). For some agents, modulation of the cellular target or pathway that was originally envisioned for the agent is independent of its apoptogenic effects, which may account for the prevalent cytotoxicity of some of these agents. For example, although apoptosis triggered by the natural retinoid all-*trans*-retinoic acid appears to be mediated by the activation of retinoid receptors in breast cancer cells *(63)*, induction of apoptosis by the synthetic retinoid *N*-(4-hydroxyphenyl)-retinamide appears to be independent of retinoid receptors *(64–68)*. Some nonsteroidal anti-inflammatory drugs are selective inhibitors of cyclooxygenase 2. However, two such agents, sulindac *(69–72)* and celecoxib *(73)*, can induce apoptosis through cyclooxygenase-2-independent mechanisms. In addition, tamoxifen has been reported to induce apoptosis in a manner that is independent of its antiestrogenic activity *(74–76)*.

We identified representatives from various classes of chemopreventive agents from recent *in vitro* studies with sufficient evidence to provide a detailed account of their apoptotic mechanisms (Table 3). Most of these compounds can activate caspases through intrinsic effector mechanisms that are regulated by Bcl-2 family members (e.g., inhibition of Bcl-2 expression or induction of Bax expression) or the mitochondrial permeability transition (e.g., dissipation of mitochondrial inner transmembrane potential). Other agents [e.g., all-*trans*-retinoic acid *(77)*, sulindac *(69)*, and epigallocatechin gallate *(78)*] may use extrinsic effectors instead.

Several of the classes of chemopreventive compounds contain agents that can promote reactive oxygen species generation or trigger oxidative stress [e.g., *N*-(4-hydroxyphenyl)retinamide *(64–68,79)*, celecoxib *(80)*, indomethacin *(81)*, epigallocatechin gallate *(82)*, curcumin *(83)*, tamoxifen *(75,84)*, capsaicin *(85–88)*, resiniferatoxin *(86,89)*, rotenone *(90,91)*, and deguelin *(92)*], which appears to be associated with apoptosis induction in various cell types *(86–91)*. Reactive oxygen species can promote divergent cellular effects depending on the extent of their production and the enzymatic or nonenzymatic mechanisms available for their dismutation in a given cell type. Thus, reactive oxygen species can serve as mitogenic stimuli, senescence promoters, or cell death mediators *(93)*. Mitochondria are the primary cellular site of reactive oxygen species production *(93–96)*, and, under certain conditions, elevated mitochondrial reactive oxygen species generation can serve as an apoptotic signal *(93,94,97)*. Many chemopreventive agents with prooxidant activity also appear to have a direct [e.g., inhibition of mitochondrial respiration *(86,92,98)*] and/or indirect [e.g., promotion of the dissipation of mitochondrial inner transmembrane potential *(74,83,85,86,89,92,98,99)*] ability to disrupt mitochondrial function to trigger the intrinsic pathway of cell death. Furthermore, enhanced ceramide production promoted by *N*-(4-hydroxyphenyl)-retinamide *(67)* and celecoxib *(80)* could also promote the production of reactive oxygen species, impair mitochondrial function, and trigger intrinsic effector mechanisms for cell degradation, considering that ceramide has been implicated in

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

Table 2. Chemopreventive agents that induce apoptosis in premalignant or malignant cells *in vitro*

Class/agent	Cell type (reference)
Retinoids	
All-*trans*-retinoic acid	Breast cancer cells *(131,132)* and myeloma cells *(133)*
9-*cis*-retinoic acid	Leukemia cells *(134)*
N-(4-hydroxyphenyl)retinamide	Leukemia cells *(135)*, squamous skin cancer cells *(65)*, cervical cancer cells *(79)*, neuroblastoma cells *(67)*, and prostate cancer cells *(68)*
Nonsteroidal anti-inflammatory drugs	
Aspirin	Gastric cancer cells *(119,136)*, leukemia cells *(137)*, and transformed T cells *(137)*
Sulindac	Hepatocarcinoma cells *(71)*, prostate cancer cells *(69,70)*, and colorectal cancer cells *(72)*
Celecoxib	Prostate cancer cells *(73)*
Exisulind	Hepatocarcinoma cells *(71)*
Polyphenols	
Resveratrol	Leukemia cells *(138,139)* and colon cancer cells *(140)*
Epigallocatechin gallate	Epidermoid cancer cells *(124)*, prostate cancer cells *(141)*, leukemia cells *(78)*, and transformed bronchial epithelial cells *(82)*
Antiestrogen/anti-androgen	
Tamoxifen	Breast cancer cells *(76,84,120,142)*, transformed breast epithelial cells *(74)*, hepatoblastoma cells *(143)*, glioma cells *(120)*, and leukemia cells *(144)*
Clomiphene	Leukemia cells *(144)*
Nafoxidine	Leukemia cells *(144)*
Vanilloids	
Capsaicin	Squamous skin cancer cells *(86)*, hepatocarcinoma cells *(121)*, transformed breast epithelial cells *(145)*, glioblastoma cells *(146)*, neuroblastoma cells *(147)*, transformed T cells *(85)*, transformed B cells *(148)*, glioma cells *(149)*, and melanoma cells *(150)*
Curcumin	Transformed T cells *(125)*, colon cancer cells *(151)*, Ehrlich's ascites carcinoma cells *(152)*, and leukemia cells *(153)*
Resiniferatoxin	Squamous skin cancer cells *(86)*, transformed T cells *(87)*, transformed B cells *(148)*
Rotenoids	
Rotenone	Leukemia cells *(154)*, transformed B cells *(90,155)*, and neuroblastoma cells *(156)*
Deguelin	Colon cancer cells *(157)*, transformed and malignant bronchial epithelial cells *(122)*, and squamous skin cancer cells *(92)*
Others	
α-Difluoromethylornithine	Gastric cancer cells *(158)*
Selenium	Prostate cancer cells *(159,160)* and glioma cells *(161)*

these processes *(94)*. Therefore, such chemopreventive agents may induce apoptosis, at least in part, by their mitochondrial toxicity. This hypothesis does not seem to be a broad conceptual leap because several novel chemotherapeutic agents are also considered mitochondriotoxic *(17)*.

We have observed that the apoptogenic effects of *N*-(4-hydroxyphenyl)retinamide *(98)*, capsaicin *(86)*, resiniferatoxin *(86)*, and deguelin *(92)* are conspicuously diminished in skin cancer cells that have been depleted of mitochondrial DNA (i.e., ρ^0 cells) and that are therefore functionally deficient in mitochondrial respiration. Other studies have demonstrated that ρ^0 cells are more resistant than their parental counterparts to the apoptogenic effects of cancer chemotherapeutic agents *(100,101)*, ceramide *(102)*, and extrinsic mediators of apoptosis, such as TNF *(103)* and TRAIL *(104)*, implying that the disruption of mitochondrial respiration by these agents is associated with cell death. The inability of the ρ^0 mitochondria to conduct bioenergetic processes appears to correspond to the mitochondrial alterations observed in various malignant tumor cells *(105–109)*, implying that certain aspects of mitochondrial function [e.g., electron transport, which is inhibited by *N*-(4-hydroxyphenyl)retinamide, capsaicin, resiniferatoxin, and deguelin] are realistic targets for chemoprevention. However, the window of opportunity for exploiting these targets to trigger apoptosis probably closes as tumor cells progress to a more malignant phenotype.

Many chemopreventive agents can also modulate genes or proteins that respond to conditions of oxidative stress to trigger apoptosis (Table 3). For example, the induction of Fas and Fas ligand is responsive to conditions of oxidative stress *(110–112)*, and all-*trans*-retinoic acid reportedly induces apoptosis by enhancing Fas ligand expression *(77)*. Oxidative stress appears to be associated with the modulation of Bcl-2 family members in several cell systems *(113–118)*. The studies summarized in Table 3 indicate that the induction of Bax or Bak or the inhibition of Bcl-2 or Bcl-X_L are commonly associated with apoptosis induction after exposure to certain chemopreventive agents [e.g., sulindac *(72)*, indomethacin (*119)*, tamoxifen *(120)*, capsaicin *(121)*, deguelin *(122)*, and genistein *(123)*]. Likewise, many of these agents can modulate nuclear factor NF-κB [e.g., tamoxifen *(75)*, epigallocatechin gallete *(124)*, curcumin *(125)*, resvertrol *(126)*, and genistein *(127)*], which has also been implicated in regulatory mechanisms associated with oxidative stress *(114,116,128,129)*. Thus, some chemopreventive agents may trigger an oxidative stress response by direct or indirect effects on the mitochondria because these organelles are important regulators of cellular redox homeostasis *(130)*.

Conclusions and Perspectives

Apoptosis is subverted during tumorigenesis, presumably through the systematic loss of regulatory control mechanisms, ultimately resulting in the generation of a malignant phenotype and resistance to chemotherapy and radiation therapy. Investigations to further elucidate the mechanisms associated with chemopreventive agent–induced apoptosis should provide increased opportunities to develop novel, selectively targeted agents or drugs for chemoprevention.

It is anticipated that the life span of human beings will continue to increase, which will inevitably be associated with an

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

Table 3. Mechanisms associated with apoptosis induction for selected chemopreventive agents *in vitro**

Class/agent	Proposed mechanisms (reference)
Retinoids	
All-*trans*-retinoic acid	Induction of tissue transglutaminase *(162,163)*, retinoid receptor activation *(63)*, caspase activation *(134)*, and the induction of Fas ligand *(77)*
N-(4-Hydroxyphenyl)retinamide	Reactive oxygen species production *(64,65,67,68,79)*, enhanced ceramide production *(67)*, disruption of mitochondrial inner transmembrane potential *(65,98,164)*, caspase activation *(98,164)*, inhibition of mitochondrial respiration *(98)*, and the activation of retinoid receptors *(165,166)*
Nonsteroidal anti-inflammatory drugs	
Aspirin	Induction of Bax *(119,136)*, cytochrome *c* release from the mitochondria *(136,137)*, induction of Bak *(119)*, and the activation of caspases *(119,136,137)*
Sulindac	Activation of death receptors and caspase 8 *(69)*, induction of Bax *(72)*, and the inhibition of BcI-X_L *(72)*
Celecoxib	Inhibition of Akt *(73)*, enhanced ceramide production *(80)*, and induction of 15-lipoxygenase-1 *(167)*
Indomethacin	Reactive oxygen species production *(81)*, induction of c-Myc *(168)*, induction of Bax and Bak *(119)*, and the activation of caspases *(119)*
Polyphenols	
Resveratrol	Activation of caspases *(126,138,139,169,170)*, inhibition of NF-κB *(126)*, and the induction of Fas ligand *(169)*
Epigallocatechin gallate	Hydrogen peroxide production *(82)*, activation of Fas receptors *(78)*, inhibition of NF-κB *(124)*, induction of p21 *(141)*, inhibition of the plasma membrane NADH oxidase *(171)*, and the activation of caspases *(78,172)*
Butyroid	
Tributyrin	Induction of Bax *(173)*, dissipation of mitochondrial inner transmembrane potential *(173)*, release of cytochrome *c* from the mitochondria *(173)*, and the activation of caspases *(173)*
Antiestrogen	
Tamoxifen	Dissipation of mitochondrial inner transmembrane potential *(74)*, caspase activation *(74,84)*, stimulation of JNK activity *(84)*, calcium mobilization *(120,143)*, induction of oxidative stress *(75,84)*, activation of nuclear factor NF-κB *(75)*, activation of nitric oxide synthase *(174)*, and the inhibition of Bcl-2 *(142)*
Flavonoid	
Genistein	Inhibition of NF-κB *(127)*, caspase activation *(123)*, induction of Bax *(123)*, calcium mobilization *(175)*, and the induction of p21 *(123,176)*
Vanilloids	
Capsaicin	Dissipation of mitochondrial inner transmembrane potential *(85–87,99)*, reactive oxygen species production *(85–88)*, calcium mobilization *(85,177)*, inhibition of the plasma membrane NADH oxidase *(87,148,178)*, Bcl-2 inhibition *(121)*, induction of Bax *(121)*, inhibition of mitochondrial respiration *(86)*, and the activation of caspases *(86,121)*
Curcumin	Inhibition of NF-κB and AP-1 *(125)*, activation of caspases *(125,152,153)*, induction of oxidative stress *(83)*, dissipation of mitochondrial inner transmembrane potential *(83)*, and the induction of Bax *(152,153)*
Resiniferatoxin	Dissipation of mitochondrial inner transmembrane potential *(86,89)*, reactive oxygen species production *(86,89)*, calcium mobilization *(177)*, inhibition of the plasma membrane NADH oxidase *(148)*, inhibition of mitochondrial respiration *(86)*, and the activation of caspases *(86)*
Rotenoids	
Rotenone	Inhibition of mitochondrial respiration *(90,154–156)*, reactive oxygen species production *(90)*, and the disruption of cell cycle progression *(90)*, and the activation of caspases *(179)*
Deguelin	Disruption of cell cycle progression *(122,157)*, disruption of cyclin-dependent kinase activity *(157)*, inhibition of phosphatidylinositol 3-kinase/Akt pathway *(122)*, dissipation of mitochondrial inner transmembrane potential *(92)*, reactive oxygen species production *(92)*, the inhibition of mitochondrial respiration *(92)*, and the activation of caspases *(92,122)*

*Please refer to the text for the criteria used to select the represented chemopreventive agents and their proposed mechanisms of action.

escalation of age-related diseases such as cancer. Numerous epidemiologic studies have provided a basis for primary cancer prevention through the elimination of carcinogens from our diet and the environment. However, given that the carcinogens responsible for many forms of cancer are unknown, it seems highly unlikely that all individuals will be able to eliminate the risk of developing cancer during their lifetimes. Our knowledge of the etiology of certain familial forms of breast, colon, and skin cancer, for example, also indicates that certain individuals are predisposed to cancer irrespective of environmental factors. Advances in the last two decades have provided very sensitive methods, such as endoscopy, blood screening tests, and mutational analysis, for the early detection of cancer that allow an opportunity for intervention to prevent the development of invasive or clinically significant disease. Likewise, the potential benefit of cancer chemoprevention appears promising given the results obtained from clinical trials, animal carcinogenesis models, and *in vitro* studies. Collectively, these considerations support a chemopreventive approach to manage the magnitude and severity of cancer at present and in the future.

REFERENCES

(1) Hong WK, Sporn MB. Recent advances in chemoprevention of cancer. Science 1997;278:1073–7.

(2) Bertram JS. The molecular biology of cancer. Mol Aspects Med 2000; 21:167–223.

(3) Berenblum I, Armuth V. Two independent aspects of tumor promotion. Biochim Biophys Acta 1981;651:51–63.

(4) Heidelberger C, Freeman AE, Pienta R, Sivak A, Bertram JS, Casto BC, et al. Cell transformation by chemical agents—a review and analysis of the literature. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutat Res 1983;114:283–385.

(5) Surh Y-J. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic compounds. Mutat Res 1999; 428:305–27.

(6) Sporn MB. Approaches to prevention of epithelial cancer during the preneoplastic period. Cancer Res 1976;36:2699–702.

(7) Umar A, Viner JL, Hawk ET. The future of colon cancer prevention. Ann NY Acad Sci 2001;952:88–108.

(8) Kelloff GJ, Lieberman R, Steele VE, Boone CW, Lubet RA, Kopelovich L, et al. Agents, biomarkers, and cohorts for chemopreventive agent development in prostate cancer. Urology 2001;57:46–51.

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

(9) Kakizoe T. Chemoprevention of cancer—focusing on clinical trials. Jpn J Clin Oncol 2003;33:421–42.
(10) Wattenberg LW. What are the critical attributes for cancer chemopreventive agents? Ann NY Acad Sci 1995;768:73–81.
(11) Smith TJ, Hong J-Y, Wang Z-Y, Yang CS. How can carcinogenesis be inhibited? Ann NY Acad Sci 1995;768:82–90.
(12) Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Boone CW, Malone WA, et al. Progress in cancer chemoprevention. Ann NY Acad Sci 1999;889: 1–13.
(13) Thun MJ, Henley SJ, Patrono C. Nonsteroidal anti-inflammatory drugs as anticancer agents: mechanistic, pharmacologic, and clinical issues. J Natl Cancer Inst 2002;94:252–66.
(14) Fabian CJ, Kimler BF. Beyond tamoxifen new endpoints for breast cancer chemoprevention, new drugs for breast cancer prevention. Ann NY Acad Sci 2001;952:44–59.
(15) Thornberry NA, Lazebnik Y. Caspases: enemies within. Science 1998; 281:1312–6.
(16) Hill LL, Ouhtit A, Loughlin SM, Kripke ML, Ananthaswamy HN, Owen-Schaub LB. Fas ligand: a sensor for DNA damage critical in skin cancer etiology. Science 1999;285:898–900.
(17) Costantini P, Jocotot E, Decaudin D, Kroemer G. Mitochondrion as a novel target of anticancer chemotherapy. J Natl Cancer Inst 2000;92: 1042–53.
(18) Reed JC. Apoptosis-targeted therapies for cancer. Cancer Cell 2002;3: 17–22.
(19) Cantley LC, Neel BG. New insights into tumor suppression: PTEN suppresses tumor formation by restraining the phosphoinositide 3-kinase/AKT pathway. Proc Natl Acad Sci USA 1999;96:4240–5.
(20) Levine AJ. p53, the cellular gatekeeper for growth and division. Cell 1997;88:323–31.
(21) Reed JC. Mechanisms of apoptosis avoidance in cancer. Curr Opin Oncol 1999;11:68–75.
(22) Gupta S, Hastak K, Ahmad N, Lewin JS, Mukhtar H. Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols. Proc Natl Acad Sci USA 2001;98:10350–5.
(23) Lu Y-P, Lou Y-R, Li XH, Xie J-G, Brash D, Huang M-T, et al. Stimulatory effect of oral administration of green tea or caffeine on ultraviolet light-induced increases in epidermal wild-type p53, p21 (WAF1/CIP1), and apoptotic sunburn cells in SKH-1 mice. Cancer Res 2000;60:4785–91.
(24) Lu Y-P, Lou Y-R, Xie J-G, Yen P, Huang M-T, Conney AH. Inhibitory effect of black tea on the growth of established skin tumors in mice: effects on tumor size, apoptosis, mitosis and bromodeoxyuridine incorporation into DNA. Carcinogenesis 1997;18:2163–9.
(25) Klaunig JE, Kamendulis LM. Mechanisms of cancer chemoprevention in hepatic carcinogenesis: modulation of focal lesion growth in mice. Toxicol Sci 1999;52:101–6.
(26) Lu Y-P, Lou Y-R, Xie J-G, Q-Y P, Liao J, Yang CS, et al. Topical application of caffeine or (-)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. Proc Natl Acad Sci USA 2002;99:12455–60.
(27) Pascale RM, Simile MM, De Miglio MR, Nufris A, Daino L, Seddaiu MA, et al. Chemoprevention by S-adenosyl-L-methionine of rat liver carcinogenesis initiated by 1,2-dimethylhydrazine and promoted by orotic acid. Carcinogenesis 1995;16:427–30.
(28) Mahmoud NN, Dannenberg AJ, Mestre J, Bilinski RT, Churchill MR, Martucci C, et al. Aspirin prevents tumors in a murine model of familial adenomatous polyposis. Surgery 1998;124:225–31.
(29) Reddy BS, Wang CX, Samaha H, Lubet R, Steele VE, Kelloff GJ, et al. Chemoprevention of colon carcinogenesis by dietary perillyl alcohol. Cancer Res 1997;57:420–5.
(30) Samaha HS, Kelloff GJ, Steele V, Rao CV, Reddy BS. Modulation of apoptosis by sulindac, curcumin, phenylethyl-3-methylcaffeate, and 6-phenylhexyl isothiocyanate: apoptotic index as a biomarker in colon cancer chemoprevention and promotion. Cancer Res 1997;57:1301–5.
(31) Yang K, Lamprecht SA, Liu Y, Shinozaki H, Fan K, Leung D, et al. Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. Carcinogenesis 2000;21:1655–60.
(32) Fong LY, Nguyen VT, Pegg AE, Magee PN. Alpha-difluoromethylornithine induction of apoptosis: a mechanism which reverses pre-established cell proliferation and cancer initiation in esophageal carcinogenesis in zinc-deficient rats. Cancer Epidemiol Biomarkers Prev 2001;10:191–9.
(33) Tanaka T, Kohno H, Sakata K, Yamada Y, Hirose Y, Sugie S, et al. Modifying effects of dietary capsaicin and rotenone on 4-nitroquinoline 1-oxide-induced rat tongue carcinogenesis. Carcinogenesis 2002;23:1361–7.
(34) Isenberg JS, Kolaja KL, Ayoubi AS, Watkins JB, 3rd, Klaunig JE. Inhibition of WY-14,643 induced hepatic lesion growth in mice by rotenone. Carcinogenesis 1997;18:1511–9.
(35) Bus PJ, Nagtegaal ID, Verspaget HW, Lamers CB, Geldof H, Van Krieken JH, et al. Mesalazine-induced apoptosis of colorectal cancer: on the verge of a new chemopreventive era. Aliment Pharmacol Ther 1999; 13:1397–402.
(36) Reinacher-Schick A, Seidensticker F, Petrasch S, Reiser M, Philippou S, Theegarten D, et al. Mesalazine changes apoptosis and proliferation in normal mucosa of patients with sporadic polyps of the large bowel. Endoscopy 2000;32:245–54.
(37) van Stolk R, Stoner G, Hayton WL, Chan K, DeYoung B, Kresty L, et al. Phase I trial of exisulind (sulindac sulfone, FGN-1) as a chemopreventive agent in patients with familial adenomatous polyposis. Clin Cancer Res 2000;6:78–89.
(38) Kelloff GJ, Johnson JR, Crowell JA, Boone CW, DeGeorge JJ, Steele VE, et al. Approaches to the development and marketing approval of drugs that prevent cancer. Cancer Epidemiol Biomarkers Prev 1995;4:1–10.
(39) Trock BJ. Validation of surrogate endpoint biomarkers in prostate cancer chemoprevention. Urology 2001;57:241–57.
(40) Greenwald P. Cancer prevention clinical trials. J Clin Oncol 2002;20:14s–22s.
(41) Kania J, Konturek SJ, Marlicz K, Hahn EG, Konturek PC. Expression of survivin and caspase-3 in gastric cancer. Dig Dis Sci 2003;48:266–71.
(42) Caulin C, Salvesen GS, Oshima G. Caspase cleavage of keratin 18 and reorganization of intermediate filaments during epithelial cell apoptosis. J Cell Biol 1997;138:1379–94.
(43) Urosevic M, Maier T, Benninghoff B, Slade H, Burg G, Dummer R. Mechanisms underlying imiquimod-induced regression of basal cell carcinoma in vivo. Arch Dermatol 2003;139:1325–32.
(44) Henderson PJF, Lardy HA. Bongkrekic acid an inhibitor of the adenine nucleotide translocase of mitochondria. J Biol Chem 1970;245:1319–26.
(45) Hengartner MO. The biochemistry of apoptosis. Nature 2000;407:770–776.
(46) Ferri KF, Kroemer G. Organelle-specific initiation of cell death pathways. Nat Cell Biol 2001;3:E255–E263.
(47) Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science 1998;281:1305–8.
(48) Li H, Zhu H, Xu CJ, Yuan J. Cleavage of Bid by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 1998;94:491–501.
(49) Lou X, Kim CN, Yang J, Jemmerson R, Wang X. Bid, a Bcl-2 interacting protein, mediates cytochrome c release from the mitochondria in response to activation of cell surface death receptors. Cell 1998;94:481–90.
(50) Li LY, Lou X, Wang X. Endonuclease G is an apoptotic DNase when released from mitochondria. Nature 2001;412:95–9.
(51) van Loo G, Schotte P, van Gurp M, Demol H, Hoorelbeke B, Gevaert K, et al. Endonuclease G: a mitochondrial protein released in apoptosis and involved in caspase-independent DNA degradation. Cell Death Differ 2001;8:1136–42.
(52) Shimizu S, Narita M, Tsujimoto Y. Bcl-2 family proteins regulate the release of apoptogenic cytochrome c by the mitochondrial channel VDAC. Nature 1999;399:483–7.
(53) Liu X, Kim CN, Yang J, Jemmerson R, Wang X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. Cell 1996;86:147–57.
(54) Chai J, Du C, Wu JW, Kyin S, Wang X, Shi Y. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. Nature 2000;406:855–62.
(55) van Loo G, van Gurp M, Depuydt B, Srinivasula SM, Rodriguez I, Alnemri ES, et al. The serine protease Omi/HtrA2 is released from mitochondria during apoptosis. Omi/HtrA2 interacts with caspase-

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

inhibitor XIAP and induces enhanced caspase activity. Cell Death Differ 2002;9:20–7.
(56) Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, et al. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 1999;397:441–6.
(57) Wu M, Xu L-G, Li X, Zhai Z, Shu H-B. AMID, an apoptosis-inducing factor-homologues mitochondrion-associated protein, induces caspase-independent apoptosis. J Biol Chem 2002;277:25617–23.
(58) Adams JM, Cory S. The bcl-2 protein family: arbiters of cell survival. Science 1998;281:1322–6.
(59) Reed JC, Jurgensmeier JM, Matsuyama S. Bcl-2 family proteins and mitochondria. Biochim Biophys Acta 1998;1366:127–37.
(60) Zoratti M, Szabò I. The mitochondrial permeability transition. Biochim Biophys Acta 1995;1241:139–76.
(61) Bernardi P. Modulation of the mitochondrial cyclosporin A-sensitive permeability transition pore by the proton electrochemical gradient. Evidence that the pore can be opened by membrane depolarization. J Biol Chem 1992;267:8834–9.
(62) Green DR, Reed JC. Mitochondria and apoptosis. Science 1998;281: 1309–12.
(63) Schneider SM, Offterdinger M, Huber H, Grunt TW. Activation of retinoic acid receptor alpha is sufficient for full induction of retinoid responses in SK-BR-3 and T47D human breast cancer cells. Cancer Res 2000;60:5479–87.
(64) Delia D, Aiello A, Lombardi L, Pelicci PG, Grignani F, Jr, Grignani F, et al. N-(4-Hydroxyphenyl)retinamide induces apoptosis of malignant hemopoietic cell lines including those unresponsive to retinoic acid. Cancer Res 1993;53:6036–41.
(65) Hail N, Jr., Lotan R. Mitochondrial permeability transition is a central coordinating event in N-(4-hydroxyphenly)retinamide-induced apoptosis. Cancer Epidemiol Biomarkers Prev 2000;9:1293–301.
(66) Oridate N, Lotan D, Xu XC, Hong WK, Lotan R. Differential induction of apoptosis by all-trans-retinoic acid and N-(4-hydroxyphenyl)retinamide in human head and neck squamous cell carcinoma cell lines. Clin Cancer Res 1996;2:855–63.
(67) Maurer B, Metelitsa L, Seeger R, Cabot M, Reynolds C. Increase of ceramide and induction of mixed apoptosis/necrosis by N-(4-hydroxyphenyl)retinamide in neuroblastoma cell lines. J Natl Cancer Inst 1999;91:1138–46.
(68) Sun S-Y, Yue P, Lotan R. Induction of apoptosis by N-(4-hydroxyphenyl)retinamide and its association with reactive oxygen species, nuclear retinoic acid receptors, and apoptosis related genes in human prostate carcinoma cells. Mol Pharmacol 1999;55:403–10.
(69) Huang Y, He Q, Hillman MJ, Rong R, Sheikh MS. Sulindac sulfide-induced apoptosis involves death receptor 5 and the caspase 8-dependent pathway in human colon and prostate cancer cells. Cancer Res 2001;61: 6918–24.
(70) Lim JT, Piazza GA, Han EK, Delohery TM, Li H, Finn TS, et al. Sulindac derivatives inhibit growth and induce apoptosis in human prostate cancer cell lines. Biochem Pharmacol 1999;58:1097–107.
(71) Rahman MA, Dhar DK, Masunaga R, Yamanoi A, Kohno H, Nagasue N. Sulindac and exisulind exhibit a significant antiproliferative effect and induce apoptosis in human hepatocellular carcinoma cell lines. Cancer Res 2000;60:2085–9.
(72) Zhang L, Yu J, Park BH, Kinzler KW, Vogelstein B. Role of BAX in the apoptotic response to anticancer agents. Science 2000;290:989–92.
(73) Hsu AL, Ching TT, Wang DS. The cyclooxygenase-2 inhibitor celecoxib induces apoptosis by blocking Akt activation in human prostate cancer cells independently of Bcl-2. J Biol Chem 2000;275:11397–403.
(74) Dietze EC, Caldwell LE, Grupin SL, Mancini M, Seewaldt VL. Tamoxifen but not 4-hydroxytamoxifen initiates apoptosis in p53(-) normal human mammary epithelial cells by inducing mitochondrial depolarization. J Biol Chem 2001;276:5384–94.
(75) Ferlini C, Scambia G, Marone M, Distefano M, Gaggini C, Ferrandina G, et al. Tamoxifen induces oxidative stress and apoptosis in oestrogen receptor-negative human cancer cell lines. Br J Cancer 1999;79:257–63.
(76) Kang Y, Cortina R, Perry RR. Role of c-myc in tamoxifen-induced apoptosis estrogen-independent breast cancer cells. J Natl Cancer Inst 1996;88:279–84.
(77) Okamura T, Masuda M, Arai Y, Ishida C, Shudou K, Mizoguchi H. All-trans retinoic acid modulates Fas antigen expression and affects cell proliferation and apoptosis in combination with anti-Fas monoclonal antibody in the human myeloma cell line, U266B1. Exp Hematol 1998; 26:501–6.
(78) Hayakawa S, Saeki K, Sazuka M, Suzuki Y, Shoji Y, Ohta T, et al. Apoptosis induction by epigallocatechin gallate involves its binding to Fas. Biochem Biophys Res 2001;285:1102–6.
(79) Oridate N, Suzuki S, Higuchi M, Mitchell MF, Hong WK, Lotan R. Involvement of reactive oxygen species in N-(4-hydroxyphenyl)-retinamide-induced apoptosis in cervical carcinoma cells. J Natl Cancer Inst 1997;89:1191–8.
(80) Chan TA, Morin P, Vogelstein B, Kinzler KW. Mechanisms underlying nonsteroidal antiinflammatory drug-mediated apoptosis. Proc Natl Acad Sci USA 1998;95:681–6.
(81) Kusuhara H, Komatsu H, Sumichika H, Sugahara K. Reactive oxygen species are involved in the apoptosis induced by nonsteroidal anti-inflammatory drugs in cultured gastric cells. Eur J Pharmacol 1999;383: 331–7.
(82) Yang GY, Liao J, Li C, Chung J, Yurkow EJ, Ho CT, et al. Effect of black and green tea polyphenols on c-jun phosphorylation and H_2O_2 production in transformed and non-transformed human bronchial cell lines: possible mechanisms of cell growth inhibition and apoptosis induction. Carcinogenesis 2000;21:2035–9.
(83) Morin D, Barthelemy S, Zini R, Labidalle S, Tillement JP. Curcumin induces the mitochondrial permeability transition pore mediated by membrane protein thiol oxidation. FEBS Lett 2001;495:131–6.
(84) Mandlekar S, Yu R, Tan TH, Kong AN. Activation of caspase-3 and c-Jun NH_2-terminal kinase-1 signaling pathways in tamoxifen-induced apoptosis of human breast cancer cells. Cancer Res 2000;60:5995–6000.
(85) Macho A, Calcado MA, Muñoz-Blanco J, Gómez-Díaz C, Gajate C, Mollinedo F, et al. Selective induction of apoptosis by capsaicin in transformed cells: the role of reactive oxygen species and calcium. Cell Death Differ 1999;6:155–65.
(86) Hail N, Jr., Lotan R. Examining the role of mitochondrial respiration in vanilloid-induced apoptosis. J Natl Cancer Inst 2002;94:1281–92.
(87) Macho A, Blázquez M-V, Navas P, Muñoz E. Induction of apoptosis by vanilloid compounds does not require de novo gene transcription and activator protein 1 activity. Cell Growth Differ 1998;9:277–86.
(88) Richeux F, Cascante M, Ennamany R, Sanchez D, Sanni A, Saboureau D, et al. Implication of oxidative stress and inflammatory process in the toxicity of capsaicin in human endothelial cells: lack of DNA strand breakage. Toxicology 2000;147:41–9.
(89) Macho A, Lucena C, Calzado MA, Blanco M, Donnay I, Appendino G, et al. Phorboid 20-homovanillates induce apoptosis through a VR1-independent mechanism. Chem Biol 2000;7:483–92.
(90) Armstrong JS, Hornung B, Lecane P, Jones DP, Knox SJ. Rotenone-induced G2/M cell cycle arrest and apoptosis in a human B lymphoma cell line PW. Biochem Biophys Res 2001;289:973–8.
(91) Isenberg JS, Klaunig JE. Role of the mitochondrial membrane permeability transition (MPT) in rotenone-induced apoptosis in liver cells. Toxicol Sci 2000;53:340–51.
(92) Hail N, Jr., Lotan R. Apoptosis induction by the natural product cancer chemopreventive agent deguelin is mediated through the inhibition of mitochondrial bioenergetics. Apoptosis. In press. 2004.
(93) Fleury C, Mignotte B, Vayssière JL. Mitochondrial reactive species in cell death signaling. Biochimie 2002;84:131–41.
(94) Skulachev VP. Mitochondrial physiology and pathology: concepts of programmed cell death of organelles, cells, and organisms. Mol Aspects Med 1999;20:139–84.
(95) Turrens JF. Superoxide production by the mitochondrial respiratory chain. Biosci Rep 1997;17:3–7.
(96) Chance B, Sies H, Boveris A. Hydroperoxide metabolism in mammalian organs. Physiol Rev 1979;59:527–605.
(97) Skulachev VP. Why are mitochondria involved in apoptosis? FEBS Lett 1996;397:7–10.
(98) Hail N, Jr., Lotan R. Mitochondrial respiration is uniquely associated with the prooxidant and apoptotic effects of N-(4-hydroxyphenyl)retinamide. J Biol Chem 2001;276:45614–21.

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

(99) Dedov VN, Mandadi S, Armati PJ, Verkhratski A. Capsaicin-induced depolarization of mitochondria in dorsal root ganglion neurons is enhanced by vanilloid receptors. Neuroscience 2001;103:219–26.
(100) Singh KK, Russell J, Sigala B, Zhang Y, Wiliams J, Keshav KF. Mitochondrial DNA determines the cellular response to cancer therapeutic agents. Oncogene 1999;18:6641–6.
(101) Joshi B, Li L, Taffe BG, Zhu Z, Wahl S, Tian H, et al. Apoptosis induction by a novel anti-prostate cancer compound, BMD188 (a fatty acid containing hydroxamic acid), requires the mitochondrial respiratory chain. Cancer Res 1999;59:4343–55.
(102) Poppe M, Reimertz C, Munstermann G, Kogel D, Prehn JH. Ceramide-induced apoptosis of D283 medulloblastoma cells requires mitochondrial respiratory chain activity but occurs independently of caspases and is not sensitive to Bcl-xL overexpression. J Neurochem 2002;82:482–94.
(103) Higuchi M, Aggarwal BB, Yeh ET. Activation of CPP32-like protease in tumor necrosis factor-induced apoptosis is dependent on mitochondrial function. J Clin Invest 1997;99:1751–8.
(104) Kim J-Y, Kim Y-H, Chang I, Kim S, Pak YK, Oh B-H, et al. Resistance of mitochondrial DNA-deficient cells to TRAIL: role of Bax in TRAIL-induced apoptosis. Oncogene 2002;21:3139–48.
(105) Dang CV, Semenza GL. Oncogenic alterations of metabolism. Trends Biochem Sci 1999;24:68–72.
(106) Cavalli LR, Liang BC. Mutagenesis, tumorigenicity, and apoptosis: are the mitochondria involved? Mutat Res 1998;398:19–26.
(107) John AP. Dysfunctional mitochondria, not oxygen insufficiency, cause cancer cells to produce inordinate amounts of lactic acid: the impact of this on the treatment of cancer. Med Hypotheses 2001;57:429–31.
(108) Warburg O. On the origin of cancer cells. Science 1956;123:309–14.
(109) Mathupala SP, Rempel A, Pedersen PL. Glucose catabolism in cancer cells. J Biol Chem 1995;270:16918–25.
(110) Bauer MKA, Vogt M, Los M, Siegel J, Wesselborg S, Schulze-Osthoff K. Role of reactive oxygen intermediates in activation-induced CD95 (APO-1/Fas) ligand expression. J Biol Chem 1998;273:8048–55.
(111) Bouvard V, Zaitchouk T, Vacher M, Duthu A, Canivet M, Choisy-Rossi C, et al. Tissue and cell-specific expression of the p53-target genes: bax, fas, mdm2 and waf1/p21, before and following ionizing irradiation in mice. Oncogene 2000;19:649–60.
(112) Kwon D, Choi C, Lee J, Kim KO, Kim JD, Kim SJ, et al. Hydrogen peroxide triggers the expression of Fas/FasL in astrocytoma cell lines and augments apoptosis. J Neuroimmunol 2001;113:1–9.
(113) Andoh T, Lee SY, Chiueh CC. Preconditioning regulation of bcl-2 and p66shc by human NOS1 enhances tolerance to oxidative stress. FASEB J 2000;14:2144–6.
(114) Aoki M, Nata T, Morishita R, Matsushita H, Nakagami H, Yamamoto K, et al. Endothelial apoptosis induced by oxidative stress through activation of NF-κB: antiapoptotic effect of antioxidant agents on endothelial cells. Hypertension 2001;38:48–55.
(115) Bojes HK, Datta K, Xu J, Chin A, Simonian P, Nunez G, et al. Bcl-xL overexpression attenuates glutathione depletion in FL5. 12 cells following interleukin-3 withdrawal. Biochem J 1997;325:315–9.
(116) Crawford MJ, Krishnamoorthy RR, Rudick VL, Collier RJ, Kapin M, Aggarwal BB, et al. Bcl-2 overexpression protects photooxidative stress-induced apoptosis of photoreceptor cells via NF-κB preservation. Biochem Biophys Res 2001;281:1304–12.
(117) Sonoda Y, Watanabe S, Matsumoto Y, Aizu-Yokota E, Kasahara T. FAK is the upstream signal protein of the phosphatidylinositol 3-kinase-Akt survival pathway in hydrogen peroxide-induced apoptosis of a human glioblastoma cell line. J Biol Chem 1999;274:10566–70.
(118) Longoni B, Boschi E, Demontis GC, Ratto GM, Mosca F. Apoptosis and adaptive responses to oxidative stress in human endothelial cells exposed to cyclosporin A correlate with BCL-2 expression levels. FASEB J 2001;15:731–40.
(119) Zhou XM, Wong BC, Fan XM, Zhang HB, Lin MC, Kung HF, et al. Non-steroidal anti-inflammatory drugs induce apoptosis in gastric cancer cells through up-regulation of bax and bak. Carcinogenesis 2001;22:1393–7.
(120) Zhang W, Couldwell WT, Song H, Takano T, Lin JH, Nedergaard M. Tamoxifen-induced enhancement of calcium signaling in glioma and MCF-7 breast cancer cells. Cancer Res 2000;60:5395–400.
(121) Jung M-Y, Kang H-J, Moon A. Capsaicin-induced apoptosis in SK-Hep-1 hepatocarcinoma cells involves Bcl-2 downregulation and caspase-3 activation. Cancer Lett 2001;165:139–45.
(122) Chun K-H, Kosmeder JW, Sun S, Pezzuto JM, Lotan R, Hong WK, et al. Effects of deguelin on the phosphatidylinositol 3-kinase/Akt pathway and apoptosis in premalignant human bronchial epithelial cells. J Natl Cancer Inst 2003;95:291–302.
(123) Li Y, Upadhyay S, Bhuiyan M, Sarkar FH. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. Oncogene 1999;18:3166–72.
(124) Ahmad N, Gupta S, Mukhtar H. Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappa B in cancer cells versus normal cells. Arch Biochem Biophys 2000;376:338–46.
(125) Cipriani B, Borsellino G, Knowles H, Tramonti D, Cavaliere F, Bernardi G, et al. Curcumin inhibits activation of Vγ9Vδ2 T cells by phosphoantigens and induces apoptosis involving apoptosis-inducing factor and large scale DNA fragmentation. J Immunol 2001;167:3454–62.
(126) Holmes-McNary M, Baldwin AS, Jr. Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. Cancer Res 2000;60:3477–83.
(127) Davis JN, Kucuk O, Sarkar FH. Genistein inhibits NF-kB activation in prostate cancer cells. Nutr Cancer 1999;35:167–74.
(128) Van Antwerp DJ. Suppression of TNF-α-induced apoptosis by NF-κB. Science 1997;274:787–9.
(129) Vollgraf U, Wegner M, Richter-Landsberg C. Activation of AP-1 and nuclear factor-kappa B transcription factors is involved in hydrogen peroxide-induced apoptotic cell death of oligodendrocytes. J Neurochem 1999;73:2501–9.
(130) Skulachev VP. Membrane-linked systems preventing superoxide formation. Biosci Rep 1997;17:347–65.
(131) Sumantran VN, Zhang R, Lee DS, Wicha MS. Differential regulation of apoptosis in normal versus transformed mammary epithelium by lutein and retinoic acid. Cancer Epidemiol Biomarkers Prev 2000;9:257–63.
(132) Toma S, Isnardi L, Raffo P, Dastoli G, De Francisci E, Riccardi L, et al. Effects of all-trans-retinoic acid and 13-cis-retinoic acid on breast-cancer cell lines: growth inhibition and apoptosis induction. Int J Cancer 1997;70:619–27.
(133) Taetle R, Dos Santos B, Akamatsu K, Koishihara Y, Ohsugi Y. Effects of all-trans retinoic acid and antireceptor antibodies on growth and programmed cell death of human myeloma cells. Clin Cancer Res 1996;2:253–9.
(134) Gianni M, Ponzanelli I, Mologni L, Reichert U, Rambaldi A, Terao M, et al. Retinoid-dependent growth inhibition, differentiation and apoptosis in acute promyelocytic leukemia cells. Expression and activation of caspases. Cell Death Differ 2000;7:447–60.
(135) Delia D, Aiello A, Meroni L, Nicolini M, Reed JC, Pierotti MA. Role of antioxidants and intracellular free radicals in retinamide-induced cell death. Carcinogenesis 1997;18:943–8.
(136) Zimmermann KC, Waterhouse NJ, Goldstein JC, Schuler M, Green DR. Aspirin induces apoptosis through release of cytochrome c from mitochondria. Neoplasia 2000;2:505–13.
(137) Pique M, Barragan M, Dalmau M, Bellosillo B, Pons G, Gil J. Aspirin induces apoptosis through mitochondrial cytochrome c release. FEBS Lett 2000;480:193–6.
(138) Tinhofer I, Bernhard D, Senfter M, Anether G, Loeffler M, Kroemer G, et al. Resveratrol, a tumor-suppressive compound from grapes, induces apoptosis via a novel mitochondrial pathway controlled by Bcl-2. FASEB J 2001;15:1613–5.
(139) Dörrie J, Gerauer H, Wachter Y, Zunino SJ. Resveratrol induces extensive apoptosis by depolarizing mitochondrial membranes and activating caspase-9 in acute lymphoblastic leukemia cells. Cancer Res 2001;61:4731–9.
(140) Mahyar-Roemer M, Katsen A, Mestres P, Roemer K. Resveratrol induces colon tumor cell apoptosis independently of p53 and precede by epithelial differentiation, mitochondrial proliferation and membrane potential collapse. Int J Cancer 2001;94:615–22.
(141) Gupta S, Ahmad N, Nieminen AL, Mukhtar H. Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (-)-epigallocatechin-3-gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. Toxicol Appl Pharmacol 2000;164:82–90.

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016

(142) Zhang GJ, Kimijima I, Onda M, Kanno M, Sato H, Watanabe T, et al. Tamoxifen-induced apoptosis in breast cancer cells relates to down-regulation of bcl-2, but not bax and bcl-X_L, without alteration of p53 protein levels. Clin Cancer Res 1999;5:2971–7.
(143) Kim JA, Kang YS, Jung MW, Lee SH, S LY. Involvement of Ca^{2+} influx in the mechanism of tamoxifen-induced apoptosis in HepG2 human hepatoblastoma cells. Cancer Lett 1999;147:115–23.
(144) Hayon T, Dvilansky A, Oriev L, Nathan I. Non-steroidal antiestrogens induce apoptosis in HL60 and MOLT3 leukemic cells;involvement of reactive oxygen radicals and protein kinase C. Anticancer Res 1999;19:2089–93.
(145) Kang H-J, Soh Y, Kim M-S, Lee E-J, Surh Y-J, Kim H-RC, et al. Roles of JNK-1 and p38 in selective induction of apoptosis by capsaicin in ras-transformed human breast epithelial cells. Int J Cancer 2003;103:475–82.
(146) Lee YS, Nam DH, Kim J-A. Induction of apoptosis by capsaicin in A172 human glioblastoma cells. Cancer Lett 2000;161:121–30.
(147) Lee YS, Kwon EJ, Jun DQ, Park SH, Kang YS, Huh K, et al. Redox status-dependent regulation of cyclooxygenases mediates the capsaicin-induced apoptosis in human neuroblastoma cells. J Environ Pathol Toxicol Oncol 2002;21:113–20.
(148) Wolvetang EJ, Larm JA, Moutsoulas P, Lawen A. Apoptosis induced by inhibitors of the plasma membrane NADH-oxidase involves Bcl-2 and calcineurin. Cell Growth Differ 1996;7:1315–25.
(149) Bíró T, Brodie C, Modarres S, Lewin NE, Ács P, Blumberg PM. Specific vanilloid responses in C6 rat glioma cells. Brain Res Mol Brain Res 1998;56:89–98.
(150) Morré DJ, Sun E, Geilen C, Wu LY, de Cabo R, Krasagakis K, et al. Capsaicin inhibits plasma membrane NADH oxidase and growth of human and mouse melanoma lines. Eur J Cancer 1996;32A:1995–2003.
(151) Rashmi R, Santhosh-Kumar TR, Karunagaran D. Human colon cancer cells differ in their sensitivity to curcumin-induced apoptosis and heat shock protects them by inhibiting the release of apoptosis-inducing factor and caspases. FEBS Lett 2003;538:19–24.
(152) Pal S, Choudhuri T, Chattopadhyay S, Bhattacharya A, Datta GK, Das T, et al. Mechanisms of curcumin-induced apoptosis of Ehrlich's ascites carcinoma cells. Biochem Biophys Res 2001;288:658–65.
(153) Pan MH, Chang WL, Lin-Shiau SY, Ho CT, Lin JK. Induction of apoptosis by garcinol and curcumin through cytochrome c release and activation of caspases in human leukemia HL-60 cells. J Agric Food Chem 2001;49:1464–74.
(154) Mills KI, Woodgate LJ, Gilkes AF, Walsh V, Sweeney MC, Brown G, et al. Inhibition of mitochondrial function in HL60 cells is associated with an increased apoptosis and expression of CD14. Biochem Biophys Res 1999;263:294–300.
(155) Wolvetang EJ, Johnson KL, Krauer K, Ralph SJ, Linnane AW. Mitochondrial respiratory chain inhibitors induce apoptosis. FEBS Lett 1994; 339:40–4.
(156) Kitamura Y, Inden M, Miyamara A, Kakimura J, Tanigucgi T, Shimohama S. Possible involvement of both mitochondria- and endoplasmic reticulum-dependent caspase pathways in rotenone-induced apoptosis in human neuroblastoma SH-SY5Y cells. Neurosci Lett 2002;333:25–8.
(157) Murillo G, Kosmeder JW, II, Pezzuto JM, Metha RG. Deguelin inhibits the growth of colon cancer cells through the induction of apoptosis and cell cycle arrest. Eur J Cancer 2002;38:2446–54.
(158) Takahashi Y, Mai M, Nishioka K. α-Difluoromethylornithine induces apoptosis as well as anti-angiogenesis in the inhibition of tumor growth and metastasis in a human gastric cancer model. Int J Cancer 2000;85:243–7.
(159) Jiang C, Wang Z, Ganther H, Lu J. Caspases as key executors of methyl selenium-induced apoptosis (anoikis) of DU-145 prostate cancer cells. Cancer Res 2001;61:3062–70.
(160) Zhong W, Oberley TD. Redox-mediated effects of selenium on apoptosis and cell cycle in the LNCaP human prostate cancer cell line. Cancer Res 2001;61:7071–8.
(161) Sundaram N, Pahwa AK, Ard MD, Lin N, Perkins E, Bowles AP, Jr. Selenium causes growth inhibition and apoptosis in human brain tumor cell lines. J Neurooncol 2000;46:125–33.
(162) Joseph B, Lefebvre O, Mereau-Richard C, Danze PM, Belin-Plancot MT, Formstecher P. Evidence for the involvement of both retinoic acid receptor- and retinoic X receptor-dependent signaling pathways in the induction of tissue transglutaminase and apoptosis in the human myeloma cell line RPMI 8226. Blood 1998;91:2423–32.
(163) Ou H, Haendeler J, Aebly MR, Kelly LA, Cholewa BC, Koike G, et al. Retinoic acid-induced tissue transglutaminase and apoptosis in vascular smooth muscle cells. Circ Res 2000;87:881–7.
(164) Suzuki S, Higuchi M, Proske RJ, Oridate N, Hong WK, Lotan R. Implication of mitochondria-derived reactive oxygen species, cytochrome c and caspase-3 in N-(4-hydroxyphenyl)retinamide-induced apoptosis in cervical carcinoma cells. Oncogene 1999;18:6380–7.
(165) Sun SY, Li W, Yue P. Mediation of N-(4-hydoxyphenyl)retinamide-induced apoptosis in human cancer cells by different mechanisms. Cancer Res 1999;59:2493–8.
(166) Lovat PE, Ranalli M, Annichiarrico-Petruzzelli M. Effector mechanisms of fenretinide-induced apoptosis in neuroblastoma. Exp Cell Res 2000; 260:50–60.
(167) Shureiqi I, Xu X, Chen D, Lotan R, Morris JS, Fischer SM, et al. Nonsteroidal anti-inflammatory drugs induce apoptosis in esophageal cancer cells by restoring 15-lipoxygenase-1 expression. Cancer Res 2001; 61:4879–84.
(168) Zhu GH, Wong BC, Eggo MC, Ching CK, Yuen ST, Chan EY, et al. Non-steroidal anti-inflammatory drug-induced apoptosis in gastric cancer cells is blocked by protein kinase C activation through inhibition of c-myc. Br J Cancer 1999;79:393–400.
(169) Clement MV, Hirpara JL, Chawdhury SH, Pervaiz S. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. Blood 1998;92:996–1002.
(170) Bernhard D, Tinhofer I, Tonko M, Hubl H, Ausserlechner MJ, Greil R, et al. Resveratrol causes arrest in the S-phase prior to Fas-independent apoptosis in CEM-C7H2 acute leukemia cells. Cell Death Differ 2000;7:834–42.
(171) Morré DJ, Bridge A, Wu LY, Morré DM. Preferential inhibition by (-)-epigallocatechin-3-gallate of the cell surface NADH oxidase and growth of transformed cells in culture. Biochem Pharmacol 2000;60:937–46.
(172) Islam S, Islam N, Kermode T, Johnstone B, Mukhtar H, Moskowitz RW, et al. Involvement of caspase-3 in epigallocatechin-3-gallate-mediated apoptosis of human chondrosarcoma cells. Biochem Biophys Res 2000; 270:793–7.
(173) Heerdt BG, Houston MA, Anthony GM, Augenlicht LH. Initiation of growth arrest and apoptosis of MCF-7 mammary carcinoma cells by tributyrin, a triglyceride analogue of the short-chain fatty acid butyrate, is associated with mitochondrial activity. Cancer Res 1999;59:1584–91.
(174) Maccarrone M, Fantini C, Ranalli M, Melino G, Agro AF. Activation of nitric oxide synthase is involved in tamoxifen-induced apoptosis of human erythroleukemia K562 cells. FEBS Lett 1998;434:421–4.
(175) Linford NJ, Yang Y, Cook DG, Dorsa DM. Neuronal apoptosis resulting from high doses of the isoflavone genistein: role for calcium and p42/44 mitogen-activated protein kinase. J Pharmacol Exp Ther 2001;299:67–75.
(176) Shao ZM, Alpaugh ML, Fontana JA, Barsky SH. Genistein inhibits proliferation similarly in estrogen receptor-positive and negative human breast carcinoma cell lines characterized by P21(WAF1/CIP1) induction, G2/M arrest, and apoptosis. J Cell Biochem 1998;69:44–54.
(177) Olah Z, Szabo T, Karai L, Hough C, Fields RD, Caudle RM, et al. Ligand-induced dynamic membrane changes and cell deletion conferred by vanilloid receptor 1. J Biol Chem 2001;276:11021–30.
(178) Morré DJ, Chueh P-J, Morré DM. Capsaicin inhibits preferentially the NADH oxidase and growth of transformed cells in culture. Proc Natl Acad Sci USA 1995;92:1831–5.
(179) Yuki K, Miyauchi T, Kakinuma Y, Murakoshi N, Suzuki T, Hayashi J, et al. Mitochondrial dysfunction increases expression of endothelin-1 and induces apoptosis through caspase-3 activation in rat cardiomyocytes in vitro. J Cardiovasc Pharmacol 2000;36:S205–8.

NOTES

This work was supported in part by a Cancer Prevention Fellowship (N.H.), sponsored by NCI grant R25 CA57780; and the Irving and Nadine Mansfield and Robert David Levitt Cancer Research Chair and USPHS Project Grant PO1 CA68233 from the NCI (R.L.).

The first and second authors have contributed equally to this work and should be considered co–first authors.

Manuscript received June 25, 2003; revised March 10, 2004; accepted March 24, 2004.

Downloaded from http://jnci.oxfordjournals.org/ at Pennsylvania State University on September 16, 2016