MINIREVIEW

Targeting Apoptosis with Dietary Bioactive Agents

KEITH R. MARTIN[1]

Nutrition and Cancer Laboratory, Department of Nutritional Sciences, The Pennsylvania State University, University Park, Pennsylvania 16802

Apoptosis, a form of programmed cell death, is a pivotal defense against the occurrence of cancer and is essential to metazoans in maintaining tissue homeostasis. Apoptosis exhibits a distinctive phenotype and involves elimination of potentially deleterious cells. Many diseases have been associated with aberrantly regulated apoptotic cell death, ultimately leading to inhibition of apoptosis and propagation of diseases such as cancer. Elucidation of the critical events associated with carcinogenesis provides the opportunity for dietary intervention to prevent cancer development through induction of apoptosis, particularly by bioactive agents or functional foods. Diet is a significant environmental factor in the overall cancer process and can exacerbate or interfere with carcinogenesis. Apoptosis occurs primarily through two well-recognized pathways in cells, including the intrinsic, or mitochondrial-mediated, effector mechanism and the extrinsic, or death receptor–mediated, effector mechanism. In addition to diet's effects on protein expression and function, evidence is also accumulating that a large number of dietary food components can exert effects on the human genome, either directly or indirectly, to modulate gene expression. In fact, many diet-related genes are involved in carcinogenesis as well as apoptosis, and thus are ultimately molecular targets for dietary chemoprevention. There are multiple steps within pathways in which dietary components can alter gene expression and phenotypes of cells and thus influence cancer outcomes (nutritional transcriptomic effect). Thus, apoptosis is an emerging therapeutic target of bioactive agents of diet. In this review, the process of apoptosis is discussed and the potential mechanistic interaction of bioactive agents, as components of functional foods, is explored within the context of apoptosis. Exp Biol Med 231:117–129, 2006

Key words: apoptosis; diet; extrinsic pathway; intrinsic pathway; bioactive agent

Introduction

Apoptosis is one of the most potent defenses against cancer, since this process eliminates potentially deleterious, mutated cells (1). The pathogenesis of many diseases, including cancer, is closely connected with aberrantly regulated apoptotic cell death. Indeed, the prolific research efforts over the last two decades regarding elucidation of the basic mechanisms that regulate apoptosis and associated mediators that trigger or inhibit cell death have laid the foundation for therapeutic strategies for combating cancer. Numerous novel approaches are currently being pursued, including gene therapy, antisense strategies, recombinant biology, and classical organic and combinatorial chemistry, to target specific apoptotic regulators (2). An obvious and efficacious approach that is gaining broader acceptance is nutritional modulation via the myriad bioactive components present in functional foods (3, 4).

An accumulating body of scientific evidence indicates that many cancers are preventable, especially because diet and nutrition are key factors in the modulation of cancer risk (5). In fact, dietary habits are estimated to contribute to at least 35%, but perhaps as many as 70%, of all human cancers (6, 7). Thus, diet is a significant environmental factor in the overall cancer process and can exacerbate or interfere with carcinogenesis through modulated gene expression, leading to altered cellular phenotypes and differing cancer outcomes (nutritional transcriptomic effect) (8, 9). In addition, genes can influence absorption, metabolism, and transport of dietary components and

This work was funded, in part, by grants from the International Life Sciences Institute (ILSI), North America; and the Huck Institute Life Sciences Consortium and Biotechnology Institute.

[1] To whom correspondence should be addressed at Department of Nutritional Science, The Pennsylvania State University, 126 Henderson Building South, University Park, PA 16802. E-mail: krm12@psu.edu

1535-3702/06/2312-0117$15.00

potentially alter the sites of mechanistic action. Furthermore, the thousands of dietary compounds consumed each day can potentially interact with the cancer process at the genetic and epigenetic levels, modifying sites of drug detoxification, DNA repair, cellular proliferation, differentiation, angiogenesis, and apoptosis (8, 9).

A greater understanding of the pivotal events associated with carcinogenesis will facilitate the use of dietary intervention as a key strategy to prevent cancer development. Experimental evidence indicates that dietary constituents, particularly phytochemicals, can modulate the complex multistage process of carcinogenesis at each of the three recognized stages of initiation, promotion, and malignant progression. As examples, promising dietary chemopreventive compounds include epigallocatechin gallate (EGCG) in green tea, quercetin in onions and tomatoes, resveratrol in grapes, curcumin in turmeric, sulforaphane and other isothiocyanates (ITCs) in cruciferous vegetables, genistein in soybeans, organosulfur compounds in garlic, and lycopene in tomatoes, among many others (10). Currently, several thousand dietary components reportedly possess chemopreventive activity, and approximately 40 are being studied clinically for efficacy in chemoprevention trials (11).

In vitro and *in vivo* studies with diet-derived compounds demonstrate modulation of tumor growth through alteration of gene expression and induction of apoptosis (12). In one study, dietary phenolic compounds, including ellagic acid and resveratrol, modulated more than 550 genes after a 48-hr exposure of hormone-dependent human prostate cells, indicating that activation by dietary compounds of multiple signaling pathways is involved in cellular proliferation and apoptosis (13). Resveratrol reduces cellular proliferation and induces apoptosis in numerous human cancer cells of various histiocytic origins (14). Others have shown that capsaicin, plants of the ginger family, curcumin, and yakuchinone exhibit the ability to suppress proliferation and clonal expansion of cancer cells through induction of apoptosis (15). Extracts and isolated compounds of dietary components have also been demonstrated to induce apoptosis and include *Allium sativum* (garlic), silibinin, aloe, and caffeic acid phenyl ester, found in porpolis (16–19). Polyphenols, as a class of dietary agents, also suppress tumorigenesis, in part through induced apoptosis (20, 21). Clearly, accumulating evidence demonstrates that phytochemicals can be chemoprotective.

Numerous animal studies demonstrate that dietary components can induce apoptosis *in vivo* as a mechanistic means of chemoprevention. For instance, curcumin, quercetin, and rutin induce apoptosis in azoxymethane-induced rat colon carcinogenesis (22–24). Dietary polyphenols from tea reduce prostate cancer in the TRAMP (transgenic adenocarcinoma of the mouse prostate) mouse model of prostate cancer, induce apoptosis in skin tumors of mice exposed to ultraviolet radiation, and protect against chemically induced hepatic tumors in mice (25–28). Polyphenols such as resveratrol, EGCG, vanilloids (including capsaicin and curcumin), and minerals such as selenium can induce apoptosis in many different cell types, including leukemia cells, colon cancer cells, epidermoid cells, prostate cells, transformed bronchial epithelial cells, and glioma cells (14, 29–31). Examples of dietary agents, their food sources, and specific mechanisms of action are given in Table 1. The pro-apoptotic effects of selenium (an indirect antioxidant) *in vitro* have been observed in prostate cancer cells, human fibroblasts, mammary gland cells, and human breast cancer cells (32, 33). Recently, extracts of tomatoes and associated phytochemical lycopene have been shown to induce apoptosis in prostate cells *in vitro* and *in vivo* (34–36). In addition to rodent and *in vitro* studies, human trials have also revealed induction of apoptosis, and a subsequent improved clinical outcome, by numerous dietary components. These results support the idea that apoptosis is a novel molecular target for chemoprevention because of its capacity to slow the progression of, reverse, or inhibit carcinogenesis, ultimately with fewer manifestations of clinically invasive disease.

The potent synergy of an undesirable proliferative stimulus and an associated defect in the pathway of apoptosis appear universal to cancer. However, apoptosis is a complex process with numerous points of potential modulation. As a result, an effective dietary chemopreventive approach would involve ingestion of bioactive components, with induction of apoptosis at any of the myriad apoptotic pathway targets, either singly or in combination. As a result, it is critical that we better understand the process of apoptosis and the molecular targets that may be modulated.

Definition and Biochemistry of Apoptosis

Deregulated apoptotic mechanisms have been implicated in numerous pathologic conditions including AIDS, allograft rejection, Alzheimer's, autoimmunity (lupus, type 1 diabetes, rheumatoid arthritis), infectious diseases, inflammation, cancer, heart failure, osteoporosis, Parkinson's disease, restenosis, stroke, and trauma (37). It is estimated that excessive or inadequate cell death contributes to approximately half of all medical illnesses. It is intriguing that key components in cellular regulation of apoptosis have been identified and thus may be targeted by therapeutic strategies. These targets include death receptors that trigger apoptosis from the cell surface, Bcl-2 proteins as integral regulators of the mitochondrial apoptotic pathway, caspases as the executioner enzymes, and endogenous caspase inhibitors (2).

Programmed cell death (PCD) is a distinct genetic and biochemical pathway of cell death essential to metazoans in maintaining tissue homeostasis without any specification of the mode. Apoptosis, however, is one specific mechanism of cell death with a distinctive phenotype, which regulates tissue homeostasis through the elimination of potentially

Table 1. Modulation of Apoptosis by Dietary Bioactive Agents

Organosulfur compounds		
Diallyl sulfide (DAS)	Allium vegetables, garlic compounds	Upregulate Bax; downregulate Bcl-2
Diallyl disulfide (DADS)	Allium vegetables, garlic compounds	Upregulate p53 and Bax; activate caspase 3; downregulate Bcl-2
Ajoene	Allium vegetables, garlic compounds	Activate caspase 3; downregulate Bcl-2; JNK, p38, ERK activation
Allicin	Allium vegetables, garlic compounds	Activate caspases 3, 8, 9; cleave PARP
S-allyl cysteine (SAC)	Allium vegetables, garlic compounds	Downregulate Bcl-2
S-allylmercaptocysteine (SAMC)	Allium vegetables, garlic compounds	Increase caspase 3 activity; JNK activation
Polyphenols		
Epigallocatechin gallate (EGCG)	Green tea, chocolate	Activate Fas; inhibit NF-κB; caspase activation; alter membrane function
Catechin	Teas	Inhibit p38, PI3K, and AP-1 activation
Genistein	Soybeans	Inhibit NF-κB; activate caspases; induce Bax
Resveratrol	Red grapes, peanuts, berries	Caspase activation; inhibit NF-κB; induce FasL
Curcumin	Turmeric, curry, mustard	Inhibit NF-κB and AP-1; caspase activation; disrupt MTP; induce Bax
Ellagic acid	Strawberries, walnuts, pecans	Increase caspase 3 activation; upregulate p53; activate MAPK, JNK, p38
Capsaicin	Chili peppers	Disrupt MTP; cyto *c* release; inhibit Bcl-2; induce Bax; caspase activation
Isothiocyanate		
Sulforaphane	Cruciferous vegetables, broccoli	Activate ERK; inhibit NF-κB; activate caspase 3; downregulate Bcl-2
Phenethyl isothiocyanate (PEITC)	Radish, cabbage	Inhibit NF-κB; activate caspase 3; cleave BID; inhibit PKC; activate p53
Allyl isothiocyanate (AITC)	Mustard	Activate caspase 8 and JNK; cleave BID; downregulate Bcl-2 & Bcl-xL
Benzyl isothiocyanate (BEITC)	Garden cress	Increase Bax/Bcl-2 ratio; activate caspase 3, JNK, p38; cyto *c* release
Glucosinolate		
Indole-3-carbinol	Cruciferous vegetables	Inhibit NF-κB, PI3K, Akt, Bcl-2, and Bcl-xL; activate caspases; induce Bax;
3,3′-Diindoylmethane	Cruciferous vegetables	induce cyto *c* release; increase TRAIL receptor, downregulate BAD
Carotenoids		
Beta carotene	Orange-yellow vegetables	Alter membrane function
Lycopene	Tomato	Induce cyto *c* release; alter MMP;
Lutein	Dark green vegetables	Induce p53; upregulate Bax; downregulate Bcl-2
Mineral		
Selenium	Cereal grains, meat, fish	Inhibit NF-κB; induce p53; inhibit PKC; alter redox status; modulate JNK

deleterious cells. The term apoptosis is Greek for "falling of the leaves"; the term describes the distinctive phenotypic phenomenon related to cellular shrinkage (38). Depending on the trigger and the mode of cell death, PCD and apoptosis can occur simultaneously or independently as elements of physiologic cell death (38). Apoptosis has further been defined as "a sequence of events based on cellular metabolism that leads to cell destruction with a specific morphology"; this definition distinguishes this process from other forms of cell death, such as autophagy, oncosis, and necrosis (39–42). Typically, apoptosis, an active energy-requiring process, is activated in single cells that are aged, dysfunctional, or damaged by external stimuli.

Phenotypically and morphologically, apoptosis is characterized by chromatin condensation, nuclear fragmentation into mono- and oligonucleosomal units, cell shrinkage, and plasma membrane blebbing (43). Ultimately, cells break into small membrane-surrounded fragments (apoptotic bodies) that are phagocytosed without inducing inflammation (44). Additional early features of apoptosis include marginalization in the nucleus, karyorrhexis, packaging of organelles, and dilatation of the endoplasmic reticulum (38). The early events occur within minutes, while final stages involving lysosomal degradation of cellular components typically are complete in hours.

Regulation of Apoptosis

Compelling evidence indicates that dietary bioactive agents may trigger apoptosis through numerous molecular targets (Fig. 1). Other inducers of apoptosis include both intra- and extracellular stimuli, such as DNA damage, disruption of the cell cycle, hypoxia, detachment of cells from their surrounding tissue, and loss of trophic signaling

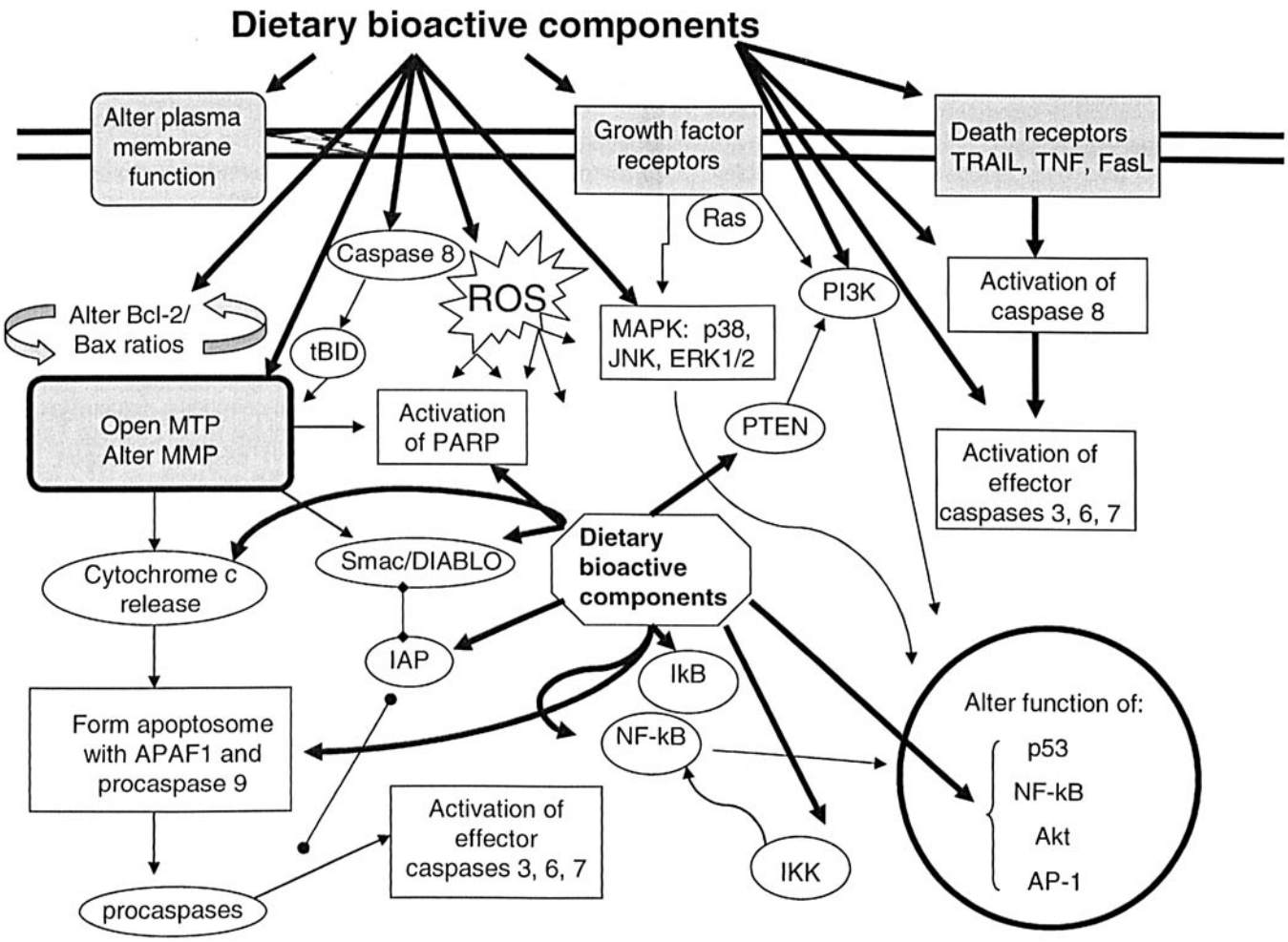

Figure 1. Potential mechanistic targets of bioactive agents in apoptosis.

(45). Apoptosis occurs primarily through two well-recognized pathways in cells (40, 46). Both effector mechanisms of apoptosis are associated with caspase activation and include the intrinsic, or mitochondrial-mediated, effector mechanism and the extrinsic, or death receptor–mediated, effector mechanism (47). In addition to mitochondria, other organelles, including the endoplasmic reticulum, Golgi apparatus, and lysosomes, may also contribute to damage sensing, pro-apoptotic signaling, and caspase activation (45, 48). The endoplasmic reticulum, as an important apoptotic control point, displays anti-apoptotic Bcl-2 and pro-apoptotic Bax and Bak proteins (46). The intrinsic pathway of apoptosis relies primarily on the permeabilization of mitochondrial membranes, with associated release of apoptogenic mitochondrial proteins, leading to activation of caspase 9 and downstream cleavage of caspases 3, 6, or 7 (39). A third pathway involving granzyme has been identified, one that directly activates caspase 3. A critical element and commonality among these pathways is the involvement of caspases and, specifically, the activation of caspase 3, the pivotal committed executioner caspase of apoptosis.

Intrinsic Mitochondrial Pathway. Although apoptosis may be mediated by the interaction between death receptors and their ligands, many dietary bioactive agents induce apoptosis through the intrinsic mitochondria-mediated pathway (10). This pathway is characterized by alterations in mitochondrial polarization and release of mitochondrial proteins, including cytochrome *c*, endonuclease G, secondary mitochondria-derived activator of caspase (Smac)/direct inhibitor of apoptosis protein (IAP) binding protein with low pI (DIABLO), Omi/HtrA2, apoptosis-inducing factor (AIF), and its homolog AIF-homologous mitochondrion-associated inducer of death (45). The release of cytochrome *c* can then trigger caspase activation and ultimately execution of apoptosis.

Mitochondrial Polarization/Depolarization. Mitochondria are intracellular organelles that generate energy for the cell and are thus known as the powerhouse of the cell. Normally the mitochondrion possesses an electrochemical gradient across the inner membrane, which is critical for proper function of the energy-yielding electron-transport chain. The mitochondrion is also a pivotal organelle for the induction of apoptosis *via* the intrinsic pathway. Increased mitochondrial permeability and dissipation of the electrochemical gradient or membrane potential

(MMP) *via* opening of the mitochondrial permeability transition pore (MTP) triggers cell death by releasing apoptogenic factors from within the mitochondria, with subsequent cytochrome *c* release, apoptosome formation, and ultimately apoptosis induction.

Dietary bioactive agents that alter mitochondrial membrane function and/or dissipate the MMP can induce apoptosis. The vanilloids curcumin, found in turmeric, and capsaicin, found in chili peppers, can open the MTP and collapse mitochondrial potential, leading to induction of apoptosis (49). The flavonoid baicilin induces apoptosis in T lymphocytes by inducing cytochrome *c* release and disrupting MMP before activation of caspase 3 (50). Herbal compounds, such as nordihydroguaiaretic acid from chaparral, can also function in this manner (51). Data also show that cytotoxic flavonoids and other dietary phenolic compounds are potent agents in collapsing hepatocyte MMP (49). Curcumin, a polyphenol, induces mitochondrial swelling and collapses the MMP, resulting in apoptosis in numerous cell types (52, 53). Epigallocatechin gallate depolarizes mitochondria in numerous human cell lines, including prostate and lung cells, leading to apoptosis (49). Beta carotene, a carotenoid found in carrots, can induce release of cytochrome *c* from mitochondria and alter mitochondrial membrane potential in different tumor cell lines derived from leukemia, colon adenocarcinoma, and melanoma cells (54). In our laboratory, we have demonstrated that lycopene, a non–provitamin A carotenoid found in tomatoes, can depolarize the mitochondria of human prostate cells, induce cytochrome *c* release, and ultimately induce apoptosis (34). We have also demonstrated that resveratrol, a polyphenol in red wine, can induce apoptosis in a salivary adenocarcinoma cell line with mutated oncogenic Ha-*ras* (55). Thus, numerous diverse dietary bioactive agents can induce apoptosis by altering mitochondrial physiology.

Alteration of Bcl-2/Bax Ratios. Failure to activate apoptosis is one of the major impediments to the successful treatment of cancer. Novel compounds that target apoptosis regulatory pathways and, specifically, the proteins involved are potentially chemopreventive. Such targets include the Bcl-2 family of proteins, since these proteins are molecular integrators of both simultaneous cellular pro-death and pro-survival signals (56). This balance dictates the decision to die or not based on the release of apoptogens from the mitochondria to the cytosol. As a result, Bcl-2 and Bcl-xL have emerged as major new chemoprevention targets.

In the intrinsic mitochondrial pathway, the Bcl-2 family of at least 18 pro- and anti-apoptotic proteins are pivotal regulators of apoptosis, all of which may be targets. Moreover, these proteins may directly or indirectly antagonize each other's functions and are important in connecting signals from the extrinsic death receptor pathway to the mitochondrial pathway. Bax, a Bcl-2 family protein, potently regulates the pro- and anti-apoptotic balance within a cell by regulating mitochondrial function (57). The location and homo- or heterodimerizations of these proteins are important in apoptosis induction and are reviewed elsewhere (58).

Dietary bioactive components can regulate intracellular location of pro-apoptotic proteins (Bcl-2, Bcl-xL) or anti-apoptotic proteins (Bax and Bak) promoting the release of cytochrome *c* from the mitochondria (10). In breast and prostate cell lines, indole-3-carbinol (I3C) and 3,3′-diindolylmethane (DIM) have been shown to affect the ratio and cellular localization of the anti-apoptotic proteins Bcl-2 and Bcl-xL and the pro-apoptotic protein Bax, producing conditions conducive to induction of apoptosis (59, 60). Sulforaphane, an ITC, can increase expression of pro-apoptotic Bax. Beta carotene, a carotenoid, decreases expression of apoptotic Bcl-2 in colon cancer cells (61). The polyphenol stilbene resveratrol decreases Bcl-2 and Bcl-xL levels and increases Bax levels (14, 62). Curcumin downregulates the apoptosis suppressor proteins Bcl-2 and Bcl-xL in several cancer cell lines, thus increasing apoptosis (20). Genistein, a polyphenol, has been shown to regulate Bcl-2 and/or Bax as a pro-apoptotic mechanism, although results have been inconsistent (63). Others have shown that genistein can increase phosphorylation of Bcl-2, upregulate pro-apoptotic Bax, and downregulate the Bcl-2 death suppressor protein, thereby altering programmed cell death by modulating Bax homodimerization (64). The garlic compounds diallyl sulfide (DAS) and diallyl disulfide (DADS) can increase Bax expression, and DAS, *S*-allylcysteine, and ajoene can decrease Bcl-2 expression (65). Additionally, DADS can also reduce Bcl-xL expression. Collectively, numerous diverse dietary bioactive agents can induce apoptosis by modulating the Bcl-2 family of proteins, making this a critical target for bioactive agents.

Bcl-2 proteins may be inhibited by other proteins with similar baculoviral inhibitor of apoptosis repeat-containing gene homology domains. These proteins heterodimerize with death suppressors, including Bcl-2 and Bcl-xL, to modulate apoptosis. The complexity of regulation of this pathway further increases as other proteins may inhibit this process, such as Bim released from disrupted microtubules, Noxa induced by p53, Hrk induced by growth factor deprivation, and Bad induced by calcineurin during calcium influx (37, 66). It is noteworthy that the recently discovered pro-apoptotic Bcl-2 family member Bcl-2 interacting protein (BID) links the intrinsic and extrinsic apoptotic pathways (67). Evidence indicates that dietary agents such as plubagin, a plant-derived naphthoquinone, and diterpenes, found in citrus peel, inhibit nox-4 activity and upregulate BID, respectively, in human kidney, brain tumor, and prostate cancer cells (68, 69).

The inhibitor of apoptosis protein (IAP) family shares a structural motif known as BIR and binds and inhibits the action of caspases, thereby blocking apoptosis. This occurs downstream of both intrinsic Bcl-2 family members and the external death receptor–mediated pathways representing important molecular targets. Bioactive agents that bind or

interfere with IAP may reverse its anti-apoptotic function (70).

Mitochondrial Cytochrome *c* Release. Cytochrome *c* is found in cells attached to the outer surface of the inner mitochondrial membrane and is largely localized in the cristae, where the protein functions in the electron-transport system. During apoptosis, cytochrome *c* is released from the cristae into the cytosol, a pivotal step in apoptosis initiation. This can be induced by various stimuli, including elevations in pore-forming pro-apoptotic Bcl-2 family proteins such as Bax, as discussed above. Once released to the cytoplasm, cytochrome *c* binds and activates apoptotic protease activating factors (Apaf-1), enabling binding and activation of procaspase 9, an initiator caspase. This process is suppressed by molecules that prevent cytochrome *c* release, including the anti-apoptotic Bcl-2 proteins. In our laboratory, we have found that lycopene delivered at physiological concentrations can induce cytochrome *c* release in human prostate cells, and, in fact, concentrations equivalent to the plasma level found in those consuming three to five daily servings of fruits and vegetables also induced this change (34). Green tea polyphenols (i.e., EGCG) induced cytochrome *c* release *in vitro* and *in vivo* in metastatic mouse mammary carcinoma cells, as well as altered Bcl-2/Bax protein ratios, increased Apaf formation, and cleaved caspase and poly(ADP-ribose) polymerase (PARP) proteins (71). Dietary ginger, including curcumin, 6-gingerol, and other diterpenes, induced apoptosis in T lymphocytes by inducing alteration of MMP and increasing cytochrome *c* release (72). The lanostanoid triterpene ganoderic acid has been shown to induce apoptosis in human hepatoma cells by decreasing Bcl-2 expression, altering MMP, inducing cytochrome *c* release, and ultimately activating caspase 3 (73). Flavonoid-rich grapeseed extract has been shown to induce apoptosis in human prostate cells by increasing cytochrome *c* release and PARP cleavage (17). Numerous examples demonstrate that dietary bioactive agents can induce mitochondrial release of cytochrome *c*.

Activation of Caspases. Caspases, comprised of 12 proteins, are a family of cysteinyl aspartate–specific proteases involved in apoptosis and are subdivided into initiator (caspases 8, 9, 10) and executioner (caspases 3, 6, 7) caspases (2). Modulating the mechanisms of caspase activation and suppression is a critical molecular target in chemoprevention, since these processes lead to apoptosis (74).

The intrinsic and extrinsic pathways converge at caspase 3. Active caspase 9 and caspase 8 of the intrinsic and extrinsic pathways, respectively, have been shown to directly cleave and activate the effector protease caspase 3. Caspase 3 cleaves and activates directly or indirectly other effector caspases, such as caspases 6 or 7. Active caspases, including caspases 3, 7, and 9, can be directly inhibited by some IAP family proteins, such as X chromosome–linked IAP (XIAP). Inhibitor of apoptosis proteins are suppressed by Smac/DIABLO, which is released from mitochondria. The transcription factor NF-κB induces expression of apoptosis suppressors, including certain IAP family genes and some anti-apoptotic Bcl-2 family genes. The kinase Akt can phosphorylate and inactivate Bad as well as caspase 9 (37). Thus, there is considerable overlap between the intrinsic and extrinsic pathways.

Dietary components can induce apoptosis through caspase activation. Resveratrol increases caspase activity (caspases 6, 3, and 9) in numerous models, including normal and hematopoietic cells (75, 76). The ITCs benzyl ITC (BITC), phenethyl ITC (PEITC), and phenylbutyl significantly induce apoptosis in cultured human and animal cell lines as well as animal tissues and cancer cell xenografts through stimulation of caspase 3–like activity and degradation of PARP (77). Phenethyl ITC, phenylmethyl isocyanate, and BITC activate caspase 3 (78). Isothiocyanate activates caspases in multiple pathways, including caspase 9 (mitochondria), caspase 8 (death receptor), and caspase 12 (endoplasmic reticulum) in conjunction with activation of caspase 3 (77). Indole-3-carbinol upregulates Bax, induces the release of cytochrome *c*, and activates caspases 3 and 9 (60). The organosulfur compounds found in garlic have shown potent pro-apoptotic activity in numerous models (69). For example, DADS, *S*-allylmercaptocysteine, and ajoene upregulate caspase 3 activity in human breast cancer cells, human colon cells, and murine melanoma cells (79–81). Allicin activates caspases 3, 8, and 9 and induces cleavage of PARP. Regarding the mineral selenium, which may be incorporated into garlic compounds, exposure of prostate cells to selenium led to DNA fragmentation and caspase-mediated cleavage of PARP associated with apoptosis (82).

Extrinsic Death Receptor Pathway. The extrinsic pathway is triggered by members of the tumor necrosis factor (TNF) receptor superfamily, which comprises almost 20 members of cytokine receptors, such as TNFR1, Fas, and TNF-related apoptosis inducing ligand (TRAIL) receptors (83, 84). These proteins recruit adapter proteins, including FADD, to their cytosolic death domains, with subsequent binding to pro-caspases, particularly caspase 8, which contains a protein interaction motif (the death effector domain, or DED) that binds a complementary domain in FADD. Next, intracellular recruitment of the death-inducing signaling complex (DISC) occurs by means of protein/protein interactions involving death domains (2). The DISC plays a central role in the extrinsic pathway by activating the initiator caspases 8 and 10 (67). As one would expect, this pathway is suppressed by DED-containing antagonists of Fas and pro-caspase 8, such as FLIP.

Inhibitor of apoptosis proteins are a family of evolutionarily conserved anti-apoptotic proteins that bind caspases 3, 7, and 9 and modulate cell division, cell cycle progression, and signal transduction (37). They are potentially clinically useful in the diagnosis and treatment of occult malignancy and, thus, are considered valid therapeutic targets (85). The I3Cs present in cruciferous vegetables can induce apoptosis

by downregulation of Bcl-2, Bcl-xL, IAP, XIAP, and FLIP (60). Resveratrol has been shown to trigger CD95 signaling-dependent apoptosis in human tumor cells (86). Dietary diterpenoids induce apoptosis in human prostate cells by upregulation of Fas ligand (69). Thus, accumulating evidence indicates that dietary bioactive agents can trigger specific aspects of the extrinsic apoptosis pathway.

Bioactive Agents in Neoplastic and Nonneoplastic Cells

Current strategies of cancer treatment, such as chemotherapy and ionizing radiation, induce apoptosis of cancer cells. Clearly, if apoptosis could be selectively induced in cancer cells by dietary components, one could use diet as an effective chemoprevention strategy. Resveratrol, a polyphenol in red wine and grapes, can selectively target tumor cells with presumably dysfunctional cell cycle checkpoints and spare normal tissue (87). This effect may be dependent on the p53 status (i.e., mutated or not) of cells exposed to resveratrol. Differential inhibition by EGCG on NF-κB activation results in cancer cells being more responsive than noncancerous cells to EGCG, and the effect is dose dependent (88). Epigallocatechin gallate affects a p57-mediated survival pathway in normal epithelial cells while inducing a pro-apoptotic pathway in oral carcinoma cells (89). It appears that low concentrations of EGCG primarily induce MAPK prosurvival genes, but at high concentrations of EGCG, both MAPK and caspase pathways are activated. Diallyl trisulfide (DATS), a garlic compound, effectively inhibits proliferation and induces apoptosis in human lung cancer cells but not in nonneoplastic lung cells (90). Ajoene induces apoptosis in human acute myeloid leukemia cells and peripheral blood mononuclear cells (PBMC) from chronic leukemia patients but not quiescent and proliferating cells from healthy donors (90, 91). Beta carotene, a carotenoid in orange vegetables, induces apoptosis preferentially in various tumor cells from human prostate, colon, breast, and leukemia. Conversely, normal cells are largely resistant to the induction of apoptosis by beta carotene (61). Other studies report that vitamin E, vitamin C, selenium, and some phytochemicals selectively induce apoptosis in cancer cells while sparing normal cells (32). Animal studies have also demonstrated that certain chemopreventive agents can induce apoptosis in tumor cells *in vivo* without affecting normal cells (45). Thus, selective targeting of dietary bioactive agents to cancer cells may be a therapeutic option.

Timing and Duration of Dietary Exposure and Apoptosis

The primary pro-apoptotic signaling events induced by dietary bioactive agents share many commonalities. However, differences have been noted regarding the temporal occurrence and duration of some events, emphasizing them as important considerations. For instance, both PEITC and EGCG induce c-Jun-N-terminal kinase (JNK) activation, alter mitochondrial potential, induce cytochrome *c* release, and activate caspases. Interestingly, PEITC mediates most of its effects in the short term (2–4 hrs), whereas EGCG exerts identical effects after 12 hrs (10). It has been suggested that prolonged exposure to some dietary agents may sustain activation of JNK, inhibit NF-κB activity, and interfere with growth factor signaling. Thus, the duration of a signal appears important in determining the biological outcome. Moreover, the effects of bioactive agents will differ depending on whether they target a signaling mechanism that is transient or sustained (12). Ultimately, these events exert strong pressure for cells to undergo apoptosis.

Since genetic mutations in cancer are cumulative, it is likely that dietary exposure will exhibit effects that are dependent, in part, on the specific mutation and relevant timing of the alteration within the cell cycle, conferring, in essence, a degree of temporal dependence. Resveratrol exhibits apparent, but not exclusive, selectivity toward tumor cells, presumably because of dysfunctional checkpoints within tumor cells, thereby potentially altering the course of tumorigenesis (92). In our laboratory, we have observed induction of apoptosis in WR-21 salivary adenocarcinoma cells possessing a mutated Ha-*ras* oncogene after overnight exposure to resveratrol (55). Gene and protein expression analyses indicated that the p53 pathway and cell cycle checkpoints were temporally modulated. In time-course studies, we observed clear cycling of gene and protein expression of p53, $p21^{cip}$, Rb, Mdm, and cyclin G at early time points (<8 hrs) but, ultimately, loss of Rb and p53 protein expression over 24 hrs, indicating collapse of G1 and G2 checkpoints. Collectively, the data support the occurrence of mitotic catastrophe after resveratrol exposure for 24 hrs, but they also demonstrate time-dependent resveratrol-mediated changes in gene and protein expression (55).

Inhibitors of Apoptosis in Carcinogenesis

Diseases associated with increased apoptosis are common. For instance, AIDS; neurodegenerative disorders including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis; and cerebellar degeneration are associated with increased apoptosis, as are myelodysplastic syndromes such as aplastic anemia. In addition, ischemic injury also can occur, inducing myocardial infarction, stroke, and reperfusion injury. Toxicant-induced liver disease can also induce widespread apoptosis as well. Collectively, increased apoptosis can contribute to numerous pathologies.

Treatment that increases cellular resistance to apoptosis may be beneficial in some instances and particularly in those associated with significant cell loss. Such treatment might entail increased hematopoiesis coupled with inhibition of apoptosis after cancer chemotherapy as a means of replenishing cell populations. Several observations indicate

that apoptosis may be mediated in a cell-specific manner, in which cells of one biological compartment are induced to die and others are not. As reported previously, there are examples of dietary components that can induce apoptosis in cancer cells without doing so in normal cells. This strategy may be useful in cancer treatment.

Reactive Oxygen Species (ROS), Oxidative Stress, and Apoptosis

Many growth factors (i.e., epidermal growth factor [EGF] and platelet-derived growth factor [PDGF]) bind their receptors and generate large increases in ROS. Oncogenic proteins involved in signaling, such as the G transducer protein *ras*, also produce elevated ROS upon stimulation, indicating broad overlap of ROS production and signal stimulation. This supports potential cross-talk among pathways and potential feedback regulation.

Many chemopreventive agents can modulate genes or proteins that respond to conditions of oxidative stress and subsequently trigger apoptosis. Induction of oxidative stress directly or indirectly exhibits effects on the mitochondria because these organelles are important regulators of cellular redox status and trigger the intrinsic apoptotic pathway (45). While phytochemicals with antioxidant activity may protect macromolecular structure, these compounds may also quench those ROS involved in apoptosis.

ROS function as second messengers for several cytokines and growth factors through alterations in cellular redox status and modifications of proteins, all of which may be affected by oxidants and antioxidants (61). Some dietary compounds, such as vitamin C in fruits, EGCG in green tea, and curcumin in turmeric, function as both oxidants and antioxidants (59). For example, soy isoflavones function as antioxidants and quench ROS needed for NF-κB activation (93). The pattern of apoptosis induction by beta carotene is consistent with the distortion of redox balance of cancer cells (61). Capsaicin and curcumin, naturally occurring phenolic and polyphenolic compounds, function as dietary antioxidants but may exert pro-oxidant activity promoting cytotoxic events or altering redox balance that regulates, in part, transcription factors (56). Beta carotene is an antioxidant that can inhibit free radical production or function as a pro-oxidant propagating free radical–induced reactions depending on the intrinsic properties and redox potential of the biological environment (61). Diallyl disulfide can induce ROS production and increase hydrogen peroxide levels, and ajoene participates in ROS-modulated apoptosis. Epigallocatechin gallate and theaflavins have been shown to inhibit apoptosis under certain conditions, such as toxicant exposure. Overall, many dietary components can paradoxically function as oxidants and antioxidants depending on the cellular milieu, which can alter cellular redox status. This may contribute to the disparate results reported in the literature.

Effects of Bioactive Agents on Plasma Membrane Function

As a result of their lipophilicity, some dietary agents can partition within membranes and potentially affect cellular function, including alteration of membrane fluidity, redox status, and lipid-protein interactions. Capsaicin can alter the plasma membrane structure and fluidity in a concentration-dependent manner (94). Curcumin promotes thiol oxidation in isolated rat mitochondria and alters membranes of red blood cells. In our studies, we have observed changes in membrane polarization using fluorescent redox-sensitive dyes, decreased lactate dehydrogenase (LDH) release, and the translocation of phosphatidylserine residues indicative of altered membrane function (34). In other studies, we have shown that beta carotene and lutein after overnight incubation interfere with protein and glucose transport in HepG2 human liver cells (95). Collectively, alteration of the plasma membrane by lipophilic dietary agents may alter function and, ultimately, the cellular signaling involved in apoptosis.

Gene Expression and Apoptosis

In the past decade, the identification of genes and gene products that regulate apoptosis coupled with increasing elucidation of mechanistic targets and modes of action have been critical in the search for compounds targeting apoptosis. Exploitation of such new apoptosis targets remains a considerable focus of attention as a means of chemotherapy (96–98). It has also become increasingly apparent that myriad genomic interrelationships with the diet exist. Moreover, genetic changes arising as a result of single point mutations, rearrangement, or copy number involving either deletions or additions can likely influence the response of an individual to various dietary components and, thus, are a critical consideration. Physiologically active dietary components can modify transcription, translation, and metabolism *in vivo* and, as a result, can simultaneously contribute to numerous, diverse processes such as cell cycle, cell signaling, and apoptosis (9). Genetic polymorphisms may be responsible, in part, for wide variations in individual responses to dietary components and subsequent activation or inactivation of pathways such as apoptosis. Studies have been undertaken to define and better characterize responses and the physiologic consequences of genetic polymorphisms (4, 8).

Cellular Signaling. The reduction of growth factor–induced proliferative signaling in many cases permits the initiation of apoptotic cascades. The proliferative signals exerted through interaction of growth factor receptors and their ligands, including insulin-like growth factor (IGF), EGF, and vascular epithelial growth factor (VEGF), strongly drive cells to proliferate in carcinogenesis. As a result, those dietary agents that can interrupt this signaling would be beneficial *via* decreased proliferative and pro-apoptotic signaling. Indeed, numerous phytochemicals,

including resveratrol, curcumin, polyphenols, and catechins, can inhibit the aforementioned growth factor signaling pathways (20, 99). In fact, the TNF family member Apo2L/TRAIL receptor has received considerable recent attention as a therapeutic target, since many cancer cells appear sensitive to Apo2L/TRAIL–induced apoptosis (83). Epigallocatechin gallate, a polyphenol in tea, directly blocks EGF binding to its receptor and interrupts signaling.

The MAPK kinase signaling cascades include extracellular signal-related protein kinases (ERKs), JNKs/stress-activated protein kinases (SAPKs), and p38 kinases. The ERKs transmit signals initiated by growth promoters, including EGF, PDGF, and fibroblast GF (FGF) and may ultimately foster cell growth and survival (63). The polyphenols curcumin, EGCG, and resveratrol downregulate phosphorylation and ligand binding of growth factor receptors including EGF, FGF, and PDGF (12). Consequently, this quenches MAPK signaling, transcription factor activation (i.e., AP-1), and ultimately gene expression. It is noteworthy that many cells require such signals to avoid apoptosis, and, as a result, interruption of this signaling encourages induction of apoptosis in many cell types. For example, the indirect inhibition of PI3-Akt anti-apoptotic signals might contribute to cell death through modulation by diet (10). The MAPKs are activated by translocation to the nucleus, where they phosphorylate numerous substrates, including the transcription factors AP-1 and NF-κB. Activation of both are linked to carcinogenesis and tumor promotion (63).

Indeed, numerous mutations can occur in tumor suppressor genes involved in induction of apoptosis, and these include *p53, p19ARF, Rb, PTEN, TRAIL,* and *CD95/Fas* (100). Numerous oncogenes may also be activated through mutation to inhibit or circumvent the inherent controls of apoptosis, and these include *Bcl-2, MDM2, IAPs, NF-κB, Akt, PI3K, Ras, Myc,* and *FLIP* (100). Blocking the expression of genes, and in particular oncogenic *ras,* is currently an active pharmacological approach for cancer therapy (101). Clearly mutations in genes that regulate apoptosis pathways are common in most cancers, emphasizing the importance of apoptosis in carcinogenesis and protection of these genes against DNA damage (45).

Many dietary agents can affect cellular signaling. Resveratrol stimulates complex formation between p53, ERK, and p38 kinase, with enhanced phosphorylation, stabilization, and activation of p53 in epidermal cells (92). Indole-3-carbinol and DIM inhibit the MAPK pathway, which may inhibit cancer cell survival. Curcumin reduces the activity of p38 MAPK, and EGCG inhibits tyrosine kinase and MAPK activation in transformed cells but not normal cells. Capsaicin markedly activates JNK-1 and p38 MAPK signaling in Ha-*ras*–transformed human breast epithelial cells (56). In cells with mutated oncogenic Ha-*ras,* green and black tea polyphenols potently inhibited ERK phosphorylation and AP-1 activity (102). Allyl ITC (AITC), BITC, and PEITC increased activity of JNK in HL-60 cells. MAPK, ERK, and p38 kinase were activated by PEITC in HT29 and PC3 cells. BITC activated p38 kinase in human head and neck squamous cell carcinoma lines (77). Regarding garlic compounds, DADS induced ROS and JNK, *S*-allylmercaptocysteine induced JNK-1 activation and *jun* kinase activity, and ajoene activated MAPKs (JNK, p38, ERK1/2) in different cell types (65). Indole-3-carbinol inhibits signaling through protein kinase B and binding of NF-κB to DNA (103).

Transcription Factors. *NF-κB.* Activation of NF-κB promotes survival and cellular proliferation, and downregulation sensitizes cells to apoptosis. Many phytochemicals inhibit NF-κB activity, most notably curcumin, green tea, 6-gingerol, and resveratrol (20). Phenethyl ITC, sulforaphane, and curcumin strongly inhibit lipopolysaccharide-induced NF-κB activation and consequently intensify pro-apoptotic signals (10). Lastly, NF-κB is a key transcription factor involved in integration of multiple survival signaling pathways, including upregulation of Bcl-xL, IAPs (XIAP and cIAP-2), and the antagonist of death receptor signaling Flip (99).

It is widely accepted that NF-κB, as well as its regulators IKK and IκB, are associated with survival from apoptosis and other physiologic processes (104). Experimental data indicate that genistein, I3C, curcumin, EGCG, and apigenin inhibit activation of NF-κB in different cell lines derived from cancer tissues (93). Moreover, genistein has been shown to inhibit NF-κB activation and DNA binding activity in prostate cells. Genistein inhibits phosphorylation of IκB and the translocation of NF-κB subunits to the nucleus in epithelial and myeloid cells, possibly through inhibition of MEKK1 kinase activity (93). Thus, this occurs, in part, through decreased phosphorylation of IκB and inactivation of NF-κB (93). Indole-3-carbinol also significantly inhibited NF-κB DNA binding activity, with induction of apoptosis following in prostate cells. Curcumin has been shown to inhibit IKK, alter AP-1 activity, and suppress constitutive and inducible NF-κB activation signaling. Exposure to EGCG significantly inhibited, in a time- and dose-dependent manner, activation and translocation of NF-κB to the nucleus by suppressing the degradation of IκB. The EGCG also inhibited activation of IKK and phosphorylation of IκB. The EGCG concurrently stabilized p53 and negatively regulated NF-κB activity, altered the ratio of Bax to Bcl-2, and induced apoptosis. Apigenin also downregulated NF-κB activity (93). Resveratrol potently inhibited NF-κB activation and resultant gene expression through inhibition of IκB kinase activity (105). Furthermore, data indicate that resveratrol may suppress phosphorylation and p65 subunit translocation to the nucleus (106).

p53 Transcription Factor. p53 is a sequence-specific transcription factor and critical tumor suppressor gene that is the most frequently mutated in human cancer (107). p53 transactivates genes that mediate apoptosis and has roles in DNA repair, senescence, and cell cycle arrest (107). There is

broad consensus that the primary physiologic role of p53 in DNA damage–induced apoptosis is to function as a transcriptional activator of genes encoding apoptosis effectors. p53 directly activates transcription of several genes encoding members of the Bcl-2 family, but it also mediates cell death through a variety of mechanisms, including downregulation of anti-apoptotic genes such as Map4 and survivin and upregulation of pro-apoptotic genes such as Bax, IGF-BP3, DR5, Fas, and Apaf-1, as well as various other apoptosome components representing potentially key therapeutic targets (108–110). p53 has also been demonstrated to exhibit a direct apoptogenic role in the mitochondria, where it translocates and interacts with Bcl-xL and Bcl-2 proteins to induce mitochondrial permeabilization (111). Given the central nature of p53 in the apoptotic response, it is not surprising that perturbations of proteins known to regulate p53 also affect the apoptotic program. Moreover, p53 deficiency leads to inappropriate survival of cells with DNA damage and therefore predisposes one to develop neoplasia.

Recently, p63 and p73 proteins have also been identified that bind p53 response elements and transactivate p53-associated genes and, as a result, induce apoptosis. Furthermore, there is extrinsic overlap of p53 and multiple transcriptional targets, in which p53 can activate at least two proteins in the intrinsic pathway, including Bax and p53-apoptosis inducing factor (112). Reactive oxygen species have been strongly correlated with p53-mediated apoptosis. Upon overexpression of p53, ROS levels rise, and mitochondrial apoptosis is induced as described above. Inhibition of ROS-mediated apoptosis has also been reported in smooth muscle cells (113). Resveratrol, a polyphenol in grapes, induces stabilization and activation of p53 (92). Epigallocatechin gallate dose-dependently increases p53 expression in prostate cells with wild-type p53 but not DU145 prostate cells with mutant p53 (114).

AP-1 Transcription Factor. In addition to intrinsic genetic variability, inhibition of growth factor pathways and pathways associated with antagonism of apoptosis has been shown to be beneficial in numerous cell models. Specifically, the AP-1 activation pathway is oncogenic and antagonizes apoptosis in neoplasia. Numerous reports indicate NF-κB activation fosters cell survival through maintenance of cellular proliferation and decreased sensitization to apoptotic signaling. This occurs through alteration of gene expression with upregulation of NF-κB, Bcl-2, Bcl-XL, cIAP, survivin, cyclin D1, TRAF-1, and TRAF-2 (115). Numerous dietary components have been shown to inhibit AP-1 and NF-κB activation, as well as other anti-apoptotic transcription factors, and include resveratrol, curcumin, and green tea (106, 116, 117). Topical application of capsaicin and curcumin suppresses TPA-induced activation of NF-κB and AP-1 in mouse epidermis.

Key Interactions of Dietary Bioactive Components

Accumulating evidence supports the notion that provision of a combination of dietary bioactive agents is more effective than treatment with a single dietary component. Thus, it is reasonable to assume that combinations of components could be designed to either target multiple pathways or exhibit potentiation or synergy of specific single pathways. For example, consumption of the tea polyphenol EGCG in combination with curcumin markedly increased cytochrome *c* release, activation of caspases (caspases 3, 8, and 9), and cleavage of BID to a greater extent than either agent alone in human prostate cells (73, 118, 119). In human prostate cancer cells, a combination of low-dose curcumin and TRAIL ligand increased apoptosis more in combination than either did alone. Resveratrol has been shown to enhance apoptosis of certain drugs (63). The organosulfur compounds *S*-allylcysteine and lycopene independently reduced chemically induced carcinogenesis in rats, but the combination was markedly more effective in altering apoptosis-associated proteins in gastric cancer (120). In human leukemia cells, ellagic acid, a polyphenol in fruits, and quercetin, a polyphenol, interacted synergistically to induce apoptosis through increased p53 phosphorylation and induction of MAPK, JNK, and p38 pathways (121). Moreover, inclusion of a third compound (viz., resveratrol found in grapes) further enhanced the pro-apoptotic activity (122).

The interaction of components of the diet clearly requires more focus and research. It appears that numerous molecular targets exist *in vivo* and collectively converge on several signaling pathways. This affords the possibility of using combinatorial therapy, with dietary agents that affect many targets each leading to the induction of apoptosis.

Summary

Accumulating evidence clearly indicates that apoptosis is a critical molecular target for dietary bioactive agents for chemoprevention of cancer. Apoptosis, however, is a complex process comprised of intrinsic and extrinsic pathways, with numerous specific targets within each arm. It is encouraging, however, that single bioactive dietary agents can directly and indirectly influence most, if not all, of the myriad targets within apoptosis. Additionally, many of these dietary agents appear to exhibit some degree of specificity for neoplastic cells while sparing normal cells. Moreover, the protective effects of some single agents are potentiated and/or synergized by other dietary agents, supporting the notion that a combinatorial approach will be especially effective in chemoprevention. While encouraging, there are many considerations that remain, such as the issue of appropriate dose of each agent, appropriate timing and duration of exposure, importance of cell type specificity, relative bioavailability of each agent, and potentially adverse side effects and interactions. Clearly

more research is needed to fully identify phytochemical-specific molecular targets and to understand the underlying molecular mechanisms of action of the myriad bioactive dietary chemopreventive agents.

1. Reed J. Mechanisms of apoptosis avoidance in cancer. Curr Opin Oncol 11:68–75, 1999.
2. Fischer U, Schulze-Osthoff K. Apoptosis-based therapies and drug targets. Cell Death Differentiation 12:942–961, 2005.
3. Finley J. Proposed criteria for assessing the efficacy of cancer reduction by plant foods enriched in carotenoids, glucosinolates, polyphenols, and selenocompounds. Ann Bot 95:1075–1096, 2005.
4. Milner J. Functional foods: the US perspective. Am J Clin Nutr 71: 1654S–1659S, 2000.
5. Forman M, Hursting S, Umar A, Barrett J. Nutrition and cancer prevention: a multidisciplinary perspective on human trials. Annu Rev Nutr 24:223–254, 2004.
6. Willett W. Diet, nutrition, and avoidable cancer. Environ Health Perspect 103:165–170, 1995.
7. Peto R, Doll R, Buckley J, Sporn M. Can dietary beta carotene materially reduce human cancer rates? Nature 290:201–208, 1981.
8. Milner J. Molecular targets for bioactive food components. J Nutr 134:2492S–2498S, 2004.
9. Davis C, Milner J. Frontiers in nutrigenomics, proteomics, metabolomics and cancer prevention. Mutat Res 551:51–64, 2004.
10. Chen C, King A. Dietary cancer-chemopreventive compounds: from signaling and gene expression to pharmacological. Trends Mol Med 26:318–328, 2005.
11. Kelloff G, Lieberman R, Steele V, Boone C, Lubet R, Kopelvitch L, Malone W, Crowell J, Sigman C. Chemoprevention of prostate cancer: concepts and strategies. Eur Urol 35:342–350, 1999.
12. Manson M, Farmer P, Gescher A, Steward W. Innovative agents in cancer prevention. Recent Results Cancer Res 166:257–275, 2005.
13. Narayanan B, Narayanan N, Stoner G, Bullock B. Interactive gene expression pattern in prostate cancer cells exposed to phenolic antioxidants. Life Sci 70:1821–1839, 2002.
14. Lee E, Min H, Park H, Chung H, Kim S, Han Y, Lee S. G2/M cell cycle arrest and induction of apoptosis by a stilbenoid, 3,4,5-trimethoxy-4′-bromo-cis-stilbene, in human lung cancer cells. Life Sci 75:2829–2839, 2004.
15. Surh Y. Molecular mechanisms of chemopreventive effects of selected dietary and medicinal phenolic substances. Mutat Res 428:305–327, 1999.
16. Borek C. Antioxidant health effects of aged garlic extracts. J Nutr 131: 1010S–1015S, 2001.
17. Dhanalakshmi S, Singh R, Agarwal C, Agarwal R. Silibinin inhibits constitutive and TNF alpha-induced activation of NF-κB and sensitizes human prostate carcinoma DU145 cells to TNF alpha-induced apoptosis. Oncogene 21:1759–1767, 2002.
18. Furukawa F, Nishikawa A, Chihaza T, Shimpo K, Beppu H, Kuruya H, Lee I, Hirose M. Chemopreventive effects of *Aloe arborescens* on N-nitrobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters. Cancer Lett 178:117–122, 2002.
19. Usia T, Banskota A, Tezuka Y. Constituents of Chinese propolis and their antiproliferative activities. J Nat Prod 65:673–676, 2002.
20. Dorai T, Aggarwal B. Role of chemopreventive agents in cancer therapy. Cancer Lett 215:129–140, 2004.
21. Lambert J, Hong J, Yang G, Liao J, Yang C. Inhibition of carcinogenesis by polyphenols: evidence from laboratory investigations. Am J Clin Nutr 81:284S–291S, 2005.
22. Sanaha H, Kelloff G, Steele V, Rao C, Reddy B. 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 57:1301–1305, 1997.
23. Yang K, Lamprecht S, Liu Y, Shinozaki H, Fan K, Leung D. Chemoprevention studies of the flavonoids quercetin and rutin in normal and azoxymethane-treated mouse colon. Carcinogenesis 21: 1655–1660, 2000.
24. Reddy B, Wang C, Samaha H, Lubet R, Steele V, Kelloff G. Chemoprevention of colon carcinogenesis by dietary perillyl alcohol. Cancer Res 57:420–425, 1997.
25. Gupta S, Hastak K, Ahmad N, Lewin J, Mukhtar H. Inhibition of prostate carcinogenesis in TRAMO mice by oral infusion of green tea polyphenols. Proc Natl Acad Sci U S A 98:10350–10355, 2001.
26. Lu Y, Lou Y, Li X, Xie J, Brash D, Huang M. Stimulatory effect of oral administration of green tea or caffeine on ultraviolet light-induced increases in epidermal wild-type p53, p21, and apoptotic sunburn cells in SKH-1 mice. Cancer Res 60:4785–4791, 2000.
27. Lu Y, Lou Y, Xie J, Yen P, Huang M, Conney A. Inhibitory effect of black tea on the growth of established skin tumors in mice: effects on tumor size, apoptosis, necrosis, and bromodeoxyuridine incorporation into DNA. Carcinogenesis 18:2163–2169, 1997.
28. Klaunig J, Kamendulis L. Mechanisms of cancer chemoprevention in hepatic carcinogenesis: modulation of focal lesion growth in mice. Toxicol Sci 52:101–106, 1999.
29. Sundaram N, Pahwa A, Ard M, Lin N, Perkins E, Bowles A. Selenium causes growth inhibition and apoptosis in human brain tumor cell lines. J Neurooncol 46:125–133, 2000.
30. Pan M, Chang W, Lin-Shiau S, Ho C, Lin J. Induction of apoptosis by garcinol and curcumin through cytochrome *c* release and activation of caspases in human leukemia HL-60 cells. J Agr Food Chem 49:1464–1474, 2001.
31. Gupta S, Ahmad N, Nieminen A, Mukhtar H. Growth inhibition, cell cycle dysregulation, and induction of apoptosis by green tea constituent epigallocatechin gallate in androgen-sensitive and androgen-insensitive human prostate carcinoma cells. Toxicol Appl Pharmacol 164:82–90, 2000.
32. Borek C. Dietary antioxidants and human cancer. Integrative Cancer Ther 3:333–341, 2004.
33. Dong Y, Zhang H, Hawthorn L, Ganther H, Ip C. Delineation of the molecular basis for selenium-induced growth arrest in human prostate cancer cells by oligonucleotide array. Cancer Res 63:52–59, 2003.
34. Hantz H, Young L, Martin K. Physiologically attainable concentrations of lycopene induce mitochondrial apoptosis in LNCaP human prostate cancer cells. Exp Biol Med 230:171–179, 2005.
35. Kim H, Bowen P, Chen L, Duncan C, Ghosh L, Sharifi R, Christov K. Effects of tomato sauce consumption on apoptotic cell death in prostate benign hyperplasia and carcinoma. Nutr Cancer 47:40–47, 2003.
36. Hwang E, Bowen P. Cell cycle arrest and induction of apoptosis by lycopene in LNCaP human prostate cells. J Med Food 7:284–289, 2004.
37. Reed J. Apoptosis-regulating proteins as targets for drug discovery. Trends Mol Med 7:314–319, 2001.
38. Holdenrieder S, Stieber P. Apoptotic markers in cancer. Clin Biochem 37:605–617, 2004.
39. Guimaraes C, Linden R. Programmed cell death: apoptosis and alternative death styles. Eur J Biochem 271:1638–1650, 2004.
40. Lockshin R, Zakeri Z. Apoptosis, autophagy, and more. Int J Biochem Cell Biol 36:2405–2419, 2004.
41. Shintani T, Klionsky D. Autophagy in health and disease: a double-edge sword. Science 306:990–995, 2004.
42. Nicotera P, Melino G. Regulation of the apoptosis-necrosis switch. Oncogene 23:2757–2765, 2004.
43. Rodriguez M, Schaper J. Apoptosis: measurement and technical issues. J Mol Cell Cardiol 38:15–20, 2005.

44. Lauber K, Blumenthal S, Waibel M, Wesselberg S. Clearance of apoptotic cells: getting rid of corpses. Mol Cell 14:277–287, 2004.
45. Sun S, Hail N, Lotan R. Apoptosis as a novel target for cancer chemoprevention. J Natl Cancer Inst 96:662–672, 2004.
46. Danial N, Korsmeyer S. Cell death: critical control points. Cell 116: 205–219, 2004.
47. Reed J. Apoptosis mechanisms: implications for cancer drug discovery. Oncology 18:11–20, 2004.
48. Jaattela M. Multiple cell death pathways as regulators of tumour initiation and progression. Oncogene 23:2746–2756, 2004.
49. Galati G, O'Brien P. Potential toxicity of flavonoids and other dietary phenolics: significance for their chemopreventive and anticancer properties. Free Rad Biol Med 37:287–303, 2004.
50. Ueda S, Makamura H, Masutani H, Sasada T, Takabayashi A, Yamaoka Y, Yodoi J. Baicilin induces apoptosis via mitochondrial pathway as pro-oxidant. Mol Pharmacol 38:781–791, 2001.
51. Biswal S, Datta K, Shaw S, Feng X, Robertson J, Kehrer J. Glutathione oxidation and mitochondrial depolarization as mechanisms of nordihydroquaiaretic acid-induced apoptosis in lipoxygenase-deficient cells. Toxicol Sci 53:77–83, 2000.
52. Morin D, Barthelemy S, Zini R, Labidalle S, Tillement J. Curcumin induces the mitochondrial permeability transition pore mediated by membrane protein thiol oxidation. FEBS Lett 495:131–136, 2001.
53. Kim J, He L, Lemasters J. Mitochondrial permeability transition: a common pathway to necrosis and apoptosis. Biochem Biophys Res Comm 304:463–470, 2003.
54. Palozza P, Serini S, Torsello A, Di Nicuolo F, Maggiano N, Ranelletti F, Wolf F, Calviello G. Mechanism of activation of caspase cascade during beta carotene-induced apoptosis in human tumor cells. Nutr Cancer 47:76–87, 2003.
55. Young L, Hantz H, Martin K. Resveratrol modulates gene expression associated with apoptosis, proliferation, and cell cycle in cells with mutated human *c*-Ha-*ras*, but does not alter *c*-Ha-*ras* mRNA or protein expression. J Nutr Biochem 16:663–674, 2005.
56. Hail N. Mechanisms of vanilloid-induced apoptosis. Apoptosis 8:251–262, 2003.
57. Cory S, Adams J. Killing cancer cells by flipping the Bcl-2/Bax switch. Cancer Cell 8:5–6, 2005.
58. Petros A, Olejniczak E, Fesik S. Structural biology of the Bcl-2 family of proteins. Biochim Biophys Acta 1644:83–94, 2004.
59. Manson M. Cancer prevention—the potential for diet to modulate molecular signalling. Trends Mol Med 9:11–18, 2003.
60. Aggarwal B, Ichikawa H. Molecular targets and anticancer potential of indole-3-carbinol and its derivatives. Cell Cycle 4:1201–1215, 2005.
61. Palozza P. Can beta carotene regulate cell growth by a redox mechanism? An answer from cultured cells. Biochim Biophys Acta 1740:215–221, 2005.
62. Billard C, Izard J, Roman V, Kern C, Mathiot C, Meritz F, Kolb J. Comparative antiproliferative and apoptotic effects of resveratrol, epsioln-viniferin, and vine-shots derived polyphenols (vineatrols) on chronic B lymphocytic leukemia cells and normal human lymphocytes. Leuk Lymphoma 43:1991–2002, 2002.
63. Bode A, Dong Z. Targeting signal transduction pathways by chemopreventive agents. Mutat Res 555:33–51, 2004.
64. Li Y, Upadhyay S, Bhuiyan M, Sarkar F. Induction of apoptosis in breast cancer cells MDA-MB-231 by genistein. Oncogene 18:3166–3172, 1999.
65. Wu X, Kassie F, Mersch-Sundermann V. Induction of apoptosis in tumor cells by naturally occurring sulfur-containing compounds. Mutat Res 589:81–102, 2005.
66. Hershko T, Ginsberg D. Up-regulation of Bcl-2 homology (BH3)-only proteins by E2F1 mediates apoptosis. J Biol Chem 279:8627–8634, 2004.
67. Perik P, de Vries E, Gietema J, van der Graaf W, Sleijfer D, Suurmeijer A, Van Veldhuisen D. The dilemma of the strive for apoptosis in oncology. Crit Rev Oncol Hematol 53:101–113, 2005.
68. Ding Y, Chen Z, Liu S, Che D, Vetter M, Chang C. Inhibition of Nox-4 activity by plumbagin, a plant-derived bioactive naphthoquinone. J Pharm Pharmacol 57:111–116, 2005.
69. Huang D, Shen Y, Wu C, Huang Y, Kung F, Teng C, Guh J. Investigation of extrinsic and intrinsic apoptosis pathways of new clerodane diterpenoids in human prostate cancer in PC-3 cells. Eur J Pharmacol 503:17–24, 2004.
70. Salveson G, Duckett C. IAP proteins: blocking the road to death's door. Nat Rev Mol Cell Biol 3:401–410, 2002.
71. Baliga M, Meleth S, Katiyar S. Growth inhibitory and antimetastatic effect of green tea polyphenols on metastasis-specific mouse mammary carcinoma 4T1 cells in vitro and in vivo systems. Clin Cancer Res 11:1918–1927, 2005.
72. Miyoshi N, Nakamura Y, Ueda Y, Abe M, Ozawa Y, Uchida K, Osawa T. Dietary ginger constituents, galanals A and B, are potent apoptosis inducers in human T lymphoma Jurkat cells. Cancer Lett 199:113–119, 2003.
73. Li C, Chen P, Chang U, Kan L, Fang W, Tsai K, Lin S. Ganoderic acid X, a lanostanoid triterpene, inhibits topoisomerases and induces apoptosis of cancer cells. Life Sci 77:252–265, 2005.
74. Philchenkov A. Caspases: potential targets for regulating cell death. J Cell Mol Med 8:432–444, 2004.
75. Ferry-Dumazet H, Garnier O, Mamani-Matsuda M, Vercauten J, Belloc F, Billiard C, Dupouy M, Thiolat D, Kolb J, Marit G, Reiffers J, Mossalayi MD. Resveratrol inhibits the growth and induces the apoptosis of both normal and leukemic hematopoietic cells. Carcinogenesis 23:1327–1333, 2002.
76. Ulrich S, Wolter F, Stine J. Molecular mechanisms of the chemopreventive effects of resveratrol and its analogs in carcinogenesis. Mol Nutr Food Res 49:452–461, 2005.
77. Zhang Y. Cancer-preventive isothiocyanates: measurement if human exposure and mechanism of action. Mutat Res 555:173–190, 2004.
78. Owuor E, Kong A. Antioxidants and oxidants regulated signal transduction pathways. Biochem Pharmacol 64:765–770, 2002.
79. Shirin H, Pinto J, Kawabata Y, Soh J, Delohery T, Moss S, Murty V, Rivlin R, Holt P, Weinstein I. Antiproliferative effects of *S*-allylmercaptocysteine on colon cancer cells when tested alone or in combination with sulindac sulfide. Cancer Res 61:725–731, 2001.
80. Nakagawa H, Tsuta K, Kiuchi K, Senzaki H, Tanaka K, Hioki K, Tsubura A. Growth inhibitory effects of diallyl disulfide on human breast cancer cell lines. Carcinogenesis 22:891–897, 2001.
81. Ledezma E, Apitz-Castrto R, Cardier J. Apoptotic and anti-adhesion effect of ajoene, a garlic derived compound, on the murine melanoma B16F10 cells. Cancer Lett 206:35–41, 2004.
82. Jiang C, Wang Z, Lu J. Distinct effects of methylseleninic acid versus selenite on apoptosis, cell cycle, and protein kinase pathways in DU-145 human prostate cancer cells. Mol Cancer Ther 1:1059–1066, 2002.
83. Bouralexis S, Findlay D, Evdokiou A. Death to the bad guys: targeting cancer via Apo2L/TRAIL. Apoptosis 10:35–51, 2005.
84. Reed J. Dysregulation of apoptosis in cancer. J Clin Oncol 17:2941–2953, 1999.
85. Schimmer A. Inhibitor of apoptosis proteins: translating basic knowledge into clinical practice. Cancer Res 64:7183–7190, 2004.
86. Clement M, Hirpara J, Chwadhury S, Pervaiz S. Chemopreventive agent resveratrol, a natural product derived from grapes, triggers CD95 signaling-dependent apoptosis in human tumor cells. Blood 92: 996-1002, 1998.
87. Gautam S, Xu Y, Dumaguin M, Janakiraman N, Chapman R. Resveratrol seleetively inhibits leukemia cells: a prospective agent for ex vivo bone marrow purging. Bone Marrow Transplant 25:639–645, 2000.

88. 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 376:338–346, 2000.
89. Hsu S, Lewis J, Borke J, Singh B, Dickinson D, Caughman G, Athar M, Drake L, Aiken AC, Huynh CT, Das BR, Osaki T, Schuster GS. Chemopreventive effects of green tea polyphenols correlate with reversible induction of p57 expression. Anticancer Res 21:3743–3748, 2001.
90. Sakamoto K, Lawson L, Milner J. Allyl sulfides from garlic suppress the in vitro proliferation of human A549 lung tumor cells. Nutr Cancer 29:152–156, 1997.
91. Dirsch A, Gerbes A, Vollmar A. Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor κB. Mol Pharmacol 53:402–407, 1998.
92. Roemer K, Mahyar-Roemer M. The basis for the chemopreventive action of resveratrol. Drugs Today 38:571–580, 2002.
93. Sarkar F, Li Y. Cell signaling pathways altered by natural chemopreventive agents. Mutat Res 555:53–64, 2004.
94. Meddings J, Hogaboam C, Tran K, Reynolds J, Wallace J. Capsaicin and its analogs induce ion channels in planar lipid biolayers. Biochim Biophys Acta 6:2134–2136, 1991.
95. Martin K, Smith J, Failla M. Beta carotene and lutein protect HepG2 human liver cells against oxidant-induced damage. J Nutr 126:2098–2106, 1996.
96. Lotan R. Retinoids and apoptosis: implications for cancer chemoprevention and therapy. J Natl Cancer Inst 87:1655–1657, 1995.
97. Holzman D. Apoptosis provides new targets for chemotherapy. J Natl Cancer Inst 88:1098–1100, 1996.
98. Fesus L, Szondy Z, Uray I. Probing the molecular program of apoptosis by cancer chemopreventive agents. J Cell Biochem 22:151–161, 1995.
99. Cummings J, Ward T, Ranson M, Dive C. Apoptosis pathway-targeted drugs—from the bench to the clinic. Biochim Biophys Acta 1705:53–66, 2004.
100. Johnstone R, Rueflir A, Lowe S. Apoptosis: a link between cancer genetics and chemotherapy. Cell 108:153–164, 2002.
101. Adjei A. Blocking oncogenic *ras* signaling for cancer therapy. J Natl Cancer Inst 93:1062–1074, 2001.
102. Chung J, Huang C, Meng X, Dong Z, Yang C. Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-*ras*-transformed cells: structure-activity relationship and mechanisms involved. Cancer Res 59:4610–4617, 1999.
103. Howells L, Gallacher-Horley B, Houghton C. Indole-3-carbinol inhibits Akt/PKB and induces apoptosis in the human breast tumor cell line MDA MB468, but not in the nontumorigenic HBL100 line. Mol Cancer Ther 1:1161–1172, 2002.
104. Courtois G. The NF-kappaB signaling pathway in human genetic diseases. Cell Mol Life Sci 62:1682–1691, 2005.
105. Holmes-McNary M, Baldwin A. Chemopreventive properties of trans-resveratrol are associated with inhibition of activation of the IkappaB kinase. Cancer Res 60:3477–3483, 2000.
106. Ashikawa K, Majumbar S, Banerjee S, Bharti A, Shishodia S, Aggarwal B. Piceatannol inhibits TNF-induced NF-κB activation and NF-κB mediated gene expression through suppression of IkappaBalpha kinase and p65 phosphorylation. J Immunol 169:6490–6497, 2002.
107. Levine A. p53, the cellular gatekeeper for growth and division. Cell 88:323–331, 1997.
108. Hajra K, Liu J. Apoptosome dysfunction in human cancer. Apoptosis 9:691–704, 2004.
109. Slee E, O'Connor D, Lu X. To die or not to die: how does p53 decide? Oncogene 23:2809–2818, 2004.
110. Woods D, Vousden K. Regulation of p53 function. Exp Cell Res 264: 56–66, 2001.
111. Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P, Moll U. p53 has a direct apoptogenic role at the mitochondria. Mol Cell 11:577–590, 2003.
112. Harms K, Nozell S, Chen X. The common and distinct target genes of the p53 family transcription factors. Cell Mol Life Sci 61:822–842, 2004.
113. Johnson T, Yu Z, Ferrans V, Lowenstein R, Finkel T. Reactive oxygen species are downstream mediators of p53-dependent apoptosis. Proc Natl Acad Sci U S A 93:11848–11852, 1996.
114. Gupta S, Afaq F, Mukhtar H. Involvement of nuclear factor-kappa B, Bax, and Bcl-2 in induction of cell cycle arrest and apoptosis by apigenin in human prostate carcinoma cells. Oncogene 21:3727–3738, 2002.
115. Garg A, Aggarwal B. Nuclear transcription factor-κB as a target for cancer drug development. Leukemia 16:1053–1068, 2002.
116. Manna S, Sah N, Newman R, Cisneros A, Aggarwal B. Oleandrin suppresses the activation of nuclear transcription factor-κB, activator protein-1, and *c*-Jun N-terminal kinase. Cancer Res 60:3838–3847, 2000.
117. Manna S, Mukhopadhyay A, Aggarwal B. Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-κB, activator protein-1, and apoptosis. J Immunol 164:6509–6519, 2000.
118. Khafif A, Schantz S, Chou T, Edelstein D, Sacks P. Quantitation of chemopreventive synergism between (-)-epigallocatechin-3-galate and curcumin in normal, premalignant, and malignant human oral epithelial cells. Carcinogenesis 19:419–424, 1998.
119. Deeb D, Xu Y, Jiang H. Curcumin (diferuloyl-methane) enhances tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in LNCaP prostate cancer cells. Mol Cancer Ther 2:95–103, 2003.
120. Velmurugan B, Mani A, Nagini S. Combination of *S*-allylcysteine and lycopene induces apoptosis by modulating Bcl-2, Bax, Bim, and caspases during experimental gastric carcinogenesis. Eur J Cancer Prev 14:387–393, 2005.
121. Mertens-Talcott S, Percival S. Ellagic acid potentiates the effect of quercetin on p21 waf1/cip1, p53, and MAP kinases without affecting intracellular generation of reactive oxygen species in vitro. J Nutr 135: 609–614, 2005.
122. Mertens-Talcott S, Percival S. Ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis and cause transient cell cycle arrest in human leukemia cells. Cancer Lett 218: 141–151, 2005.