Targeting Apoptosis with Dietary Bioactive Agents

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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...
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 histiocyte 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 components, with induction of apoptosis at any of the 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...
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, oncrosis, 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

<table>
<thead>
<tr>
<th>Table 1. Modulation of Apoptosis by Dietary Bioactive Agents</th>
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<tbody>
<tr>
<td><strong>Organosulfur compounds</strong></td>
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<tr>
<td>Diallyl sulfide (DAS)</td>
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<td>Allium vegetables, garlic compounds</td>
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<td>Upregulate Bax; downregulate Bcl-2</td>
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<tr>
<td>Diallyl disulfide (DADS)</td>
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<tr>
<td>Allium vegetables, garlic compounds</td>
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<tr>
<td>Upregulate p53 and Bax; activate caspase 3; downregulate Bcl-2</td>
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<tr>
<td>Ajoene</td>
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<td>Allium vegetables, garlic compounds</td>
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<tr>
<td>Activate caspase 3; downregulate Bcl-2; JNK, p38, ERK activation</td>
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<tr>
<td>Allicin</td>
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<tr>
<td>Allium vegetables, garlic compounds</td>
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<tr>
<td>Activate caspases 3, 8, 9; cleave PARP; downregulate Bcl-2</td>
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<tr>
<td><strong>S-allyl cysteine (SAC)</strong></td>
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<td>Allium vegetables, garlic compounds</td>
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<td>Increase caspase 3 activity; JNK activation</td>
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<td><strong>S-allylmercaptocysteine (SAMC)</strong></td>
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<td>Allium vegetables, garlic compounds</td>
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<td>Increase caspase 3 activity; JNK activation</td>
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<td><strong>Polyphenols</strong></td>
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<td>Epigallocatechin gallate (EGCG)</td>
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<td>Green tea, chocolate</td>
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<td>Activate Fas; inhibit NF-κB; caspase activation; alter membrane function</td>
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<td>Catechin</td>
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<td>Teas</td>
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<td>Inhibit p38, PI3K, and AP-1 activation</td>
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<td>Genistein</td>
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<td>Soybeans</td>
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<td>Inhibit NF-κB; activate caspases; induce Bax</td>
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<td>Resveratrol</td>
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<td>Red grapes, peanuts, berries</td>
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<td>Caspase activation; inhibit NF-κB; induce FasL</td>
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<td>Curcumin</td>
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<td>Turmeric, curry, mustard</td>
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<td>Inhibit NF-κB and AP-1; caspase activation; disrupt MTP; induce Bax</td>
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<td>Ellagic acid</td>
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<td>Strawberries, walnuts, pecans</td>
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<td>Increase caspase 3 activation; upregulate p53; activate MAPK, JNK, p38</td>
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<td>Capsaicin</td>
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<td>Chili peppers</td>
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<td>Disrupt MTP; cyto c release; inhibit Bcl-2; induce Bax caspase activation</td>
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<td><strong>Isothiocyanate</strong></td>
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<td>Sulforaphane</td>
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<td>Cruciferous vegetables, broccoli</td>
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<td>Activate ERK; inhibit NF-κB; activate caspase 3; downregulate Bcl-2</td>
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<tr>
<td>Phenethyl isothiocyanate (PEITC)</td>
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<td>Radish, cabbage</td>
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<td>Inhibit NF-κB; activate caspase 3; cleave BID; inhibit PKC; activate p53</td>
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<td>Allyl isothiocyanate (AITC)</td>
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<td>Mustard</td>
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<td>Activate caspase 8 and JNK; cleave BID; downregulate Bcl-2 &amp; Bcl-xL</td>
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<td>Benzyl isothiocyanate (BEITC)</td>
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<td>Garden cress</td>
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<td>Increase Bax/Bcl-2 ratio; activate caspase 3, JNK, p38; cyto c release</td>
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<td><strong>Glucosinolate</strong></td>
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<td>Indole-3-carbinol</td>
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<td>Cruciferous vegetables</td>
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<tr>
<td>Inhibit NF-κB, PI3K, Akt, Bcl-2, and Bcl-xL; activate caspases; induce Bax; induce cyto c release; increase TRAIL receptor, downregulate BAD</td>
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<td>3,3’-Diindoylmethane</td>
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<td>Cruciferous vegetables</td>
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<td><strong>Carotenoids</strong></td>
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<td>Beta carotene</td>
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<td>Orange-yellow vegetables</td>
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<td>Alter membrane function</td>
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<td>Lycopene</td>
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<td>Tomato</td>
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<td>Induce cyto c release; alter MMP; Induce p53; upregulate Bax; downregulate Bcl-2</td>
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<td>Lutein</td>
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<td>Dark green vegetables</td>
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<tr>
<td><strong>Mineral</strong></td>
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<td>Selenium</td>
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<tr>
<td>Cereal grains, meat, fish</td>
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<tr>
<td>Inhibit NF-κB; induce p53; inhibit PKC; alter redox status; modulate JNK</td>
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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
prostate cells, induce 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 baicillin 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 (13C) 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 conductive 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 ajene 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 the organosulfur compounds found in garlic have shown potent pro-apoptotic activity in numerous models (69).

Dietary components can induce apoptosis through caspase activation. Resveratrol increases caspase activity (caspases 6, 3, and 9) in numerous models, including normal human and hematopoietic cells (75, 76). The ITCs benzyl ITC (BITC), phenethyl ITC (PEITC), and phenylbutyl significantly induce apoptosis in cultured hamster testis and liver 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 superfamly, 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 processes leading to apoptosis, 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-kB 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.
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-xB 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-xB 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, p21cip, 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 phosphatidylinerse 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). Epi-gallocatechin 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 epithelial 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 ajene 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 down-regulation 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 apoptosisomponents 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, clAP, 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.

51. Biswal S, Datta K, Shaw S, Feng X, Robertson J, Keher J. Glutathione oxidation and mitochondrial depolarization as mechanisms of nordihydroguaiaretic acid-induced apoptosis in lipoxyn-

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