Nutraceuticals, Apoptosis, and Disease Prevention

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CHEMOPREVENTION

Chemoprevention is the use of small molecules, including dietary or herbal chemicals, to prevent diseases, as opposed to chemotherapeutics, where chemicals, mostly synthetic, are used to remove or alleviate the symptom of diseases. The concept of chemoprevention, although prevalent in the East for thousands of years, has not gained scientific recognition in the West until recently. Large-scale clinical studies have demonstrated the efficacy of using tamoxifen, raloxifene, both estrogen receptor antagonists, and fenretinide, a synthetic retinoid, in protecting women from breast cancer.[1–3] The report by the Chemoprevention Working Group to the American Association for Cancer Research was a watershed that signaled the acceptance of chemoprevention as a viable alternative means in cancer control.[4] Therefore, it is of interest to explore the possibility of using phytochemicals or other dietary chemicals as chemopreventive agents. Further, the study of the biological effects of these phytochemicals at cellular level provides the molecular basis for their anti-disease function and helps to establish the platform for generating more potent chemopreventive and even chemotherapeutic agents.

APOPTOSIS

Programmed cell death (apoptosis), first described in 1842[5] and rekindled 30 y ago,[6] was originally defined by a set of morphologic changes including chromatin condensation, nuclear fragmentation, membrane blebbing, and cell shrinkage. With an increasing appreciation of the fundamental importance of apoptosis in life process, we have witnessed an explosion of literature output since the 1990s, with close to 70 000 relevant papers in PubMed. At the molecular level, apoptosis represents a collection of intricate pathways with more than 100 different proteins actively participating in activities from signal transduction, zymogen-type cascade, to precision surgical execution of key cytoskeletal structures and command center DNA within the marked cell. These events lead to DNA fragmentation, blebbing, the formation of apoptotic bodies and ultimately to cell death. Further, the dying cell is engulfed by phagocytes due to the exposure of phosphatidylserine and changes in surface sugars.[7–10]

Figure 1 illustrates this apoptosis saga. The two major pathways that initiate apoptosis are extrinsic (receptor mediated) and intrinsic (mitochondrial mediated). In addition, mitogenic and stress responsive pathways are involved in the regulation of apoptotic signaling. Noteworthy is the cross talk between these pathways. The fine tuning of the balance between the pro- and anti-apoptotic factors within each of these pathways in a cell leads to programmed cell death or survival.

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The Mitochondrial-Mediated Pathway

Mitochondria are truly the arbitrator of life and death for a cell.[11] Various stressors such as inflammation, radiation (ultraviolet or x-rays), heavy metals, ozone, heat shock, and acidification are inducers of apoptosis, and they are known to be involved in the generation of reactive oxygen species (ROS) from mitochondria.[12,13]

For activation of the mitochondrial-mediated death pathway, the critical step is the increase of the membrane permeability that causes mitochondrial swelling, rupture of the outer membrane, and release of proapoptotic factors from the intermembranous space.[8–11] This is achieved by 1) an opening of the permeability transition pore, 2) an increase of the Bax/Bcl-2 ratio, or 3) ROS-induced damage of mitochondrial membrane. The opening of the permeability transition pore at the contact sites of inner and outer mitochondrial membranes destroys the electrochemical gradient and uncouples the respiratory chain with concomitant cessation of adenosine triphosphate synthesis.[10,14] The Bax/Bcl-2 ratio is a rheostat that indicates the relative amount of antiapoptotic (Bcl-2, Bcl-x, Bfl-1, and Bad-P) and proapoptotic (Bax, Bid, Bik, Bim, and Bad) proteins within the Bcl-2 superfamily. This ratio controls the permeability of mitochondrial membranes via oligomerization mechanism.[9,15] Caspase-2 also may be involved in the permeabilization of mitochondria.[16]

Proapoptotic factors released from mitochondria after membrane potential collapse include procaspases, cytochrome c, apoptosis protease activating factor-1 (Apaf-1), endonuclease-G, and apoptosis-inducing factor. Cytochrome c, Apaf-1, adenosine triphosphate, and procaspase-9 form a supramolecular complex termed *apoptosome*, which activates caspase-9 through autocatalysis. The mitochondrial-activated caspase-9 and the death receptor-activated caspase-8 (see below) cleave procaspase-3 and generate the active caspase-3, which serves as the "central executioner of apoptosis."[17] Caspase-3 activates other caspases, cleaves cytoskeletal proteins (e.g., fodrin and gelsolin), or activates the caspase-activated DNase. In particular, caspase-3 cleaves an inhibitor of caspase-activated DNase that allows caspase-activated DNase to enter the nucleus and to fragment nuclear DNA. Apoptosis-inducing factor and endonuclease-G operate independent of caspases and may represent proapoptotic factors of an ancestral cell death pathway.[10]

The caspase family proteins (cysteine aspartate-specific proteases) exhibit proteolytic specificity for aspartate residue. Caspases-2, -3, -6, -7, -8, -9, and -10 are sequentially activated during apoptosis via a zymogen-type cascade. Caspases-8, -9, and -10 are considered initiator caspases responsible for activating the downstream effector caspases (-3, -6, and -7). Procaspases represent the inactive forms (zymogens) of caspases, which are cleaved at internal aspartate residues by other caspases, which results in their activation.[10,18] Interactions of caspases are performed by specific domains called *caspase-associated recruitment domains*.[9]

The caspase pathway is regulated by inhibitors of apoptosis protein (IAPs), which bind to and inhibit the activation of procaspases and the activity of mature caspases. During apoptosis, inhibitory effects of IAPs are neutralized by the second mitochondria-derived activator of caspase, direct IAP binding pro-

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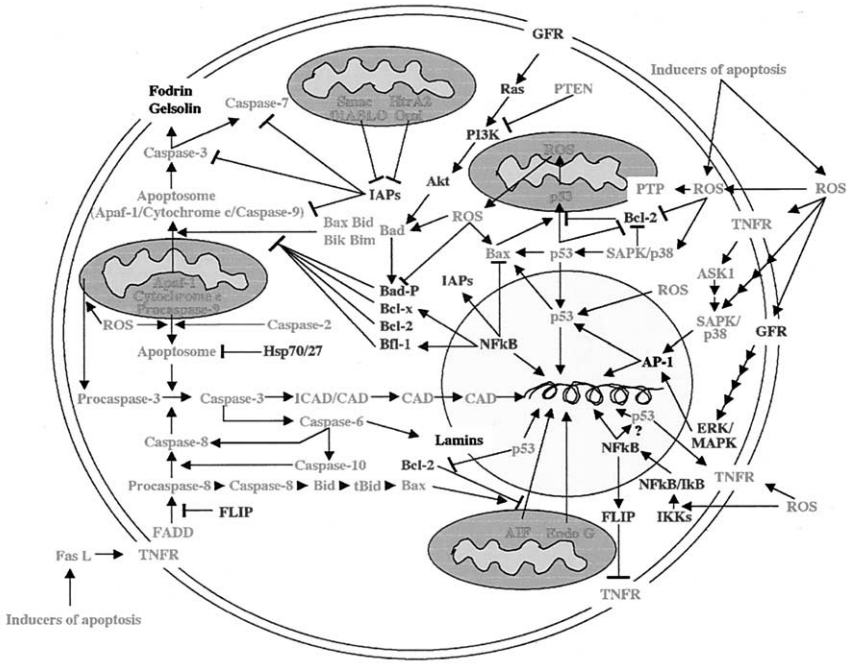

FIG. 1. Apoptotic signaling network. Proapoptotic and antiapoptotic (or proto-oncogenic) components are indicated. Precursors for proapoptotic factors are also indicated. The mechanism of activation and inhibition is explained in the text. AIF, apoptosis-inducing factor; AP-1, activator protein-1; Apaf-1, apoptosis protease activating factor-1; ASK1 apoptosis signal-regulating kinase-1; Bad-P, phosphorylated Bad; CAD, caspase-activated DNase; Caspases, cysteine aspartate-specific proteases; DIABLO, direct IAP binding protein with low pI; Endo G, endonuclease-G; ERK, extracellular-regulated protein kinase; FADD, Fas-associated protein with death domain; FasL, Fas ligand; FLIP, FADD-like interleukin-1β–converting enzyme inhibitory protein; GFR, growth factor receptors; Hsp70 and Hsp27, heat shock proteins 70 and 27; HtrA2/Omi, high-temperature requirement protein-A2; IAPs, inhibitors of apoptosis protein; ICAD, inhibitor of CAD; IkB, NFkB inhibitory protein; IKKs, IkB kinases; MAPK, mitogen-activated protein kinase; NFkB, nuclear factor κB; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PTP, permeability transition pore; ROS, reactive oxygen species; SAPK, stress-activated protein kinase; Smac, second mitochondria-derived activator of caspase; tBid, truncated form of twice a day; TNFR, tumor necrosis factor receptor.

tein with low pI, and/or high-temperature requirement protein-A2, which are released from mitochondria.

Activation of second mitochondria-derived activator of caspase, direct IAP binding protein with low pI, and/or high-temperature requirement protein-A2 represents another layer of regulation of the apoptotic program. Another mechanism of regulation is performed by heat-shock proteins 70 and 27, which bind to Apaf-1 and cytochrome-c, respectively, and block apoptosome formation and thus block the mitochondrial-mediated apoptosis.[19] Other factors such as acyl coenzyme A–binding protein and polypyrimidine tract-binding protein released from mitochondria also may play certain role in apoptosis.[10]

Interestingly, mitochondria may be composed of a network called *mitochondrial reticulum*[20] rather than of single ellipsoid organelles. There is increasing evidence that various mammalian cells contain a mitochondrial reticulum,[20,21] which may favor a progressive signaling of apoptosis. Besides mitochondria, other organelles such as endoplasmic reticulum may play an active role in apoptosis due to caspase-12 activation.[22]

The Death Receptor Pathway

The cell surface death receptors belong to the superfamily of tumor necrosis factor receptors and are activated by tumor necrosis factor family ligands.[23,24] The well-characterized APO-1 receptor (also called Fas or CD95) is activated by binding of Fas ligand, which leads to its trimerization and the recruitment of Fas-associated protein with death domain. These conformational changes result in binding of procaspases-8 and -10 to a supramolecular complex called *death-inducing signaling complex*. Fas ligand, Fas-associated protein with death domain, and caspase-8 form a death-inducing signaling complex via the interactions between the death effector domains of these proteins. Caspase-8 in turn activates caspase-3, the caspase executioner. Caspase-8 activation can be blocked by Fas-associated protein with death domain–like interleukin-1β–converting enzyme inhibitory protein (FLIP).[25] Conversely, caspase-8 can also activate Bid, a proapoptotic member of the Bcl-2 family, by converting it to its truncated form.[10,15]

Other Extrinsic Pathways

Mitotic or stress-activated pathways such as the extracellular-regulated protein kinase (ERK) and Akt, or stress-activated protein kinase (SAPK) and p38, play roles in apoptosis. Extracellular-regulated protein kinase is an isoform of mitogen-activated protein kinase, which is activated predominantly by mitogens but also by ROS.[26] Activation of growth factor receptors lead to the activation of the Ras-Raf-MEK-extracellular-regulated protein kinase cascade, which is involved in activation of different transcription factors such as activator protein-1 and others.[26,27] The receptor pathway leading to activation of Akt via Ras and phosphoinositide 3-kinase (PI3K) may result in the suppression of apoptosis because Akt is involved in phosphorylation of Bad to its antiapoptotic isoform, Bad-P.[10,27,28] Block of the PI3K/Akt pathway may be achieved via the action of the phosphatase and tensin homolog deleted on chromosome 10, a cellular antagonist of PI3K.[29]

Nuclear factor κB (NF-κB), an important stress trans-acting factor, may play an antiapoptotic role. NF-κB is activated through phosphorylation of NF-κB inhibitory protein by kinases of the inhibitory protein and its subsequent proteolysis by protea-

somes.[30,31] NF-κB induces antiapoptotic genes such as FLIP, IAP, Bcl-x, and Bfl-1 and suppresses the proapoptotic gene Bax.[31] However, the picture is still fuzzy because NF-κB activity has been correlated with the activation of apoptosis-associated genes such as Fas ligand and p53.[27,32]

The stress-activated members of the mitogen-activated protein kinase family such as SAPK and p38 are activated by ROS or inflammatory signals, which then activate kinases such as apoptosis signal-regulating kinase-1 (ASK-1). Apoptosis signal-regulating kinase-1 can activate both SAPKs through activation of stress-activated protein kinase/extracellular-signal regulated kinase kinase 1 (SEK1) and the p38s through mitogen-activated protein kinase kinase 3 (MKK3) and MKK6. SAPK and p38 may be involved in apoptosis due to their role in the activation of activator protein-1, an important transactivator not only for proapoptotic genes such as tumor necrosis factor-α and Fas ligand but also for proto-oncogenes.[26,33,34]

The Unique p53

P53 is considered to be "a cellular gatekeeper for growth and division" by controlling critical cell cycle checkpoints.[35] P53 mediates apoptosis through activation of APO-1/Fas and other death receptors and/or up- and downregulation of Bax and Bcl-2, respectively.[36,37] P53 is also involved in mitochondrial ROS generation, which may cause the release of apoptotic factors.[38–40] ROS in turn is involved in the activation of stress responsive pathways such as p38/SAPK. ROS may inhibit Bcl-2 and activate p53.[27]

APOPTOSIS AND DISEASE

Apoptosis is involved in a whole array of normal physiologic processes, including immune defense, tissue homeostasis, and development, and any tilt of the balance between life and death within an organism can lead to disease. Thus, the loss of essential cells of postmitotic tissues due to enhanced cell death may play an important role in a number of functional deficiencies and degenerative diseases such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, myocardial infarction, arteriosclerosis, chronic inflammation, rheumatoid arthritis, sterility, or cataract.[41–44] However, apoptosis can be considered a proactive self-defense mechanism of a living organism to weed out dysfunctional cells such as the precursors of metastatic cancer cells without creating secondary oxidative stress due to inflammation.[45]

Indeed, defect in apoptosis mechanism is recognized as an important cause of carcinogenesis.[31] A dysregulation of proliferation alone is not sufficient for cancer formation; a suppression of apoptotic signaling is needed.[29,31] Cancer cells acquire resistance to apoptosis by overexpression of antiapoptotic proteins (Bcl-2, IAPs, and FLIP) and/or by the downregulation or mutation of proapoptotic proteins (Bax, Apaf-1, caspase-8, and death receptors). Overexpression of antiapoptotic Bcl-2 and Bcl-xL probably occurs in more than 50% of all cancers,[31] e.g., in prostate cancer cells.[46] Moreover, many cancers show pathologic overexpression of FLIP (e.g., in stomach cancer[47]) and IAPs (e.g., in gastric cancer[48]), which interferes with apoptosis induction at the level of the death receptors and caspases, respectively.[9,29] According to its role in induction or suppression of antiapoptotic (FLIP, IAP, Bcl-x, and Bfl-1) or proapoptotic (Bax) factors,[31] a hyperactivity of NF-κB is observed in certain cancers,[49] which suggests an antiapoptotic role of NF-κB. In fact, inhibiting NF-κB activity results in enhanced apoptosis by chemotherapy.[50]

Because chemotherapy and irradiation act primarily by inducing apoptosis of body cells, both normal and malignant, a defect in the apoptotic pathway may increase the resistance of cancer cells to these treatments. Alternatively, tumor resistance to apoptosis is due to inactivation of proapoptotic genes. Enhanced mutation rates of Bax, Apaf-1, and caspase-8 have been found in various types of cancer. Moreover, death receptors are downregulated, mutated, or inactivated in many tumors.[29] Lack of Fas ligand–mediated killing has been linked to in vivo tumor promotion of lung cancer in mouse.[51] Another mechanism of suppression of death receptor–mediated apoptosis in cancers might be the expression of soluble receptors that act as competitive inhibitors for ligands of the tumor necrosis factor family. Elevated levels of those receptors have been found in the sera of cancer patients.[29]

On the one hand, p53 play a central role in apoptosis of cancer cells because p53 mutations increase the resistance to chemotherapy,[52,53] possibly due to a decrease of Bax.[36] On the other hand, reintroduction of p53 into p53 mutant tumor cells can result in apoptosis by chemotherapy, which underscores the apoptotic function of p53.[50] In addition, the PI3K/AKT pathway may be hyperactivated in some tumors because Ras, the catalytic subunit of PI3K, and Akt are overexpressed in several cancers. In contrast, phosphatase and tensin homolog deleted on chromosome 10, the cellular antagonist of PI3K, is frequently downregulated in various cancer types.[29,50]

Strategies to overcome apoptosis resistance in cancer cells may include treatment with or upregulation of proapoptotic factors (e.g., caspases, apoptosis-inducing factor, endonucleases, Apaf-1, cytochrome-c, Bax, Bid, p53, death receptors and/or its ligands, apoptosis signal-regulating kinase-1, second mitochondria-derived activator of caspase/direct IAP binding protein with low pI, high-temperature requirement protein-A2, phosphatase and tensin homolog deleted on chromosome 10) and/or inhibition or downregulation of antiapoptotic factors (e.g., Bcl-2, Bcl-xL, Bfl-1, IAPs, heat-shock proteins 70 and 27, Akt, NF-κB).[29,31]

NEUTRACEUTICALS AND APOPTOSIS

Nutraceuticals, mostly phytochemicals derived from dietary or medicinal plants such as soya bean, garlic, ginger, tea, and others, may have chemopreventive activity, as suggested by epidemiologic and animal model studies. Their ability to reduce cancer incidence in these studies is likely related to apoptosis. The potential of using nutraceuticals as chemopreventive reagents has prompted a surge of in vitro study of their biological effects in cultured human cells. In this section, we review the recent studies on the effects of nutraceuticals in cultured human cells, in particular apoptosis. Due to the vast amount of information in the literature, we cannot cover all nutraceuticals. We selected phytochemicals that belong to the following structural classes: carotenoids, flavonoids, stilbenes, sulfur-containing compounds, or other phenolic compounds.

Carotenoids

Recent epidemiologic studies have shown good correlation between dietary intake of tomato and reduced risk of cancer and cardiovascular diseases.[54] Tomato is rich in various carotenoids. Lycopene (Figure 2A) is the precursor of β-carotene in tomato, which accumulates after the lycopene cyclase gene is downregulated during ripening.[55] Lycopene and β-carotene can induce apoptosis in prostate cancer cells[56] and malignant lymphoblast cells[57] at a concentration range of 3 to 30 μM within 24 h. The carotenoid-induced apoptosis shows typical DNA fragmentation, poly ADP-ribose polymerase (PARP) cleavage, and caspase-3 activation.[57]

However, in the case of insulin-like growth factor-1–stimulated growth of MCF-7 mammary cancer cells, the inhibitory effect of lycopene may be independent of apoptosis.[58] Although it is certain that carotenoids have antiproliferative activity, it is unclear what the direct molecular targets of lycopene and β-carotene are. β-Carotene has been shown to affect NF-κB binding activity,[59] whereas lycopene causes an increase in connexin-43 mRNA and

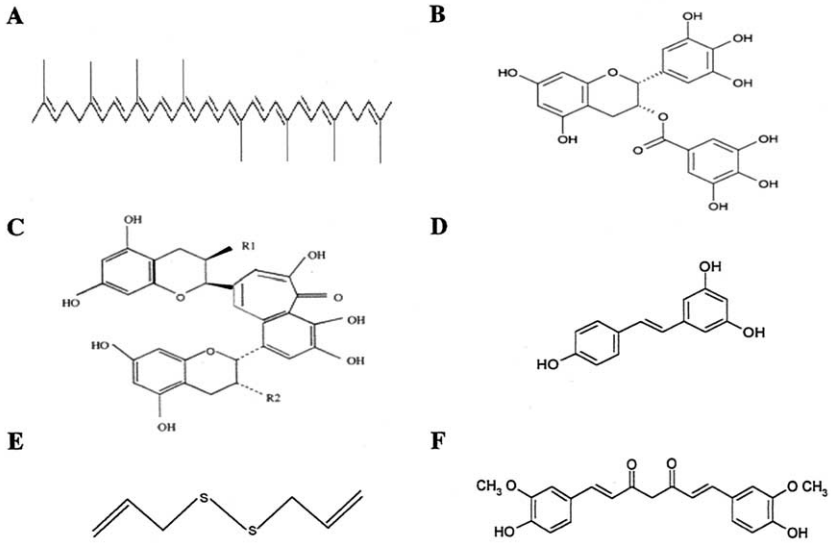

FIG. 2. Some representative members of carotenoids, flavonoids, stilbenes, sulfur-containing compounds, or other polyphenolic compounds are illustrated: (A) lycopene; (B) (−)-epigallocatechin gallate; (C) black tea polyphenols: theaflavin (R1 = OH, R2 = OH), theaflavin-3-monogallate (R1 = OH, R2 = gallate), theaflavin-3′-monogallate (R2 = OH, R1 = gallate), theaflavin-3-3′-digallate (R1 = R2 = gallate); (D) resveratrol (3,5,4′-trihydroxy-*trans*-stilbene); (E) diallyl disulfide; (F) curcumin.

stimulates gap junction communication[60] at a concentration (0.1 μM) much lower than that required for apoptosis. Thus, it is likely that the biological effects of carotenoids are pleiotropic, and their chemopreventive activity may not be solely due to apoptosis.

Flavonoids

Flavonoids are a group of more than 4000 polyphenolic compounds that occur naturally in foods of plant origin. These compounds possess a common phenylbenzopyrone structure (C6-C3-C6), and they are categorized according to the saturation level and opening of the central pyran ring, mainly into flavones, flavonols, isoflavones, flavonols, flavanone, and flavanonols.[61] Among them, tea polyphenols, quercetin, and genistein have been widely studied for their potential chemopreventive applications.

Although epidemiologic studies have not yielded a clear positive correlation between tea consumption and cancer risk reduction, there is no doubt that tea extracts or tea polyphenols have promising anticancer effects in animal models.[62,63] In addition to cancer, tea polyphenols may have protective effect for cardiovascular and inflammatory diseases.[62,64] (−)-Epigallocatechin gallate (EGCG; Figure 2B) and other catechins were first shown to be apoptotic in human lymphoid leukemic cells[65] and human carcinoma cells.[66] Similar observation has since been extended to lung tumor cell lines,[67] colon cancer cells, breast cancer cells and virally transformed human fibroblasts,[68] prostate cancer cells,[69] stomach cancer cells,[70] brain tumor cells,[71] head and neck squamous carcinoma,[72] and cervical cancer cells.[73] The effective dosages of EGCG for apoptosis in these cells are in the range of 20 to 100 μM, and the time course varies from 10 to 30 h. Based on the study of using p53-dominant negative mutant or p53 knockout cells, it is thought that intrinsic and extrinsic apoptotic pathways are involved in the action of EGCG.[74–76]

Although the binding of Fas to an EGCG-immobilized column has been demonstrated,[77] it is not clear whether this binding leads to the activation of Fas. Modulation of many other genes involved in cell cycling and signal transduction has also been reported, including Cip/p21, mitogen-activated protein kinase family kinases, proto-oncogenes such as c-fos, c-jun, and c-Ras, and transcription factors such as activator protein-1 and NF-κB at the mRNA or protein level.[72,78,79] The precise molecular action of EGCG in inducing apoptosis remains to be investigated. The finding that the EGCG-induced apoptosis in certain cells can be inhibited by exogenously added catalase[80] also raises the question as to whether the action of EGCG may be mediated, at least in part, by the secondary product such as H_2O_2 that is generated during culture incubation. Importantly, the apoptotic effect of EGCG exhibits striking preference toward transformed cancer cells than toward their matched normal counterparts.[66,68,74,81] However, some studies have also shown that EGCG can induce apoptosis in normal cells such as human endothelial ECV304[82] and vascular smooth muscle cells.[76]

Black tea extract is potent in inhibiting tumorigenesis in animal models, including skin,[83] lung,[84] colon,[85] esophagus,[86] and mammary gland.[87] Three major black tea polyphenols, theaflavin (TF-1), theaflavin-3-gallate and theaflavin-3′-gallate (TF-2), and theaflavin-3, 3′-digallate (TF-3), are derived from green tea through fermentation (Figure 2C). Black tea polyphenols induce apoptosis in human stomach cancer cells,[88] virally transformed human fibroblasts,[89] and hepatoma cells.[90] In the case of simian virus-40–transformed WI38 cells, TF-2 is more potent than TF-1 and TF-3,[89] but in human carcinoma cells, TF-3 is more potent.[91] The mechanism on how theaflavins induce apoptosis has not been investigated. We reported that TF-2 is 100-fold more potent in inhibiting cancer cell growth than are normal cells.[89]

Genistein, quercetin, rutin, and other food flavonoids have been shown to inhibit carcinogenesis in animal models.[92] They all induce apoptosis in tumor cells at the dosage range of 40 to 100 μM.[93–96] Similar to tea polyphenols, it appears that these flavonoids also can differentially induce apoptosis in cancer cells, but not in their normal counterparts.[94]

Stilbenes

Resveratrol (3,5,4′-trihydroxy-*trans*-stilbene; Figure 2D), a phytoalexin present in grapes, peanuts, and pines, has antioxidant and anti-inflammatory activities[97] and is the active ingredient in Leguminoseae that inhibits cellular events associated with tumor initiation, promotion, and progression in a mouse skin cancer model.[98] Its potential as a cancer chemopreventive agent has been exten-

sively reviewed recently.[99–102] The possible role of resveratrol, a phytoestrogen, in cardiovascular protection has been reviewed recently.[103,104] In vitro, resveratrol induces apoptosis and inhibits the growth of various human tumor cells, including oral squamous carcinoma,[105] promyelocytic leukemia,[106] human breast cancer cells,[107] prostate cancer cells,[108,109] esophageal carcinoma cells,[110] pancreatic cancer cells,[111] and monocytic leukemia cells.[112] The dosage of resveratrol used in various studies has varied between 10 and 300 μM, with apoptosis appearing between 24 and 96 h. Induction of p53 at the mRNA and protein levels is the most commonly observed effect of resveratrol and is considered the major cause for apoptosis. We found that resveratrol does not exhibit a clear differential growth inhibitory effect toward transformed human fibroblasts.[113] Interestingly, a resveratrol analog, 3,4,5,4′-tetrahydroxystilbene, is more potent than resveratrol in inducing apoptosis of transformed cells, but has no effect on normal counterparts at much higher concentrations.[113]

Sulfur-Containing Compounds

The use of garlic as anticancer agent has been reviewed.[114–116] The allyl-sulfur compounds derived from garlic have significant antiproliferate activity against human cancers.[117,118] Diallyl-sulfide and diallyl-disulfide (Figure 2E) induce apoptosis in non–small cell lung cancer cells[119] and in prostate cancer and breast cancer cells.[117] Another garlic component, Z-ajonene, also induces apoptosis of HL-60 cells.[120] Changes in the ratio of Bax to Bcl-2 have been observed in cells treated with these compounds.

Edible Brassica plants (e.g., broccoli and cauliflower) contain substantial amounts of glucosinolates, which are converted into isothiocyanates such as sulforaphane. Animal studies have suggested that isothiocyanates have cancer chemopreventive activity.[121] In vitro studies have shown that sulforaphane induces apoptosis in colon cancer cells,[122,123] prostate cancer cells,[124] and leukemia cells.[125] In these studies, the increase of Bax in the treated cells was offered as the explanation for the effect of sulforaphane on apoptosis.

Other Phenolic Compounds

Caffeic acid phenethyl ester, an active phenolic component extracted from honeybee propolis, blocked tumorigenesis in a two-stage model of mouse skin cancer that was promoted by treatment with 12-*O*-tetradecanoylphorbol-13-acetate.[126] Caffeic acid phenethyl ester induced apoptosis in HL-60 leukemic cells[127] and mouse epidermal JB6 Cl 41 cells.[128] In contrast, caffeic acid inhibited ceramide-induced increase in NF-κB activity and apoptosis.[129] Clearly further research is needed to delineate the action of caffeic acid phenethyl ester in different experimental systems.

Curcumin (Figure 2F), a diferuloylmethane, is a major active polyphenolic component of the food flavor turmeric (*Curcuma longa*) that has been shown to be a potent inhibitor of the initiation and promotion of chemical carcinogen-induced skin carcinogenesis in mice.[130] Topical application of curcumin on mouse skin inhibited chemically induced skin carcinogenesis.[131] Curcumin induced apoptosis in colon carcinoma cells,[132,133] leukemic cells,[134] prostate cancer cells,[135] melanoma cells,[136] and breast cancer cells.[137] By using p53-null mutant or a stable transfectant whose p53 expression is under tight tetracycline control, it has been established that curcumin induces apoptosis in tumor cells via a p53-dependent pathway.[138]

NUTRACEUTICALS AND DISEASE PREVENTION

Current research on the use of nutraceuticals as potential cancer chemopreventive agents has been limited largely to the study of their antiproliferative and apoptotic effects in human cancer cells. Because apoptosis, as determined by DNA fragmentation, terminal deoxyuridine triphosphate nick end labeling (TUNEL) assay, annexin V, or caspase-3 activation, is measured 10 to 40 h after treatment, the molecular events preceding these measurements are unclear. For EGCG, modulation of mitogen-activated protein kinase and NF-κB may be involved.[139,140] To understand how a nutraceutical induces apoptosis or other beneficial biological activities, it is crucial to know its molecular targets as a function of time course. In addition to chemical identification of these targets, the dynamic gene expression profile of the effect of the nutraceutical, generated by array and proteomic approaches, are invaluable.[141,142] For a nutraceutical to be useful, it may be desirable that its antiproliferative and apoptotic effects are limited to transformed cancer cells. It is certainly of interest to understand the molecular basis for the differential apoptotic effects of a nutraceutical such as EGCG or TF-2. Because many phytochemicals such as theaflavins, catechins, and carotenoids are photosensitive and chemically active, the stability of these compounds during experimental operations should be a concern in future nutraceutical research. The possibility that chemicals derived from the nutraceutical during culture incubation or cell metabolism are biologically active should be considered. For example, it is important to ascertain whether the effect of EGCG on apoptosis is mediated solely by H_2O_2.[80]

With our increased understanding of the chemistry and biology of nutraceuticals, the nutraceutical research will shift more into the area of chemoprevention. With that in mind, we offer the following considerations on the future use of nutraceuticals for disease prevention. 1) Designer nutraceuticals: To further increase the efficacy of a promising nutraceutical, one can use it as a chemical template for combinatorial synthesis. For example, we have proposed the synthesis of resveratrol analogs as a means to generate more effective compounds. Among the eight trans-stilbene derivatives that we synthesized, two are more potent than resveratrol in inducing apoptosis of cancer cells but have no detrimental effect on their normal counterparts.[89] 2) Multitargeted diseases: Carcinogenesis, atherogenesis, neurodegeneration, and other age-related diseases share certain common molecular bases that can be exploited.[143] With the molecular targets of nutraceuticals being known, it may be possible to develop more refined chemicals that specifically target those commonly shared sites. 3) Synergistic regimen: With the understanding of the molecular action of each nutraceutical, one can test possible synergistic effects on chemoprevention by using two or more nutraceuticals or derivatives.

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