

REVIEW

Apoptosis by dietary factors: the suicide solution for delaying cancer growth

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Apoptosis, a form of programmed cell death, plays a fundamental role in the maintenance of tissues and organ systems by providing a controlled cell deletion to balanced cell proliferation. The last decade has witnessed an exponential increase in the number of studies investigating how different components of the diet interact at the molecular and cellular level to determine the fate of a cell. It is now apparent that many dietary chemopreventive agents with promise for human consumption can also preferentially inhibit the growth of tumor cells by targeting one or more signaling intermediates leading to induction of apoptosis. In this brief review, we summarize the available evidence for dietary chemopreventive substances as inducers of apoptosis in cancer cells. These emerging data suggest that some of these dietary agents especially those which humans could be persuaded to consume may be utilized in the prevention and management of cancer.

Introduction

Apoptosis is a preferential way of elimination of damaged cells. Since our study in 1997, in which we provided evidence that (–)-epigallocatechin gallate (EGCG), the major constituent of green tea, causes induction of apoptosis and cell cycle arrest in many types of cancer cells without affecting normal cells, many subsequent studies have shown the effect of many dietary constituents on the induction of apoptosis of cancer cells (1,2). Apoptosis, since then, has undergone extensive scrutiny as a potential target for cancer chemoprevention. Apoptosis is characterized by a set of morphologic changes including chromatin condensation, nuclear fragmentation, membrane blebbing and cell shrinkage. At the molecular level, apoptosis represents a collection of intricate pathways with >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. Failure of tumor cells to undergo apoptosis translates into malignant potential and chemotherapeutic resistance.

Carcinogenesis is a multistage process consisting of apparently three major steps: initiation, promotion and progression and in humans it takes many years for the journey of normal cells into complete malignancy. An offshoot of this thinking is to explore whether this journey can be slowed by the use of dietary substances. Using this approach, it has become clear that there could be many opportunities to intervene in the development of cancer. Chemoprevention, a promising strategy to prevent cancer is the use of either natural or synthetic substances or their combination to block, reverse or retard the process of carcinogenesis. Therefore, it is of interest to explore the possibility

Abbreviations: Apaf-1, apoptotic protease-activating factor 1; Bid, Bcl-2 interacting domain; c-FLIP, FADD-like interleukin-1 β -converting enzyme inhibitory protein; DIABLO, direct IAP-binding protein with low pI; EGCG, (–)-epigallocatechin gallate; FADD, Fas-associated protein with death domain; IAP, inhibitors of apoptosis protein; MMP, mitochondrial membrane potential; PARP, poly (ADP-ribose) polymerase; Smac, second mitochondria-derived activator of caspase; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand; UV, ultraviolet.

of using phytochemicals or other dietary agents as chemopreventive agents. Further, the study of the biological effects of these phytochemicals at cellular level provides the molecular basis for their antidisease function and helps to establish the platform for generating more potent chemopreventive regimens with potential for chemotherapeutic efficacy. The purpose of this review is to briefly outline the concept of apoptosis as a novel target for cancer chemoprevention by dietary chemopreventive agents. Instead of providing an extensive literature on each aspect discussed, we elected to highlight selected examples and cited only few references.

Pathways of apoptosis

The two major pathways that initiate apoptosis are extrinsic (death 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 some of these pathways. The fine-tuning of the balance between the pro- and antiapoptotic factors within each of these pathways in a cell leads to programmed cell death or survival. Figure 1 illustrates the saga of apoptosis and the induction of apoptosis by dietary chemopreventive agents.

The death receptor-mediated pathway

The extrinsic pathway of apoptosis is activated at the cell surface when a specific ligand binds to its corresponding cell-surface death receptor (Figure 1). Death receptors, for example, tumor necrosis factor (TNF) receptor, TNF-related apoptosis-inducing ligand (TRAIL) receptor and Fas belong to the TNF receptor superfamily. The well-characterized APO1 receptor (also called Fas or CD95) is activated by binding of Fas ligand that leads to its trimerization and the recruitment of Fas-associated protein with death domain (FADD). These conformational changes result in binding of procaspases-8 and -10 to a supra-molecular complex called death-inducing signaling complex. (3). Caspase-8 in turn activates caspase-3, the caspase executioner. Caspase-8 activation can be blocked by cellular FADD-like interleukin-1 β -converting enzyme inhibitory protein (c-FLIP). Conversely, caspase-8 can also activate Bcl-2 interacting domain (Bid), a proapoptotic member of the Bcl-2 family, by converting it to its truncated form.

The mitochondrial-mediated pathway

The role of mitochondria in apoptosis is complex and has been extensively reviewed (4). Because the activation of mitochondria has been considered as the ‘point of no return’ in the apoptotic process, the manipulation of mitochondrial activation with proapoptotic intentions has been envisaged as a potential therapeutic approach. In the mitochondrial pathway, activation of mitochondria is accompanied by the translocation of cytochrome *c* from the mitochondrial intermembrane space into the cytoplasm. Proapoptotic factors released from mitochondria after membrane potential collapse include procaspases, cytochrome *c*, apoptotic 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’, that activates caspase-9 through autocatalysis. The mitochondrial-activated caspase-9 and the death receptor-activated caspase-8 cleave procaspase-3 and generate the active caspase-3 that serves as the ‘central executioner of apoptosis’. Caspase-3 activates other caspases, cleaves cytoskeletal or activates the caspase-activated DNase. The caspase pathway is regulated by inhibitors of apoptosis protein (IAP) that 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 (Smac), direct IAP-binding

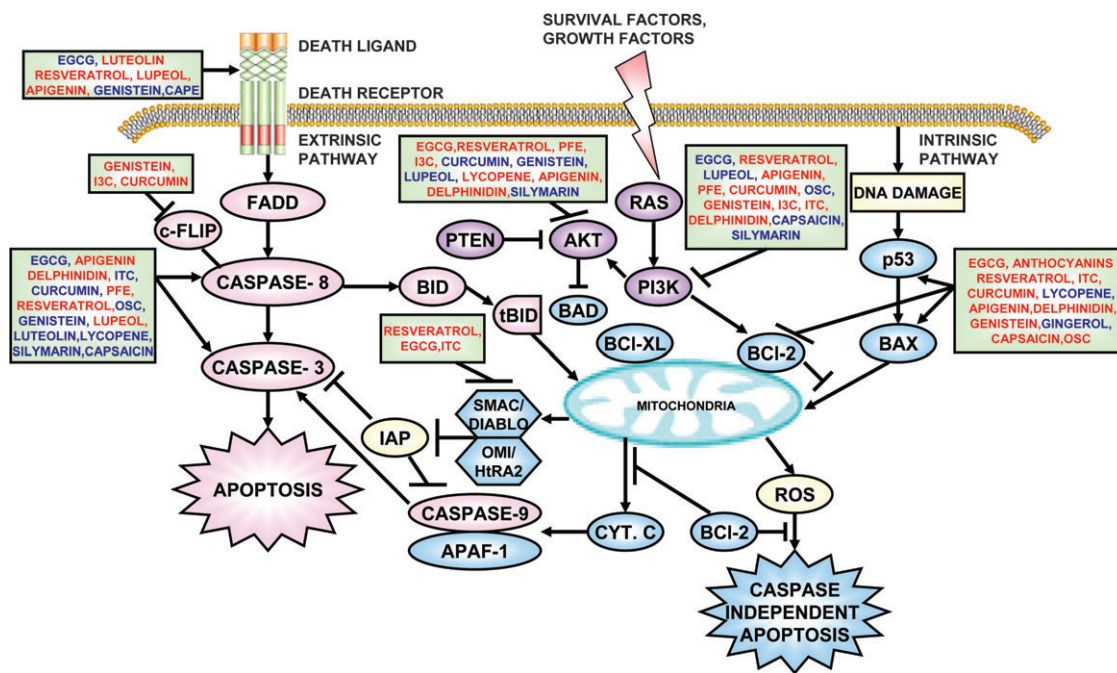


Fig. 1. Induction of apoptosis by dietary chemopreventive agents. The extrinsic pathway is initiated by ligation of transmembrane death receptors (CD95, TNF receptor and TRAIL receptor) to activate membrane-proximal (activator) caspase-8 via the adaptor molecule FADD. This in turn cleaves and activates effector caspase-3. Dietary agents block the death receptor and also target the caspases blocking the caspase cascade. This pathway can be regulated by c-FLIP, which inhibits upstream activator caspases and IAPs, that affects both activator and effector caspases. The intrinsic pathway requires disruption of the mitochondrial membrane and the release of mitochondrial proteins into the cytoplasm. Stress signals elicited by the dietary chemopreventive compounds regulate the proapoptotic proteins and antiapoptotic proteins, leading to the release of cytochrome *c* from the mitochondrial inner membrane. Cytochrome *c* forms an apoptosome with Apaf-1 and caspase-9, thereby initiating the apoptotic caspase cascade, whereas Smac/DIABLO and high-temperature requirement protein-A2 bind to and antagonize IAPs. The activated caspases catalyze the dissolution of intracellular structure that leads to apoptotic cell death. The Bcl-2 family proteins regulate apoptosis as they form complexes that enter the mitochondrial membrane, regulating the release of cytochrome *c* and other proteins. The activation of the caspase cascade occurs by the TNF family receptor and it also causes activation of Bid that activates mitochondria-mediated apoptosis. Bax is activated and releases cytochrome *c* and other mitochondrial proteins. Dietary agents can also block growth factor-mediated antiapoptotic signals through the direct inhibition of the binding of growth factors to the receptor or inhibition of the downstream phosphatidylinositol 3-kinase (PI3K)-Akt pathway. Blue color of dietary chemopreventive agents denotes that both the *in vivo* and *in vitro* effects have been demonstrated and red color denotes that only *in vitro* effects have been demonstrated.

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

Dietary intervention and apoptosis

In spite of substantial progress in the development of anticancer therapies, the incidence of cancer is still increasing worldwide. Recently, chemoprevention by the use of naturally occurring dietary substances is considered as a practical approach to reduce the ever-increasing incidence of cancer. The intervention of multistage carcinogenesis by modulating intracellular signaling pathways may provide molecular basis of chemoprevention with a wide variety of dietary phytochemicals. It has been estimated that by making modifications in the diet, more than two-thirds of human cancers could be prevented (5). The defect in apoptosis mechanism is recognized as an important cause of carcinogenesis. A dysregulation of proliferation alone is not sufficient for cancer development as suppression of apoptotic signaling is also required. Cancer cells acquire resistance to apoptosis by overexpression of antiapoptotic proteins and/or by the downregulation or mutation of proapoptotic proteins. A greater understanding of the pivotal events involved in carcinogenesis will facilitate the use of dietary constituents as a key strategy to prevent cancer development. Various studies indicate that dietary constituents, particularly phytochemicals, can modulate the complex multistage process of carcinogenesis (6,7). The promising dietary chemopreventive compounds with demonstrated effects in more than one tumor model include (–)-EGCG in green tea; resveratrol in grapes; luteolin in fruits like mango, strawberry and grape; delphinidin in pigmented fruits like pomegranate and strawberry; curcumin in turmeric; sulforaphane and other isothio-

cyanates in cruciferous vegetables; organosulfur compounds in garlic; lycopene in tomato; quercetin in onion and tomato; silymarin in milk thistle and genistein in soybeans among many others (8). Table I shows various dietary agents that have been reported to induce apoptosis of cancer cells.

Generally, the growth rate of preneoplastic or neoplastic cells exceeds that of normal cells due to dysregulation of their cell-growth and cell-death machineries. Therefore, an excellent approach to inhibit the promotion and progression of carcinogenesis and to remove genetically damaged, preinitiated or neoplastic cells from the body is by induction of apoptosis or cell cycle arrest by human acceptable doses of dietary chemopreventive compounds. Resveratrol selectively target tumor cells with presumably dysfunctional cell cycle checkpoints and spare normal tissue (9). This effect may be dependent on the p53 status of cells exposed to resveratrol. EGCG has been shown to affect a p57-mediated survival pathway in normal epithelial cells while inducing a proapoptotic pathway in oral carcinoma cells (10). Ajoene induces apoptosis in human acute myeloid leukemia cells and peripheral blood mononuclear cells from chronic leukemia patients but not quiescent and proliferating cells from healthy donors (11,12). Diallyl trisulfide, effectively inhibits proliferation and induces apoptosis in human lung cancer cells but not in non-neoplastic lung cells (11). Beta-carotene, a carotenoid in orange vegetables, induces apoptosis preferentially in various tumor cells from human prostate, colon, breast and leukemia. Many more examples of dietary substances inducing apoptosis of cancer cells are available. Conversely, normal cells are largely resistant to the induction of apoptosis by beta-carotene (13).

Table I. Dietary agents shown to induce apoptosis of cancer cells *in vitro* or during chemopreventive intervention

Dietary agent	Major dietary source	Target/mechanism	References
EGCG	Green tea	Activation of Fas	(16)
		Enhancement of TRAIL-induced apoptosis	(20)
		Activation of caspases	(34)
		Release of cytochrome <i>c</i> from mitochondria, inhibition of Bcl-2	(34, 35, 38)
Curcumin	Turmeric	Reduction in tumor growth and increase in apoptotic markers in mice	(34)
		TRAIL-induced apoptosis, activation of caspases	(18)
		Release of cytochrome <i>c</i> from mitochondria	(36)
Genistein	Soybean	Increase in the number of apoptotic tumor cells in mice	(61)
		Degradation of c-FLIP	(24)
Indole-3-carbinol	Cruciferous vegetables	Induced MMP change, caspase-3 activation and PARP cleavage	(29)
		Downregulation of Bcl-2 and Bcl-XL expression, activation of caspase-3	(40)
		Downregulation of survivin, IAP1, IAP2, X chromosome-linked IAP and FLIP	(23, 25)
Resveratrol	Grape, red wine	Downregulation of survivin, IAP1, IAP2, X chromosome-linked IAP, Bcl-2, TNF receptor-associated factor 1 and c-FLIP	(55)
		Release of cytochrome <i>c</i> from mitochondria, activation of caspases, induction of p53-dependent transcriptional activation	(15)
Isothiocyanates	Cruciferous vegetables	Sensitizes TRAIL-induced apoptosis	(19)
		Decrease in survivin, increase in Smac/DIABLO	(63)
		Activation of caspases	(39)
Luteolin	Celery, green pepper and peppermint	Activation of p53 activity, induction of apoptosis in lung tissues, effect on AP-1 and p53	(52)
		Sensitizes TRAIL-induced apoptosis	(21)
Lycopene	Tomato	Induction of TRAIL, along with Bid cleavage and the activation of caspases	(22)
		Decrease in the expression of surviving	(56)
Anthocyanins	Pomegranate	Release of cytochrome <i>c</i> from mitochondria	(33)
		Effect on p53, activation of caspases, decrease in PCNA, increase in Bax	(54)
Delphinidin	Pigmented fruits and vegetables such as strawberry and pomegranate	Bcl-XL downregulation, mitochondrial release of cytochrome <i>c</i> , activation of caspases	(37)
		Shift in the ratio of Bax to Bcl-2	(43)
		PARP cleavage, nuclear condensation and fragmentation, induced MMP change	(31)
Lupeol	Mango, olive and grape	Activation of caspases, increase in Bax, decrease in Bcl-2, upregulation of Bid, Bak, downregulation of Bcl-xL, inhibition of UVB-mediated apoptosis in mice	(48)
		Increase in the expression of Fas receptor and FADD, activation of caspases	(14)
Caffeic acid phenethyl ester	Honey	Fas activation, induction of p53, Bax and activation of caspases	(17, 68)
		Activation of caspases and PKCdelta	(41, 64)
Apigenin	Parsley, celery and lettuce	Activation of caspases and PARP cleavage	(42)
		Decrease in the apoptotic sunburn cells, increase in p53, p21	(67)
Silymarin	Milk thistle	Induced MMP change, release of cytochrome <i>c</i> from mitochondria, downregulation of Bcl-2 and enhancement of Bax	(47)
		Dissipation of the mitochondrial inner transmembrane potential, activation of caspase-3, induced apoptosis of prostate tumor cells in nude mice	(30)
Gingerol	Ginger	Increase in protein expression of p53, p21 and Bax	(46)
		Induction of p53, Bax and downregulation of Bcl-2, cytochrome <i>c</i> release, activation of caspases	(49)
Capsaicin	Red pepper		
Organosulfur compounds	Garlic and onion		

This list provides selected examples.

Effect of dietary agents on the death receptor-mediated pathway

Fas. Fas (APO1, CD95) is an integral cell membrane protein and a member of the TNF family of receptors. We have recently shown that lupeol, a triterpene present in fruits and vegetables, specifically caused a significant increase in the expression of Fas receptor with a significant increase in the expression of FADD protein. The small interfering RNA-mediated silencing of the Fas gene and inhibition of caspase-6, caspase-8 and caspase-9 by their specific inhibitors confirmed that lupeol specifically activates the Fas receptor-mediated apoptotic pathway in androgen-sensitive prostate cancer cells (14). Resveratrol, a naturally occurring phytoalexin in grapes, has been shown to trigger CD95 signaling-dependent apoptosis in human prostate cancer cells (15). It has also been reported that high level of FAS activity in prostate cancer cells was dose dependently inhibited by EGCG and this inhibition was paralleled by inhibition of cell growth and induction of apoptosis (16). Caffeic acid phenethyl ester, a phenolic antioxidant, induced apoptosis via Fas activation in human breast cancer cells with induction of p53, Bax and activation of caspases (17).

TNF-related apoptosis-inducing ligand. TRAIL, called APO2 ligand, is also a member of the TNF family. Binding of TRAIL to DR4 or DR5 receptors activates the TRAIL death-receptor pathway of apoptosis. Curcumin, the yellow pigment in the spice turmeric, differentially sensitizes malignant glioma cells to TRAIL-induced apoptosis and also causes cleavage of procaspases-3, -8 and -9 and release of cytochrome *c* from mitochondria (18). Recent studies demonstrated that resveratrol is a potent sensitizer of tumor cells for TRAIL-induced apoptosis through p53-independent induction of p21- and p21-mediated cell cycle arrest (19). EGCG was found to enhance TRAIL-induced apoptosis in hepatocellular carcinoma cell lines (20). Pretreatment with a non-cytotoxic concentration of luteolin, a dietary flavonoid commonly found in some medicinal plants, significantly sensitized TRAIL-induced apoptosis in both TRAIL-sensitive and TRAIL-resistant cancer cells (21). Luteolin markedly induced the expression of TRAIL, along with Bid cleavage and the activation of caspase-8, -10, -9 and -3. In addition, suppression of DR5 expression with small interfering RNA efficiently reduced luteolin-induced caspase activation and apoptosis in HeLa cells (22).

Cellular FADD-like interleukin-1 β -converting enzyme inhibitory protein. c-FLIP inhibits death receptor-induced apoptosis by binding to FADD and procaspase-8. The indole-3-carbinol present in cruciferous vegetables can induce apoptosis by downregulation of Bcl-2, Bcl-xL, IAP, X chromosome-linked IAP and c-FLIP (23). Phenoxodiol, a synthetic analog of the plant isoflavone genistein, exerts its effect mainly by the induction of apoptosis through multiple mechanisms resulting in degradation of antiapoptotic proteins including c-FLIP (24). An indole compound, 3,3'-diindolylmethane, derived from cruciferous vegetables led to significant downregulation of the c-FLIP that was predominantly mediated by the ubiquitin-proteasome degradation system (25).

Effect of dietary agents on the mitochondrial-mediated pathway

Mitochondrial permeability transition. 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 that is critical for proper function of the energy-yielding electron transport chain. Increased mitochondrial permeability and dissipation of the electrochemical gradient or mitochondrial membrane potential (MMP) via opening of the mitochondrial permeability transition pore triggers cell death by releasing apoptogenic factors from within the mitochondria, and release of mitochondrial proteins including cytochrome *c*, endonuclease G, Smac/DIABLO, high-temperature requirement protein-A2 and apoptosis-inducing factor. Dietary bioactive agents that alter mitochondrial membrane function and/or dissipate the MMP can induce apoptosis. Curcumin induces mitochondrial swelling and collapses the MMP, resulting in apoptosis in numerous cell types (26). Beta-carotene, a carotenoid found in carrots, can induce release of cytochrome *c* from mitochondria and alter MMP in different tumor cell lines derived from leukemia, colon adenocarcinoma and melanoma cells (27). Black tea extract has recently been shown to cause tumor cell apoptosis by loss in mitochondrial transmembrane potential, cytochrome *c* release and caspase activation in Ehrlich's ascites carcinoma cells (28). Genistein, an isoflavone from soybeans, induced MMP change, caspase-3 activation and poly (ADP-ribose) polymerase (PARP) cleavage in anaplastic large-cell lymphoma cells (29). Capsaicin, the major pungent ingredient in red peppers, induced apoptosis in prostate cells by a mechanism involving reactive oxygen species generation, dissipation of the mitochondrial inner transmembrane potential and activation of caspase-3. Subcutaneous injection of capsaicin in nude mice suppressed PC-3 tumor growth in all tumors investigated and induced apoptosis of tumor cells (30). Delphinidin, an anthocyanidin in pigmented fruits and vegetables, has been shown to cause nuclear condensation and fragmentation, PARP cleavage and loss of MMP of apoptotic cells in uterine carcinoma and colon adenocarcinoma cells (31).

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. Once released to the cytoplasm, cytochrome *c* binds and activates 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 antiapoptotic Bcl-2 proteins. Nordihydroguaiaretic acid markedly induced the release of cytochrome *c* from mitochondria into the cytosol (32). 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 (33). EGCG induced cytochrome *c* release *in vitro* and *in vivo* in metastatic mouse mammary carcinoma cells, as well as altered Bax:Bcl-2 protein ratios, increased Apaf formation and cleaved caspase and PARP proteins (34). In pancreatic cancer cells, EGCG invokes Bax oligomerization and depolarization

of mitochondrial membranes to facilitate cytochrome *c* release into cytosol. EGCG-induced downregulation of IAP family member X chromosome-linked IAP is thought to be helpful to facilitate cytochrome *c*-mediated downstream caspase activation (35). Curcumin has been shown to inhibit mitochondrial release of cytochrome *c* in human breast cancer cell lines (36). It has been reported that ellagitannins of pomegranate fruit caused induction of apoptosis via intrinsic pathway through Bcl-XL downregulation with mitochondrial release of cytochrome *c* into the cytosol, activation of initiator caspase-9 and effector caspase-3 in human colon cancer cells (37).

Activation of caspases. The apoptotic program is executed by a family of highly conserved cysteinyl aspartate-specific proteases known as caspases that dismantle the cell in an orderly fashion by cleaving a large number of cellular substrates. They form the 'engine of the apoptotic pathway'. Caspases-8, -9 and -10 are considered initiator caspases responsible for activating the downstream effector caspases-3, -6 and -7. Modulating the mechanisms of caspase activation and suppression is a critical molecular target in chemoprevention, since these processes lead to apoptosis. We have recently reported that treatment of prostate cancer cells with luteol resulted in a significant decrease in the expression of procaspases-6, -8 and -9 (14). In melanoma cell lines, EGCG treatment caused activation of caspases-3, -7 and -9 (38). Curcumin induced the cleavage of procaspases-3, -8 and -9 in malignant glioma cells (18). Isothiocyanates caused activation of caspases-3, -8, -9 and -12 in human leukemia HL60 cells (39). Activation of caspase-3 and cleavage of the caspase-3 substrate, PARP, were seen in hepatoma cells after exposure to genistein (40). Apigenin, a plant-derived flavanoid, induced apoptosis in monocytic and lymphocytic leukemia cell lines has been reported to be mediated by the activation of caspase-9 and -3 and activation of PKCdelta (41). Silymarin, a flavonoid antioxidant from milk thistle, has been shown to cause prominent caspases-9 and -3 activation as well as PARP cleavage, accompanied by a strong apoptotic death and growth inhibition of leukemia cells (42).

Alteration of Bax:Bcl-2 ratios. Members of the Bcl-2 family of proteins are critical regulators of the apoptotic pathway. Bcl-2 is an upstream effector molecule in the apoptotic pathway and is identified as a potent suppressor of apoptosis. Bcl-2 has been shown to form a heterodimer complex with the proapoptotic member Bax, thereby neutralizing its proapoptotic effects. Therefore, the ratio of Bax:Bcl-2 is a decisive factor and plays an important role in determining whether cells will undergo death or survival. We have recently reported significant dose-dependent shift in the ratio of Bax to Bcl-2 following treatment of prostate cancer cells with pomegranate fruit extract, indicating the induction of an apoptotic process (43). Green and black teas also caused induction of apoptosis accompanied with upregulation in Bax and decrease in Bcl-2 proteins in prostate cancer cells (44). EGCG treatment resulted in down-modulation of antiapoptotic protein Bcl-2 and upregulation of proapoptotic Bax in melanoma cells (38). Capsaicin caused apoptosis in prostate cancer cells with an increase of p53, p21 and Bax (45). Dietary ginger constituents induced mitochondrial transmembrane potential alteration, cytochrome *c* release, downregulation of Bcl-2 and induction of Bax protein expression in human T lymphoma Jurkat cells (46). Diallyl disulfide, a component of garlic, induced apoptosis in estrogen receptor-positive and -negative human breast cancer cells correlated with upregulation of Bax and downregulation of Bcl-xL. A change in intracellular ratio of Bax:Bcl-2 was observed in non-small cell lung cancer cells treated with diallyl sulfide, diallyl disulfide and garlic extract (47). We have recently reported that treatment of delphinidin caused activation of caspases, increase in Bax, decrease in Bcl-2, upregulation of Bid and Bak and downregulation of Bcl-xL in HaCaT cells and inhibition of ultraviolet (UV) B-mediated apoptosis in SKH-1 hairless mice (48).

p53. p53, also known as tumor protein 53, is a transcription factor that regulates the cell cycle and apoptosis and hence functions as

a tumor suppressor. It is considered to be 'a cellular gatekeeper for growth and division' by controlling critical cell cycle checkpoints. Normally, it induces apoptosis by activating caspases-9, -8, -7 and -3. The loss of p53 decreases caspase activation and therefore the cell does not undergo apoptosis. The 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 antiapoptotic genes such as Map4 and survivin and upregulation of proapoptotic genes such as Bax, IGFBP-3, DR5, Fas and Apaf-1, as well as various other apoptosome components representing potentially key therapeutic targets. Resveratrol suppressed tumor promoter-induced cell transformation and markedly induced apoptosis, transactivation of p53 activity and expression of p53 protein in JB6 C1 41 cells (49). It has been recently shown that the treatment of prostate carcinoma cells PC-3 (p53^{-/-}) with EGCG caused an increase in p53 protein that exacerbated both G₁ arrest and apoptosis. The cells lacking p53 continued to cycle and did not undergo apoptosis upon treatment with similar concentrations of EGCG, thus establishing the action of EGCG in a p53-dependent manner (50). Resveratrol also caused significant increase in the expression of the p53 in melanoma cells (51). The *N*-acetylcysteine conjugates of benzyl and phenethyl isothiocyanates have been reported to cause activation of p53 activity and induction of apoptosis in A/J mice (52). 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 (53). There was attenuation of smoke-induced total p53 and phosphorylated p53 and increase in the expression of p21 (Waf1/Cip1), Bax-1 and cleaved caspase-3 and decrease in cyclin D1 and proliferating cell nuclear antigen (PCNA) in the gastric mucosa of ferrets on lycopene supplementation in a dose-dependent fashion (54).

Survivin. Survivin is a member of the IAPs that is expressed at high levels in most human cancers and may facilitate evasion from apoptosis and aberrant mitotic progression. It has been shown to increase tumor resistance to various apoptotic stimuli, primarily through caspase-dependent mechanisms, although it can also block apoptosis in a caspase-independent fashion. Conversely, antagonizing survivin in tumor cells induces apoptosis. Resveratrol caused downregulation of survivin expression and sensitization for TRAIL-induced apoptosis (19). Indole-3-carbinol causes downregulation of survivin, IAP1, IAP2, X chromosome-linked IAP, Bcl-2, TNF receptor-associated factor 1 and c-FLIP in myeloid and leukemia cells (55). Recently, luteolin was found to decrease the expression of survivin in human hepatoma cells (56).

Studies supporting apoptosis as a target by dietary agents in in vivo systems

Recently, in breast cancer xenograft study, mice treated with green tea and tamoxifen showed high levels of apoptosis in tumor tissue (57). We have reported that treatment with oral infusions of a polyphenolic extract isolated from green tea, at doses equivalent to the human consumption of six cups of green tea/day, resulted in statistically significant delay in prostate cancer development and also increased overall survival. The chemopreventive intervention in prostate carcinogenesis with the green tea extract also caused a significant induction of apoptosis in prostate cancer cells (58). Oral pretreatment of SKH-1 mice with lyophilized green tea solids for 2 weeks enhanced the UV-induced increases in the number of p53-positive cells, p21-positive cells and apoptotic sunburn cells in the epidermis (59). Topical applications of caffeine or EGCG inhibited carcinogenesis and selectively increased apoptosis in UVB-induced skin tumors in mice (60). Treatment of EGCG-rich GTP in drinking water to 4T1 cells bearing BALB/c mice resulted in reduction of tumor growth accompanied with increase in apoptotic markers in tumors (34). Dietary administration of sulindac, curcumin and phenylethyl-3-methylcaffeate was associated with a statistically significant increase in the number of apoptotic tumor cells relative to that in animals fed the control diet

from chemically induced colon tumors in rats (61). In the male F344 rat model for chemically induced colon tumors, quercetin and rutin given as a dietary supplement increased the frequency of apoptotic cells and caused a redistribution of these cells along the colon crypt axis in the focal area of dysplasia relative to that in animals fed the control diet (62). Resveratrol enhanced apoptosis in UVB exposure-mediated skin tumors by downregulation of survivin and upregulation of proapoptotic Smac/DIABLO protein (63). Apigenin has been shown to inhibit apoptosis of human prostate carcinoma tumor xenograft in athymic nude mice (64). Dietary supplementation with EGCG diminished the frequency and expansion of hepatic lesions by stimulating the induction of apoptosis in transformed hepatocytes in a mouse model for chemically induced liver carcinogenesis (65). Silibinin caused decrease in the apoptotic sunburn cells together with an increase in p53- and p21-positive cell population in UVB-induced carcinogenesis in SKH-1 hairless mouse epidermis (66). During chemically induced colon carcinogenesis, male F344 rats fed a diet supplemented with perillyl alcohol, a monoterpene isolated from lavender, exhibited a statistically significant reduction in the incidence and multiplicity of invasive adenocarcinomas of the colon. Histopathologic evaluation of the treated colons indicated that the chemopreventive activity of perillyl alcohol was mediated through the induction of apoptosis in tumor cells (67). The flavonoids quercetin and rutin, when given as a dietary supplement increased the frequency of apoptotic cells and caused a redistribution of these cells along the colon crypt axis in the focal area of dysplasia relative to that in animals fed the control diet in male F344 rat model for chemically induced colon tumors (62). Caffeic acid phenethyl ester has been reported to inhibit intestinal carcinogenesis and this was associated with increased apoptosis and proliferation (68).

Conclusions and perspectives

Dietary chemopreventive compounds offer great potential in the fight against cancer by inhibiting the carcinogenesis process through the regulation of cell defensive and cell-death machineries. An issue of utmost importance is whether the journey of normal cell to full-blown malignancy can be slowed by one or more dietary agents. Apoptosis is a complex process comprising of extrinsic and intrinsic pathways with numerous specific targets. Accumulating evidence clearly indicates that apoptosis is a critical molecular target for dietary bioactive agents for chemoprevention of cancer. The understanding of the critical events associated with carcinogenesis provides the opportunity for dietary intervention to prevent cancer development through induction of apoptosis. It is encouraging that dietary agents can directly and indirectly influence most, if not all of the various targets of apoptosis. Additionally, many of these dietary agents appear to exhibit some degree of specificity for neoplastic cells while sparing normal cells. The last decade has seen an extraordinary increase in our understanding of apoptosis, and its contribution to cancer and cancer therapy. The chemopreventive agents when administered as an adjunct to radiation therapy or chemotherapy may improve their efficacy by increasing tumor response, decreasing toxicity and sensitize cancer cells to chemoradiation when patients become unresponsive to standard therapy. This could, in fact, improve quality of life and possibly increase the survival time of patients. Furthermore, the molecular mechanisms that control and execute apoptotic cell death are coming into focus. Although there is much more to learn, our current understanding of apoptosis provides new avenues for cancer diagnostics, prognosis and therapy. In the coming years, it seems probably that rational strategies to manipulate cell suicide programs will produce new therapies that are less toxic and mutagenic than current treatment regimens. Different therapeutic avenues have certainly been opened by the knowledge acquired on apoptosis and despite the long way ahead, before they become a therapeutic option, there is room for optimism.

A careful and well-designed plan of clinical development ensures that apoptosis-targeted strategies are probably integrated into the anti-cancer armamentarium in the next decade. For development of a

potent dietary agent to a clinically viable drug requires detailed consideration of *in vivo* pharmacokinetics, product quality, feasibility of use by humans, intermediate biomarkers and targeted patients or healthy populations. It is also possible that such useful agents can be used as an adjuvant to enhance the efficacy of other known chemotherapeutic regimens. Investigations to further elucidate the mechanisms associated with dietary agents-induced apoptosis should provide increased opportunities to develop novel, selectively targeted agents or drugs for personalized approaches for delaying cancer growth.

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