Elucidating the Role of Nutraceuticals in Overexpressing Antiapoptotic Proteins in Prostate Cancer

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NUTRACEUTICALS AND HEALTH

Nutraceuticals are naturally derived, bioactive compounds that have health-promoting, disease-preventing, or medicinal properties and have an impact on human genes that control cellular metabolisms.[1,2] Fruits, vegetables, common beverages, grains, marine products, medicinal plants, and herbs possess diversified pharmacologic properties and contain nutraceuticals with the potential to protect against heart diseases and stroke and to prevent human cancers.[3–11] Herbs and medicinal plants have been used throughout the world for centuries to treat many diseases, and 80% of the world population relies on botanical preparations as medicines for their health needs.[12,13] The biological activity of a natural product is very often believed to be the result of the combined action of several of its constituents. However, in most cases, the active ingredient of the natural product has not been completely characterized. An estimated 25% of all modern pharmaceutical drugs are derived from herbs, including aspirin (from white willow bark), the heart medication digitalis (foxglove), and the cancer treatment drug, Taxol (pacific yew tree). Approximately 15 million Americans take herbs at the same time as prescription medications.[14] Epidemiologic studies have shown that the environmental factors, especially food components, have a major impact on hormone-related cancer prevention, and a low intake of fruits and vegetables is associated with high mortality in cardiovascular disease.[15,16] One class of substances suggested to be responsible for these cancer protective effects is the isoflavones, which are abundant in soy products.[17–19] Many of the herbs, foods, and spices contain flavonoids, phytoestrogens, and unidentified phytochemicals with estrogenic activity in prostate patients.[20,21] Although flavonoids and phytoestrogens are generally considered to be non-nutritive agents, interest in these nutraceuticals has arisen because of their potential role in the prevention of human cancer.[22] Chemoprevention through the consumption of nutraceuticals, e.g., resveratrol from grapes,[23,24] lycopene from tomato,[25] and genistein from soy,[26] may reduce morbidity and mortality in cancer. The foods and herbs that possess anticancer activity include garlic, soybeans, cabbage, ginger, licorice, onions, flax, turmeric, cruciferous vegetables, tomatoes, peppers, brown rice, wheat, and the umbelliferous vegetables such as carrot, celery, cilantro, parsley, and parsnips.[27] Natural products and their isolated constituents have been shown to possess strong chemopreventive activity in animal models.[28–30] The effect of nutraceuticals on apoptotic pathways, signaling pathways, or different targets in cancer would be helpful in the design and development of novel cancer-preventive agents.

PROGRAMMED CELL DEATH (APOPTOSIS) AND BCL-2

Apoptosis, or programmed cell death, is a genetically controlled process of cell suicide that plays a pivotal role in maintaining homeostasis and preventing disease.[31,32] Apoptosis is a tightly controlled mechanism, and its dysregulation has been shown to play a key role in a number of human diseases including cancer, neurodegenerative diseases, and autoimmune diseases. Currently all chemotherapeutic drugs kill tumor cells by activating an endogenous biochemical pathway for cell suicide, known as programmed cell death or apoptosis.[33] However, tumor cells develop defects in the regulation of genes that control apoptosis, rendering them resistant to the induction of apoptosis by chemotherapeutics. The Bcl-2 families of proto-oncogenes are one of the critical regulators of apoptosis whose expression frequently becomes altered in many human cancers.[34] Bcl-2 was first discovered as the gene on chromosome 18q21 at the breakpoint of the t(14,18) chromosomal translocation found in B-cell follicular lymphomas.[35] This translocation places the *bcl-2* gene next to the immunoglobulin heavy-chain enhancer, leading to the overexpression of Bcl-2. Overexpression of Bcl-2 also occurs in many other types of human tumors, including cancers of the prostate, colon, and lung, and has been associated with chemoresistance and radioresistance in some types of malignancy.[33] The Bcl-2 family proteins constitute one of the important classes of apoptosis and regulatory proteins. These include the pro-apoptotic (Bax, Bad, Bak, Bcl-Xs, Bim, Bik) and antiapoptotic (Bcl-2, Bcl-X_L, Bcl_w, Mcl_1) proteins.[34] Several of these Bcl-2 family proteins are capable of physically interacting with each other through a complex network of homo- and heterodimers. Bcl-2 inhibits cell death in response to diverse stimuli and inhibits mitochondrial and nuclear manifestations of apoptosis. The interactions of apoptotic and antiapoptotic proteins and signaling events leading to apoptosis are shown in Figure 1. The Bcl-2 family regulates apoptosis through a cascade of reactions, as shown in the pathway, and altering the mitochondrial function.[36] Phosphorylation of Bcl-2 releases cytochrome-c from mitochondria, which induces the activation of cysteine proteases that cleave after aspartic acid (caspases). Multiple proteases have been identified as caspases. A *Caenorhabditis elegans* ced-4 homolog, Apaf-1, was identified that interacts with cytochrome-c to activate caspases.[37] Multiple proteins regulate the process of cytochrome-c release. Bcl-2 and Bcl-X_L regulate the pore opening by inhibiting Bax-mediated release of cytochrome-c. When activated, caspases induce a cascade that can result in a pattern of programmed cell death or apoptosis, including the cleavage of poly–adenosine diphosphate ribose polymerase.[38,39] Understanding these pathways responsible for the progression of cancer can help to select targets for drug discovery. It is well known that p53

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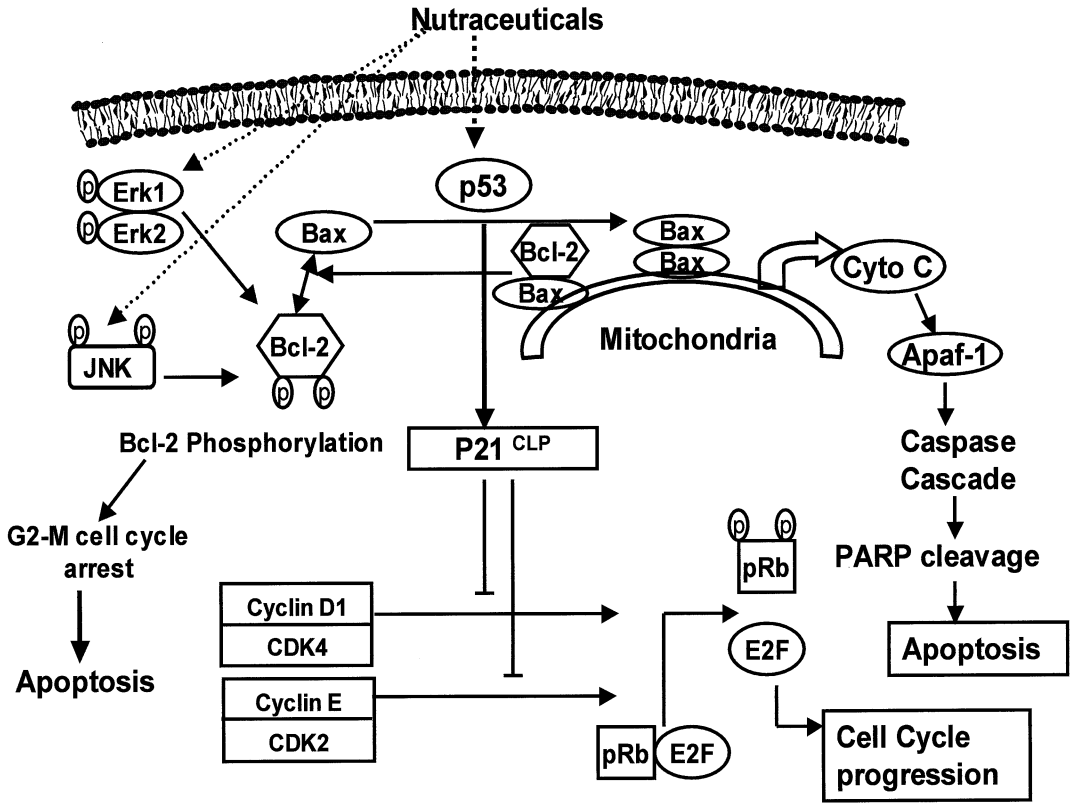

FIG. 1. Targeting molecular pathways using nutraceuticals. Cyto c, cytochrome c; PARP, poly–adenosine diphosphate ribose polymerase.

is one of the most frequent mutant tumor-suppressor genes and that loss of its function leads to many cancers.[40] In response to signals generated by a variety of stresses (nutraceuticals, ultraviolet light, DNA damage), p53 is activated to turn on the cell cycle regulator p21, which in turn binds to and inhibits cyclin-dependent kinases, causing hyperphosphorylation of retinoblastoma, thus preventing the release of E2F and blocking the cell cycle progression.[41] Nutraceuticals have been reported to induce apoptosis through p53-dependent and p53-independent pathways, as shown in Figure 1. Although many potential targets exist, the focus here is on targeting overexpressed proteins Bcl-2 by using nutraceuticals.

OVEREXPRESSION OF BCL-2 AND RESISTANCE TO CHEMOTHERAPY

Overexpression of the tumor-suppressor gene *bcl-2* plays an important role in cellular resistance to apoptosis.[42] Lin et al. found that overexpression of antiapoptotic Bcl-2 and Bcl-X_L proteins may play a role in the development of resistance to cancer therapy.[43] Functional overexpression of Bcl-2 has been reported to confer an antiapoptotic potential in a variety of cell types. The role of Bcl-2 in epithelial cell cycle control and its interactions with other cell cycle regulators is not clearly understood. Its expression has been correlated with the hormone- and chemoresistant phenotype in advanced prostate cancer.[44] Granville et al. found that overexpression of Bcl-2 in HL-60 cells prevents apoptosis-related events including caspase-3 and -6 activations and poly–adenosine diphosphate ribose polymerase cleavage by photodynamic therapy.[45] Overexpression of HER2 in estrogen receptor–positive human breast tumors has been associated with resistance to endocrine therapy. HER2 overexpression in MCF-7 cells accompanied by the upregulation of antiapoptotic Bcl-2 and Bcl-X_L proteins have been reported.[46] Transgenic mouse generated with the proto-oncogene Bcl-2 protects cells of the hematolymphoid system from the consequences of ionizing radiation and increase the radioresistance.[47] Raffo et al. reported that overexpression of Bcl-2 can protect human prostate cancer cells from apoptotic stimuli in vitro and in vivo.[48] Bcl-2 overexpression has been shown to protect prostate cancer cells from many different apoptotic stimuli, including hormone ablation, radiotherapy. and chemotherapy.[44,49] Several genes have been shown to be involved in the mechanism and progression of prostate cancer.[50] The role of the phosphatidylinositol 3-kinase (PI3) kinase/nuclear factor-κB pathway that leads to the upregulation of antiapoptotic protein and other possible mechanisms involved in prostate cancer progression has been reviewed recently.[51]

BCL-2 MODULATORS

I and my colleagues previously showed that licochalcone-A, a weak phytestrogen isolated from *Glycyrrhiza glabra* (licorice) root extract, represses the antiapoptotic protein Bcl-2 in breast and leukemic cell lines.[52] DiPaola et al. found that 13-cis retinoic acid and interferon can downregulate Bcl-2 protein in the peripheral blood mononuclear cells of prostate patients.[53] A clinical study with 18-base, fully phosphorothioate Bcl-2 antisense oligonucleotide administered to patients represses the Bcl-2 level in patients with non-Hodgkin's lymphoma.[54] Bcl-2 antisense oligonucleotide has been shown to reduce tumor in castrated athymic mice.[55]

CURCUMIN

Recent studies have shown that curcumin (diferuloylmethane), an agent that has very little or no cytotoxicity in humans, represses antiapoptotic proteins (Bcl-2 and Bcl-X_L) and downregulates nuclear factor-κB activity in human multiple myeloma cell lines,

renal cells, prostate cancer cell lines, and acute myelogenous leukemia cell lines[56–59]; however, Bcl-2 and Bcl-X_L–transfected cells were relatively resistant to induced apoptosis.[60] Curcumin has never been shown to phosphorylate Bcl-2 proteins.

TEA POLYPHENOLS

(−)-Epigallocatechin, one of the green tea polyphenols, has been shown to inhibit growth of p53 wild-type and mutant breast cancer cell lines (MCF-7 and MDA-MB-231), suggesting that the (−)-epigallocatechin–triggered apoptosis is independent of p53 status and the decrease antiapoptotic protein Bcl-2 and also increases apoptotic protein Bax level.[61] Chung et al. studied the effect of (−)-epigallocatechin-gallate in prostate cancer cell lines and concluded that apoptosis is not associated with altering the expression of Bcl-2, Bcl-X_L, and BAD in DU-145 cells.[62]

CARNOSOL

Carnosol, a phenolic compound extracted from the herb rosemary, has been reported to have anticancer activity in acute lymphoblastic leukemia lines by decreasing Bcl-2 levels, suggesting that carnosol may be useful as a novel chemotherapeutic agent against B-lineage leukemias and possibly other types of cancers that express high levels of the protective protein, Bcl-2.[63]

CAROTENOIDS

Recent studies have shown that carotenoids (lycopene, β-carotene, zeaxanthin, and astaxanthin) inhibit the proliferation of breast cancer cell lines, cause cell cycle arrest at the G2/M phase, and decrease the expression of the *bcl-2* gene in the breast cancer cell line MCF-7.[64] Kucuk et al. investigated the effects of lycopene supplementation in patients with prostate cancer and studied the expression of Bcl-2 and Bax and found that Bcl-2 and Bax levels did not differ significantly in their studies.[65]

OTHER POLYPHENOLS, INDOLES, PECTINS, AND FLAVONOIDS

Lee et al. studied the cytotoxicity of several structurally related flavonoids including luteolin, nobiletin, wogonin, baicalein, apigenin, myricetin, and fisetin in the human leukemia cell line HL-60 and found that wogonin and fisetin were the most potent apoptotic inducers. However, antiapoptotic proteins Bcl-2, Bcl-Xl, and Bad remained unchanged in wogonin- and fisetin-treated HL-60 cells.[66]

Iwashita et al. investigated the growth inhibitory activity of several flavonoids, including apigenin, luteolin, kaempherol, quercetin, butein, isoliquiritigenin, naringenin, genistein, and daizein against B16 mouse melanoma 4A5 cells and found that isoliquiritigenin and butein, belonging to the chalcone group, markedly suppress the growth of B16 melanoma cells and induce cell death.[67] Gupta et al. studied the effect of apigenin, a common dietary flavonoid abundantly present in fruits and vegetables, and reported that apigenin induces apoptosis in prostate cancer cell line by modulating the ratio of Bax to Bcl-2.[68] 3,3′-Diindolylmethane is a major in vivo derivative of the putative anticancer agent indole 3-carbinol, which is present in vegetables of the Brassica genus, has been shown to modulate Bcl-2 protein in breast cancer cell lines.[69] Indole 3-carbinol has been shown to downregulate Bcl-2 protein levels in breast and prostate cancer cell lines.[70,71] The soy isoflavone, genistein, modulates cell cycle progression and induces apoptosis in HER-2/neuoncogene–expressing human breast epithelial cells and modulates Bcl-2 proteins.[72] Avivi-Green et al. studied the in vivo effect of citrus pectins and butyrate in rat colon cancer models and found that Bcl-2 expression is downregulated in colonocytes.[73] Su et al. studied the effect of genistein, biochanin-A, and daidzein in hepatocarcinoma cell lines and correlated cytotoxicity with the downregulation of Bcl-2 and Bcl-X_L expression.[74]

TABLE I.

BCL-2–MODULATING NUTRACEUTICALS

S NO	Nutraceuticals	Cancer cell lines	References
1	Apigenin	Prostate	68
2	Butein	Melanoma	67
3	Carotenoids	Breast	64
4	Carnosol	Leukemia	63
5	Curcumin	Multiple myeloma; prostate, acute myelogenous leukemia	56–59
6	Diindolylmethane	Breast	70
7	Decosahexaenoic acid	Colon	76
8	Epigallo catechin gallate	Breast	61
9	Genistein	Breast	72
10	Indol 3-carbinol	Breast and prostate	70,71
11	Licochalcone-A	Breast	52
12	ω-3 fatty acid	Colon	75
13	Pectins and butyrate	Colon	73

OMEGA-3 FATTY ACIDS

Consumption of ω-3 fatty acids have been shown to slow the growth of cancer in xenograft animal models, increase the efficacy of chemotherapy, and reduce the side effects of the chemotherapy. ω-3 Fatty acids also have been shown to decrease nuclear factor-κB activation and Bcl-2 expression in colon cancer cell lines.[75] Docosahexaenoic acid, a long-chain polyunsaturated fatty acid, is a major constituent of nutrients rich in ω-3 polyunsaturated fatty acids. Docosahexaenoic acid inhibits the growth of CaCo-2 cells, induces apoptosis, and inactivates antiapoptotic *bcl-2* family genes.[76] The Bcl-2 modulating nutraceuticals are listed in Table I.

Bcl-2 PHOSPHORYLATION

Phosphorylation of Bcl-2 protein is a post-translational modification and is an important event in apoptosis.[77] Exposure of most of the tumor cell lines to cytotoxic agents phosphorylates Bcl-2 and induces apoptosis.[78–80] Recent evidence has indicated that antiapoptotic functions of Bcl-2 can be regulated by its phosphorylation. Hyperphosphorylation of Bcl-2 induced by paclitaxel and other microtubule-active drugs are strictly dependent on targeting microtubules that in turn cause mitotic arrest. In addition to serine-70, microtubule-active agents promote phosphorylation of serine-87 and threonine-69, thereby inactivating Bcl-2.[77,81] Pathan et al. reported that phosphorylated Bcl-2 protein is associated in M-phase–arrested cells with Pin1, a mitotic peptidyl prolyl isomerase known to interact with substrates of Cdc2 during mitosis.[82] Because the region in Bcl-2 containing serine-70 and serine-87 represents a proline-rich loop that has been associated with autorepression of its antiapoptotic activity, the discovery of Pin1 interactions with phosphorylated Bcl-2 raises the possibility that Pin1 alters the conformation of Bcl-2 and thereby modulates its function in cells arrested with antimicrotubule drugs.[82] Thomas et al. reported that p53 mediates Bcl-2 phosphorylation in baby rat kidney cells and apoptosis via activation of the small G-family protein Cdc42/c-Jun N-terminal kinase-1 (JNK1) pathway.[83] Bcl-2 has

been phosphorylated by CDC-2 kinase, a master regulator in the G2/M phase of the cell cycle.[84] In addition to antimicrotubule agents, effective DNA-damaging agents have been shown to induce Bcl-2 phosphorylation.[85] Srivastava et al. showed that deletion of the loop region of Bcl-2 and mutation in the phosphorylation sites completely block paclitaxel-induced apoptosis and that Bcl-2 phosphorylation is important in inducing apoptosis.[81] Bcl-2 phosphorylation has been shown to phosphorylate through the activation of JNK phosphorylation.[86] Yamamoto et al. found that signal transduction enzymes ASKI/JNK is involved in Bcl-2 phosphorylation.[87] Ling et al. showed that Bcl-2 phosphorylation is tightly associated with mitotic arrest and found that it is not a determinant of apoptosis.[88] Natural estrogen metabolite 2-methoxyestradiol phosphorylates Bcl-2 and has been shown to be a strong growth inhibitor in vitro.[89] Bu et al. recently showed that 2-methoxyestradiol induces apoptosis in epithelial carcinomas by causing phosphorylation of JNK, which appears to be correlated with phosphorylation of Bcl-2.[90]

BCL-2 PHOSPHORYLATING MOLECULE FROM NATURAL PRODUCTS

Genistein, isolated from soy, inactivates Bcl-2 by phosphorylation, delays the G2/M phase of the cell cycle, induces apoptosis of human breast adenocarcinoma MCF-7 cells, and is an inhibitor of protein tyrosine kinase and topoisomerase II.[91] The natural estrogen metabolite 2-methoxyestradiol is antiangiogenic in vivo and can phosphorylate Bcl-2, and its mechanism of action is independent of the signaling enzymes JNK/SAPK.[89] Taxol, isolated from *Taxus brevefolia*, vincristine and vinblastine isolated from *Vinca rosea*, and Taxotere, a semisynthetic form of Taxol, can induce Bcl-2 phosphorylation in tumor cell lines.[78,92,93] We isolated and identified a novel polyphenol from *Glycyrrhiza glabra* (licorice) that induces apoptosis, phosphorylates antiapoptotic protein Bcl-2, and causes G2/M cell cycle arrest in breast and prostate cancer cell lines.[80] The antiapoptotic potential of Bcl-2 is now well established, but the biochemical mechanism of Bcl-2 action is poorly understood.

NEED FOR NOVEL BCL-2 MODULATORS

Bcl-2 has been described as a factor that can protect cancer cells from apoptosis.[33,37,94] The protective effect of Bcl-2 may be lost if the protein is phosphorylated or its expression is repressed.[92] Agents that affect microtubule depolymerization or prevent microtubule assembly can induce Bcl-2 phosphorylation. Currently all available Bcl-2 phosphorylating molecules in clinical use are highly toxic with side effects. In conclusion, several molecular mechanisms involved in the progression of cancer and the development of resistance to chemotherapy are associated with overexpression of Bcl-2. Therefore, the need exists for the discovery of novel Bcl-2 phosphorylating or repressing molecules. Nutraceuticals from herbal products, medicinal plants, fruits, vegetables, grains, nuts, and common beverages are promising and their elucidation is warranted.

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