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Apoptosis by dietary agents for prevention and treatment of prostate cancer

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Abstract

Accumulating data clearly indicate that induction of apoptosis is an important event for chemoprevention of cancer by naturally occurring dietary agents. In mammalian cells, apoptosis has been divided into two major pathways: the extrinsic pathway, activated by pro-apoptotic receptor signals at the cellular surface; and the intrinsic pathway, which involves the disruption of mitochondrial membrane integrity. This process is strictly controlled in response to integrity of pro-death signaling and plays critical roles in development, maintenance of homeostasis, and host defense in multicellular organisms. For chemoprevention studies, prostate cancer (PCa) represents an ideal disease due to its long latency, its high incidence, tumor marker availability, and identifiable preneoplastic lesions and risk groups. In this article, we highlight the studies of various apoptosis-inducing dietary compounds for prevention of PCa *in vitro* in cell culture, in preclinical studies in animals, and in human clinical trials.

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Introduction

Prostate cancer (PCa) is one of the most common cancers in men in the United States and is the second leading cause of male cancer death worldwide after lung cancer. The number of new PCa cases expected to be diagnosed in the United States alone in 2009 are 192 280 with an estimated 27 360 disease-related deaths (Jemal *et al*. 2009). PCa is an ideal disease for chemopreventive intervention as it grows slowly before the onset of symptoms and the establishment of diagnosis and it is usually diagnosed in men more than 50 years of age. Therefore, pharmacological or nutritional intervention could considerably impact the quality of life of patients by delaying the progression of cancer (Syed *et al*. 2007).

Multicellular organisms normally eliminate damaged cells effectively through apoptosis, a controlled cellular mechanism resulting in cell death. The concept of physiological cell death was developed by Kerr *et al*. (1972) with the publication of a seminal paper on apoptosis. The term apoptosis is derived from the Greek word describing the falling off of petals from a flower or leaves from a tree. It has become clear that apoptosis is a highly conserved mechanism that has evolved to maintain cell numbers and cellular positioning within tissues comprised of different cell compartments (Fadeel & Orrenius 2005, Khan *et al*. 2007). It involves the concerted action of a number of intracellular signaling pathways, including members of the caspase family of cysteine proteases, stored in most cells as zymogens or procaspases. Characteristic apoptotic features include cell shrinkage, membrane blebbing, chromatin condensation, and formation of a DNA ladder with multiple fragments caused by internucleosomal DNA cleavage finally ending with the engulfment by macrophages or neighboring cells, thereby avoiding an inflammatory response in surrounding tissues (Savill & Fadok 2000). Cells can undergo apoptosis via two different pathways: the intrinsic or mitochondrial-mediated pathway and the extrinsic or death receptor-mediated pathway.

The intrinsic pathway is usually activated by the loss of growth factor signals or in response to many different damaging influences, for example DNA damage, oxidative stress, hypoxia, or chemotherapeutic drugs. Intrinsically triggered apoptosis is mainly regulated by proteins of the Bcl-2 family, which control the release of pro-apoptotic factors from the mitochondrial intermembrane space. Caspase-9 is

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activated when cytochrome *c* is released into the cytoplasm from the mitochondrial intermembranous space. Activated caspase-8 and -9 activate executioner caspases, including caspase-3, which in turn cleave a number of cellular proteins that include structural proteins, nuclear proteins, cytoskeletal proteins, and signaling molecules. The Bcl-2 family of proteins plays a central role in controlling the mitochondrial pathway. More than 20 members of this family have been identified to date in humans, including suppressors (Bcl-2, Bcl-xL, Mcl-1, Bfl-1/A1, Bcl-W, and Bcl-G) and promoters (Bax, Bak, Bok, Bad, Bid, Bik, Bim, Bcl-Xs, Krk, Mtd, Nip3, Nix, Nora, and Bcl-B) of apoptosis (Iannolo *et al*. 2008). In addition to cytochrome *c*, mitochondria release a large number of other polypeptides, including AIF, Endo G, second mitochondrial activator of caspases (Smac/Diablo), and HtrA2/Omi from the intermembrane space. Smac/Diablo and Omi/HtrA2 promote caspase activation through neutralizing the inhibitory effects of inhibitor of apoptosis proteins (IAPs). In addition to cytochrome *c*, mitochondria release a large number of other polypeptides, including AIF, Endo G, Smac/Diablo, and HtrA2/Omi from the intermembrane space. Smac/Diablo and Omi/HtrA2 promote caspase activation through neutralizing the inhibitory effects of IAPs.

The extrinsic pathway is initiated by binding of the transmembrane death receptors such as Fas, tumor necrosis factor (TNF) receptor, DR3, DR4, or DR5 with their specific ligands. These cell surface receptors are activated when cross-linked by their ligands. Activation of death receptors by cross-linking with their natural ligands induces receptor clustering and formation of a death-inducing signaling complex. The complex recruits procaspase-8 via the adaptor molecule Fas-associated death domain protein (FADD), resulting in the activation of caspase-8. Next, procaspase-8 is proteolytically activated and serves as the ‘initiator’ caspase, further activating downstream effectors’ proteins such as caspases-3 and -7 to initiate cell degradation, causing inevitable apoptosis.

For the maintenance of prostate growth, the complex equilibrium between cell growth, proliferation factors, and apoptosis-inducing factors is essential. Fluctuations in this balance cause overexpression of factors causing cell survival and proliferation and loss of apoptosis leading to tumorigenesis and cancer. The deregulation of prostate growth in PCa cells is notable by apoptotic evasion, loss of differentiation, and uncontrolled proliferation. For the treatment of advanced metastatic PCa and the appearance of therapeutic resistance of prostate tumors, the challenges in the implementation of effective therapeutic strategies involve functional significance of anti-apoptotic pathways (Reynolds & Kyprianou 2006). In PCa progression, loss/suppression of apoptosis has been heavily implicated and apoptosis induction has been considered as an effective means of therapeutic approach for the treatment of prostate tumors. For the effective establishment of the efficacy of the markers of apoptosis in PCa, their evaluation should be performed in combination with numerous clinical parameters and other biochemical markers.

Various research studies have demonstrated that dietary agents may be used alone or in combination with conventional chemotherapeutic agents to prevent the occurrence and spread of cancer. Being rich sources of abundant bioactive compounds, consumption of fruits, vegetables, and whole grains may reduce the risk of cancer in some individuals. In this review, we discuss the use of dietary components present in the mostly consumed fruits, vegetables, and beverages as chemopreventive and/or chemotherapeutic agents against cancer. The rationale for selecting these compounds is that they are present in large amounts in the dietary substances and have been shown to exhibit chemopreventive and/or chemotherapeutic effects against PCa. We summarized studies on the effects of selected dietary components like (−)-epigallocatechin-3-gallate (EGCG), genistein, curcumin, resveratrol, lycopene, pomegranate, fisetin, and lupeol (Fig. 1) on PCa within the context of apoptosis (Fig. 2) *in vitro*, *in vivo*, and where available in human clinical trials.

(−)-Epigallocatechin-3-gallate

Tea, the most consumed beverage in the world next to water, is derived from the plant *Camellia sinensis* and is processed in different ways in different parts of the world to give green, black, or oolong tea. Green tea contains characteristic polyphenolic compounds, namely, EGCG, (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epicatechin (EC). There is vast amount of scientific literature, which suggests that EGCG is responsible for the majority of the potential health benefits attributed to green tea consumption (Khan *et al*. 2008*a*).

In vitro studies

We reported for the first time that EGCG (80 μM) induced apoptosis in human PCa DU145 cells (Ahmad *et al*. 1997), and recently reported that treatment of human PCa cells such as LNCaP, PC-3, and

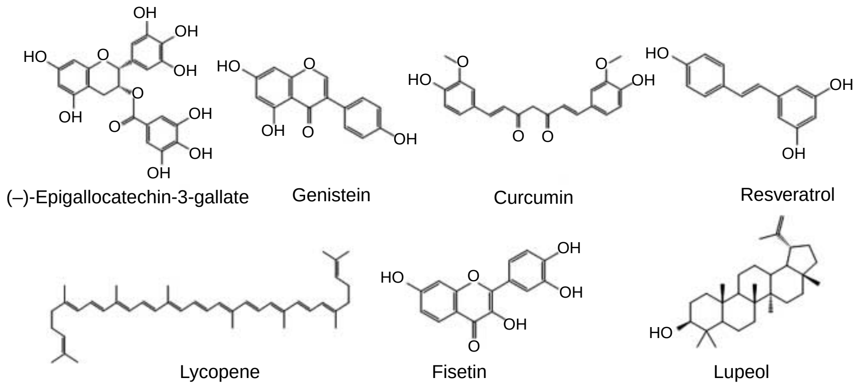

Figure 1 Chemical structures of dietary agents.

CWR22Rν1 with combination of EGCG (10–40 μM) and cyclooxygenase-2 (COX-2) inhibitor resulted in enhanced cell growth inhibition, apoptosis induction, and inhibition of NF-κB (Adhami *et al*. 2007). Study from our laboratory has also shown that EGCG (20 and 40 μM) sensitizes TRAIL-resistant LNCaP cells to TRAIL-mediated apoptosis through modulation of intrinsic and extrinsic apoptotic pathways (Siddiqui *et al*. 2008*a*). In our earlier studies, using isogenic cell lines, we have demonstrated that EGCG (20–80 μM) activates growth arrest and apoptosis in prostate carcinoma cells primarily via p53-dependent pathway that involves the function of both p21 and Bax such that downregulation of either molecule confers a growth advantage to the cells (Hastak *et al*. 2005). It has been shown that EGCG, the major green tea catechin, inhibited the growth of SV40-immortalized human prostate epithelial cells (PNT1A) as well as tumorigenic, poorly differentiated PC-3 cells, but had no effects on normal human prostate epithelial cells

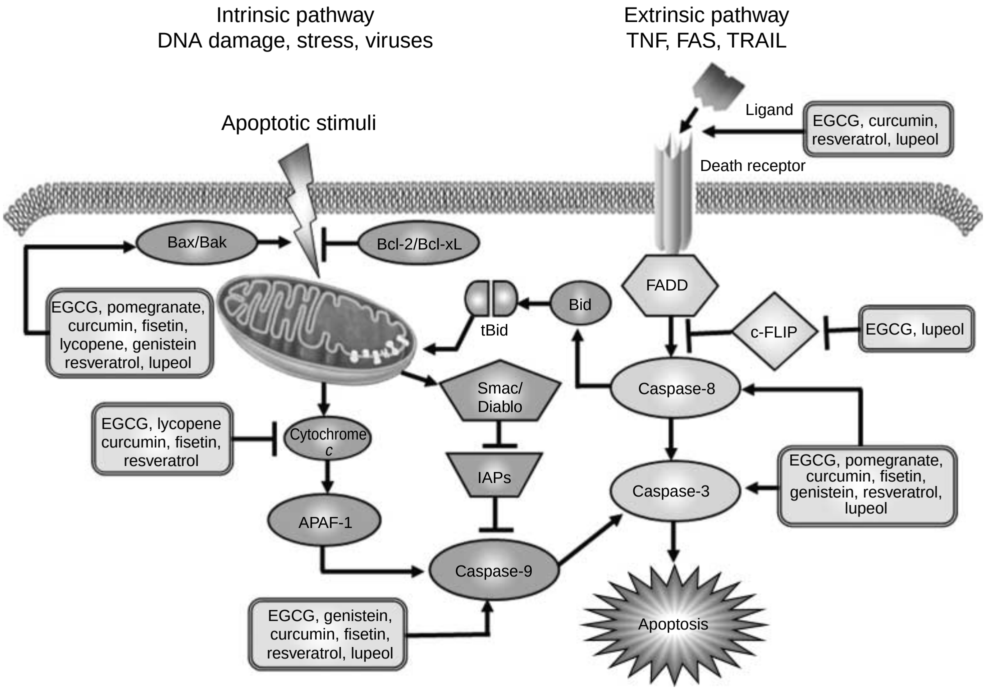

Figure 2 Effects of dietary agents on the extrinsic and intrinsic pathways of apoptosis in prostate cancer. The extrinsic pathway is triggered by death receptors and involves activation of the initiator caspase-8, which directly activates caspase-3 causing apoptosis. The intrinsic pathway is activated by different apoptotic stimuli that lead to release of cytochrome *c* from mitochondria and activation of caspase-9. This step can be inhibited by the anti-apoptotic members of the Bcl-2 family of apoptosis regulators. Cytochrome *c* interacts with APAF-1 and caspase-9 to promote the activation of caspase-3. Most dietary agents interfere with the key regulators of the apoptotic pathway.

(Caporali *et al*. 2004). It has been shown that EGCG (20–80 μM) induced apoptosis in human PCa LNCaP cells via stabilization of p53 by phosphorylation on critical serine residues and p14ARF-mediated down-regulation of murine double minute 2 (MDM2) protein, negative regulation of NF-κB activity, activation of caspases, causing a change in the ratio of Bax/Bcl-2 in a manner that favors apoptosis (Hastak *et al*. 2003). The high levels of fatty acid synthase (FAS) activity in LNCaP cells were shown to be dose dependently inhibited by EGCG (40–150 μM) and it was paralleled by decreased endogenous lipid synthesis, inhibition of cell growth, and induction of apoptosis. Treatment of nonmalignant cells exhibiting low levels of FAS activity with EGCG led to a decrease in growth rate but not to induction of apoptosis. These studies showed that EGCG inhibited FAS activity and selectively caused apoptosis in LNCaP cells but not in nontumoral fibroblasts (Brusselmans *et al*. 2003). EGCG and (+)-gallocatechin gallate at dose level of 1–100 μM potently and specifically inhibit the chymotrypsin-like activity of purified 20S proteasome and the 26S proteasome in tumor cell lysates. EGCG treatment (10 μM) also accumulated the pro-apoptotic Bax protein and induced apoptosis in LNCaP cells expressing high basal levels of Bax (Smith *et al*. 2002). Green tea catechins (100 μM) suppressed the growth and induced apoptosis through an increase in reactive oxygen species formation and mitochondrial depolarization in human PCa DU145 cells (Chung *et al*. 2001). We have shown earlier that EGCG (10–80 μM) negatively modulates PCa cell growth by the induction of apoptosis, which may be mediated by WAF1/p21-caused G_0/G_1 phase cell cycle arrest, irrespective of the androgen association or p53 status of the cells (Gupta *et al*. 2000).

In vivo studies

We have earlier reported that green tea polyphenols (GTP) containing EGCG consumption significantly inhibited PCa development and metastasis in transgenic adenocarcinoma of the mouse prostate (TRAMP) model. This was achieved by an oral infusion of GTP equivalent to six cups of green tea a day, i.e. at doses that are achievable in humans. GTP consumption was found to cause significant apoptosis of PCa cells, which possibly resulted in reduced dissemination of cancer cells, thereby causing inhibition of PCa development, progression, and metastasis to distant organ sites (Gupta *et al*. 2001). Recently, we have reported that continuous GTP infusion for 32 weeks resulted in substantial reduction in expression of NF-κB, IKKα, IKKβ, RANK, NIK, STAT-3, and osteopontin in dorsolateral prostate of TRAMP mice. There was also induction of Bax and downregulation of Bcl-2 proteins (Siddiqui *et al*. 2008*b*). In athymic nude mice, implanted with CWR22Rν1 cells, combination treatment with GTP and celecoxib resulted in enhanced tumor growth inhibition, lowering of prostate-specific antigen (PSA), insulin-like growth factor 1 (IGF1) levels, and increase in IGF-binding protein (IGFBP)-3 levels (Adhami *et al*. 2007). EGCG inhibited early stage PCa, but not late stage PCa, in the TRAMP mice. In the ventrolateral prostate, EGCG significantly reduced cell proliferation, induced apoptosis, and decreased androgen receptor (AR), IGF1, IGF1R, phospho-extracellular signal-regulated kinases 1/2, COX-2, and inducible nitric oxide synthase (Harper *et al*. 2007). It has also been reported previously that treatment of athymic nude mice implanted with CWR22Rν1 cells with GTP, water extract of black tea, and their major constituents, EGCG and theaflavins respectively, resulted in significant inhibition in growth of implanted prostate tumors. This was accompanied with induction of apoptosis as evidenced by upregulation in Bax and decrease in Bcl-2 proteins and decrease in the levels of vascular endothelial growth factor (VEGF) protein (Siddiqui *et al*. 2006). In a study, only 20% of mice developed the neoplasms who were receiving 0.3% GTC in drinking water, while 100% of TRAMP mice developed CaP in the control group. In TRAMP mice, the clusterin (*CLU*) gene was dramatically downregulated during onset and progression of CaP. In GTC-treated TRAMP mice in which tumor progression was chemoprevented, CLU mRNA and protein progressively accumulated in the prostate gland. CLU dropped again to undetectable levels in animals in which GTC chemoprevention failed and PCa developed (Caporali *et al*. 2004).

Clinical trials

The effects of green tea consumption to various stages of PCa patients have been reported in several studies, but apoptotic effects have not been reported in any of these studies. Recently, the results of a phase II clinical trial have been reported. The PCa patients were given daily doses of Polyphenon E, which contained 800 mg (−)-EGCG and lesser amounts of (−)-EC, (−)-EGC, and (−)-ECG, until time of radical prostatectomy. There was a significant reduction in serum levels of PSA, hepatocyte growth factor, and VEGF in men with PCa after brief treatment with EGCG (Polyphenon E) without the elevation in liver enzymes (McLarty *et al*. 2009). In a clinical trial, men with high-grade prostatic intraepithelial neoplasia were given either extracts of green tea or a placebo for 1 year. There were no significant side effects and lower urinary tract symptoms with 3% tumor incidence in GTC-treated men as compared to 30% in placebo-treated men (Bettuzzi *et al*. 2006). In patients of hormone-refractory PCa, green tea was found to have minimal clinical activity against the disease with nine patients reported to have progressive disease within 2 months of starting therapy and six patients developed progressive disease after additional 1–4 months of therapy (Choan *et al*. 2005). A case–control study was conducted in Hangzhou, Southeast China, in patients with histologically confirmed adenocarcinoma of the prostate. The risk of PCa declined with increasing frequency, duration, and quantity of green tea consumption (Jian *et al*. 2004). In a phase II clinical trial, decrease in PSA was observed in 2% of cohort, but there was also green tea toxicity in 69% of patients with androgen-independent PCa (Jatoi *et al*. 2003).

Genistein

Phytoestrogens are naturally occurring phenolic compounds classified as flavones, isoflavones, coumestans, and lignans. The beneficial effects of a soy diet have been attributed to isoflavones. Genistein (5,7,4′-trihydroxyisoflavone), the predominant isoflavone in human diet, is derived mainly from soybeans but is also found in other legumes, including peas, lentils, or beans.

In vitro studies

It has been recently reported that genistein (15–120 μM) inhibited proliferation and induced apoptosis of DU145 and HeLa cells and had minimal effects on normal L-O2 cells (Yuan-Jing *et al*. 2009). Genistein-combined polysaccharide (GCP) at doses of 1–200 μM has been reported to mediate growth inhibition and promote apoptosis through molecular mimicry of androgen ablation via AR downregulation and by providing an AR-independent, pro-apoptotic signal through mammalian target of rapamycin (mTOR) inhibition (Tepper *et al*. 2007). Genistein (15 μM) combined with radiation was found to cause greater inhibition in PC-3 colony formation compared to genistein or radiation alone. Treatment with genistein caused dose- and time-dependent G_2/M phase cell cycle arrest with increased WAF1/p21 and decreased cyclin B1 expression. Radiation-induced activation of NF-κB activity was strongly inhibited by genistein pretreatment. A significant increase in apoptosis was measured by an increase in cleavage of poly (ADP-ribose) polymerase (PARP) protein (Raffoul *et al*. 2006). Genistein (15–50 μM) reduced MDM2 protein and mRNA levels in human cancer cell lines of breast, colon and prostate, primary fibroblasts, and breast epithelial cells. At the post-translational level, genistein induced ubiquitination of MDM2, which led to its degradation. Treatment with genistein also led to induction of apoptosis, G_2 arrest, and inhibition of cell proliferation (Li *et al*. 2005). Both genistein (10–70 μM) and β-lapachone (1.2 μM) caused dose-dependent growth inhibition and treatment-induced apoptosis in PC-3 cells. Treatment with caspase-3 inhibitor, DEVD-FMK before exposure to genistein, significantly inhibited caspase-3 expression and treatment-induced apoptosis (Kumi-Diaka *et al*. 2004). Genistein in combination with polysaccharide (10 μM) significantly suppressed cell growth in LNCaP and PC-3 cells, which was associated with apoptosis in LNCaP cells, but not in PC-3 cells (Bemis *et al*. 2004). Inhibition of the proteasome by genistein (50–200 μM) was associated with accumulation of ubiquitinated proteins and three known proteasome target proteins, Kip1/p27, IκBα and the pro-apoptotic protein Bax in PCa LNCaP and breast cancer MCF-7 cells. Genistein-mediated proteasome inhibition was accompanied by induction of apoptosis in these tumor cells (Kazi *et al*. 2003). Polyphenols from tomatoes and soy such as genistein, quercetin, kaempferol, biochanin A, daidzein, and rutin (5–50 μM) modulated IGF1-induced *in vitro* proliferation and apoptotic resistance in the AT6.3 rat PCa cell line via inhibition of multiple intracellular signaling pathways involving tyrosine kinase activity (Wang *et al*. 2003). A cDNA microarray gene expression profile of genistein (100 μM) -treated LNCaP cells revealed that the expression of many genes, including survivin, DNA topoisomerase II, cdc-6, and MAPK-6, was downregulated. The glutathione peroxidase (*GPx*)-1 gene expression level was the most upregulated (Suzuki *et al*. 2002). It was reported that genistein (75–150 μM) inhibited the growth of LNCaP cells, which was accompanied by the suppression of DNA synthesis and the induction of apoptosis (Onozawa *et al*. 1998).

In vivo studies

In an orthotopic prostate carcinoma model of PC-3 cells in nude mice, genistein combined with prostate tumor irradiation led to a greater control of the growth of the primary tumor and metastasis to lymph nodes than genistein or radiation alone, resulting in greater survival. There was an increase in giant cells, apoptosis, inflammatory cells, and fibrosis with decreased tumor cell proliferation consistent with increased tumor cell destruction after radiation and genistein treatment (Hillman *et al*. 2004). It was also shown that MDM2 overexpression abrogated genistein-induced apoptosis *in vitro* and that genistein inhibited MDM2 expression and tumor growth in PC-3 xenografts (Li *et al*. 2005). The 2% GCP-supplemented diet significantly slowed LNCaP tumor growth, increased apoptosis, and decreased proliferation over 4 weeks in xenograft model (Bemis *et al*. 2004). Genistein regulated the expression of multiple genes involved in the control of cell growth, apoptosis, and metastasis in PC-3 cells and in experimental PC-3 bone tumors created by injecting PC-3 cells into human bone fragments previously implanted in severe combined immunodeficient mice (Li *et al*. 2004).

Clinical trials

A nonrandomized, nonblinded trial with historically matched controls from archival tissue was designed to determine the effects of acute exposure to a dietary supplement of isoflavones in men with clinically significant PCa before radical prostatectomy. Prior to surgery, 20 men consumed 160 mg/day of red clover-derived dietary isoflavones, containing a mixture of genistein, daidzein, formononetin, and biochanin A. There were no significant differences in serum PSA, Gleason score, serum testosterone, or biochemical factors between pre- and post-treatment in the subjects. Apoptosis in radical prostatectomy specimens from treated patients was significantly higher than in control subjects, specifically in regions of low to moderate grade (Jarred *et al*. 2002).

Curcumin

Curcumin (diferuloylmethane) is a major chemical component of turmeric (*Curcuma longa* Linn.) and is used as a spice to give a specific flavor and yellow color to food in the Indian subcontinent. It has been used for centuries in indigenous medicine for the treatment of a variety of inflammatory conditions and other diseases. There are diverse mechanisms that are implicated in the inhibition of tumorigenesis by curcumin and include a combination of anti-inflammatory, anti-oxidant, immunomodulatory, pro-apoptotic, and anti-angiogenic properties via pleiotropic effects on genes and cell-signaling pathways at multiple levels (Khan *et al*. 2008*a*).

In vitro studies

Curcumin (30 μM) sensitized PC-3 cells to TRAIL by inhibiting Akt-regulated NF-κB and NF-κB-dependent anti-apoptotic Bcl-2, Bcl-xL, and X-linked inhibitor of apoptosis protein (XIAP) (Deeb *et al*. 2007). Curcumin has been shown to enhance the apoptosis-inducing potential of TRAIL in PC-3 cells and LNCaP cells. On treatment with curcumin (20–40 μM), the expressions of Bcl-2, Bcl-xL, survivin, and XIAP were inhibited, and there was induction in the expressions of Bax, Bak, PUMA, Bim, Noxa, and death receptors DR4 and DR5 in both cell lines. Treatment of cells with curcumin resulted in activation of caspases-3 and -9 and drop in mitochondrial membrane potential. Combination with TRAIL further led to the enhancement of these events (Shankar *et al*. 2007*a*). It has also been reported that combination of phenethyl isothiocyanate (PEITC; 10 μM) and curcumin (25 μM) significantly increased the activity of PARP and cleavage of caspase-3 in correlation with apoptotic cell death. The phosphorylations of IκBα and Akt were significantly attenuated by the combination of PEITC and curcumin. Treatment with PEITC and curcumin caused suppression of epidermal growth factor (EGF) receptor phosphorylation and inhibition of EGF-induced phosphorylation of Akt and induction of phosphatidylinositol 3-kinase (PI3K) in PC-3 cells (Kim *et al*. 2006). Combined curcumin and TRAIL treatment led to induction of apoptosis as observed by accumulation of hypodiploid cells in sub-G_1 phase, enhanced annexin V binding, DNA fragmentation, cleavage of procaspases-3, -8, and -9, truncation of pro-apoptotic Bid, and release of cytochrome *c* from mitochondria (Deeb *et al*. 2005). Curcumin (2–5 μM) in combination with radiation showed significant enhancement of radiation-induced clonogenic inhibition and apoptosis in PC-3 cells. However, curcumin in combination with radiation showed inhibition of TNF-α-mediated NF-κB activity resulting in Bcl-2 protein downregulation. Significant activation of cytochrome *c* and caspases-9 and -3 was also observed in cells treated with a combination of curcumin and radiation (Chendil *et al*. 2004). Treatment of PCa cells with curcumin (1–100 μM) suppressed both constitutive (DU145) and inducible (LNCaP) NF-κB activation and potentiated TNF-induced apoptosis. Curcumin treatment (50–100 μM) induced apoptosis in both cell types, which correlated with the downregulation of the expression of Bcl-2 and Bcl-xL and the activation of procaspase-3 and -8 (Mukhopadhyay *et al*. 2001).

In vivo studies

Recently, curcumin has been shown to inhibit the growth of LNCaP xenografts in nude mice by inducing apoptosis, inhibiting proliferation, and also sensitized tumors to undergo TRAIL-induced apoptosis. Curcumin upregulated the expression of TRAIL-R1/DR4, TRAIL-R2/DR5, Bax, Bak, p21/WAF1, and p27/KIP1, and inhibited the activation of NF-κB and its gene products in xenografted tumors (Shankar *et al*. 2008). Combination of PEITC and curcumin showed stronger tumor growth-inhibitory effects in NCr immunodeficient (nu/nu) mice bearing subcutaneous xenografts of PC-3 cells. There was inhibition of Akt- and NF-κB-signaling pathways contributing to the inhibition of cell proliferation and induction of apoptosis (Khor *et al*. 2006). Curcumin caused a marked decrease in the extent of cell proliferation and a significant increase in the extent of apoptosis as measured by an *in situ* cell death assay in excised tumors of athymic nude mice. A significant decrease in the microvessel density as observed by the CD31 antigen staining was also observed (Dorai *et al*. 2001).

Resveratrol

Resveratrol (3,5,4′-trihydroxystilbene), a naturally occurring phytoalexin found in red wine, grapes, peanuts, mulberries, and in a variety of other plants, has anti-oxidant and anti-inflammatory properties. It was first detected in the dried roots of *Polygonum cuspidatum*, traditionally used in Chinese and Japanese medicines as an anti-inflammatory agent. It exists in two isoforms; *trans*-resveratrol and *cis*-resveratrol, where the *trans*-isomer is the more stable form (Khan *et al*. 2008*a*,*b*, Athar *et al*. 2009). It has gained considerable attention because of its potential cancer chemopreventive properties. Resveratrol has attracted considerable attention since 1997, when Jang *et al*. (1997) reported that it inhibits the carcinogenic process at the initiation, promotion, and progression stages. These studies were performed using a panel of cells originating from human lymphocytic leukemia and murine mammary and skin tumors.

In vitro studies

It has been shown that resveratrol (100 μM) sensitized docetaxel-resistant tumor cells to TRAIL by blocking CLU expression. It was further indicated that resveratrol acts as an effective tyrosine kinase inhibitor and could inhibit Src and Jak kinases, thus resulting in the loss of Stat1 activation (Sallman *et al*. 2007). Methoxy- and hydroxy-substituted resveratrol derivatives exerted cytotoxic effects in PC-3, LNCaP, and DU145 human PCa cell lines. The most potent compounds, 3,3′,4,4′,5,5′-hexahydroxy-stilbene and 3,4,4′,5-tetramethoxystilbene, induced apoptosis at concentrations lower than resveratrol and caused cell cycle arrest. It was concluded in this study that introducing additional hydroxy- and methoxy moieties to the stilbene ring of resveratrol was capable of enhancing its cytotoxic and pro-apoptotic effects in hormone-responsive and nonresponsive PCa cells (Horvath *et al*. 2007). Resveratrol pretreatment (50 μM) sensitized PC-3 and DU145 cells to agents that specifically target death receptors but not to agents that initiate apoptosis through other mechanisms. Resveratrol also altered the expression of IAPs and Bax, and decreased Akt phosphorylation in PC-3 cells leading to increased caspase activation and apoptosis (Gill *et al*. 2007). Resveratrol (0–30 μM) was found to inhibit growth and induced apoptosis in LNCaP cells, but had no effect on normal human prostate epithelial cells. The expression of Bax, Bak, PUMA, Noxa, Bim, TRAIL-R1/DR4, and TRAIL-R2/DR5 was upregulated, and the expression of Bcl-2, Bcl-xL, survivin, and XIAP was downregulated on treatment with resveratrol. There was also generation of reactive oxygen species, translocation of Bax and p53 to mitochondria, subsequent drop in mitochondrial membrane potential, release of mitochondrial proteins, activation of caspase-3 and -9, and induction of apoptosis on treatment of cells with resveratrol. The dominant negative FADD, caspase-8 siRNA, or *N*-acetyl cysteine inhibited the ability of resveratrol to sensitize TRAIL-resistant LNCaP cells. (Shankar *et al*. 2007*b*). Resveratrol (1–150 μM) induced a decrease in proliferation rate and an increase in apoptosis in LNCaP and PC-3 cells in a dose- and time-dependent manner. The resveratrol-induced apoptosis was mediated by activation of caspases-9 and -3 and a change in the Bax/Bcl-2 ratio (Benitez *et al*. 2007). Treatment of LNCaP cells with resveratrol (1–50 μM) was found to result in a significant loss of mitochondrial membrane potential, inhibition in the protein level of anti-apoptotic Bcl-2, and increase in pro-apoptotic members of the Bcl-2 family, i.e. Bax, Bak, Bid, and Bad. Resveratrol caused apoptosis of LNCaP cells via inhibition of PI3K/Akt activation and modulations in Bcl-2 family proteins (Aziz *et al*. 2006). Resveratrol (100 μM) is a potent sensitizer of tumor cells for TRAIL-induced apoptosis through p53-independent induction of p21 and p21-mediated cell cycle arrest associated with survivin depletion. Resveratrol-induced G_1 arrest was associated with downregulation of survivin expression and sensitization for TRAIL-induced apoptosis.

Resveratrol-mediated cell cycle arrest followed by survivin depletion and sensitization for TRAIL was impaired in p21-deficient cells. The downregulation of survivin using survivin anti-sense oligonucleotides sensitized cells for TRAIL-induced apoptosis (Fulda & Debatin 2004).

In vivo studies

Resveratrol delayed LNCaP tumor growth in athymic nude mice and inhibited expression of a marker for steroid hormone responses. Exposure to resveratrol also led to increased angiogenesis and inhibition of apoptosis in the xenograft (Wang *et al*. 2008). An *in vivo* experiment was performed to explore the effect of resveratrol in the TRAMP model, featuring the rat probasin promoter/SV40 T antigen. Resveratrol suppressed PCa growth and induced apoptosis through AR downregulation and suppression of the androgen-responsive glandular kallikrein 11, known to be an ortholog of the human PSA, at the mRNA level without any signs of toxicity (Seeni *et al*. 2008).

Lycopene

Lycopene is a nonprovitamin A carotenoid that gives red color to tomatoes. Humans and animals depend on dietary sources and do not synthesize lycopene. The dietary sources of lycopene include tomatoes and tomato products, apricots, pink guava, papaya, watermelon, and pink grapefruit. It has been suggested that carotenoids may modulate processes related to mutagenesis, carcinogenesis, cell differentiation, and proliferation (Khan *et al*. 2008*a*,*b*).

In vitro studies

It has been reported recently that treatment of PCa LNCaP and PC-3 cells with lycopene-based agents (100 nM) resulted in mitotic arrest with cells accumulating in G_0/G_1 phase. There was block in G_1/S transition mediated by decreased levels of cyclins D1, E, cdk-4, and suppression of retinoblastoma phosphorylation. These responses correlated with decreased IGF1R expression and activation, increased IGFBP-2 expression, and decreased AKT activation. Exposure to lycopene also induced a profound apoptotic response in LNCaP cells (Ivanov *et al*. 2007). It has been demonstrated that treatment of LNCaP cells with physiologically attainable concentrations of lycopene (0.3–3.0 μM) significantly reduced mitochondrial transmembrane potential, induced the release of mitochondrial cytochrome *c*, and increased annexin V binding, confirming induction of apoptosis (Hantz *et al*. 2005). Treatment of PCa DU145 cells with lycopene (8–32 μM) resulted in G_0/G_1 phase cell cycle arrest and induction of apoptosis in a dose-dependent manner. The rate of apoptosis was 42.4% lower in DU145 cells treated with lycopene as compared with the untreated control cells (Tang *et al*. 2005).

In vivo studies

In a study, the timing of initiation of micronutrients, and the effect of micronutrient combinations, on PCa development in Lady transgenic model was examined. Transgenic males were administered either i) a control diet, ii) control diet supplemented with human equivalent doses of vitamin E, selenium, and lycopene, or iii) control diet supplemented with vitamin E and selenium. In separate experiments, the combination of vitamin E, selenium, and lycopene was initiated at 4, 8, 20, and 36 weeks of age. There was significant reduction in PCa and liver metastasis when intervention was commenced within 8 weeks of age by a combination diet of vitamin E, selenium, and lycopene. There was a strong correlation between disease-free state with upregulation of the prognostic marker p27/Kip1 and decreased expression of proliferating cell nuclear antigen (PCNA) and significantly increased apoptotic index. A combination of vitamin E and selenium was not effective in preventing PCa, with 84.6% of animals developing PCa and 11.5% developing high-grade prostatic intraepithelial neoplasia. Early commencement of micronutrients combination of vitamin E, selenium, and lycopene was found to be beneficial in reducing PCa. Lycopene was found to be an essential component of the combination and effective for PCa prevention (Venkateswaran *et al*. 2009). The inhibitory effect of lycopene on the growth rate of DU145 tumor xenografts was studied in BALB/c male nude mice. There was 55.6 and 75.8% inhibition of tumor growth rate in mice treated with 100 and 300 mg/kg lycopene respectively as compared to the control group. Treatment with lycopene caused accumulation of DU145 cells in the G_0/G_1 phase and apoptosis in a dose-dependent manner. The rate of apoptosis was 42.4% greater in cells treated with 32 μmol/l lycopene than in control group (Tang *et al*. 2005).

Clinical trials

In a clinical study, thirty-two patients diagnosed by biopsy with PCa were given tomato sauce pasta entrees (30 mg lycopene/day) for 3 weeks before prostatectomy. Thirty-four patients with PCa that served as controls did not consume tomato sauce and underwent prostatectomy. When tumor areas with the most apoptotic cells were compared in the biopsy and resected prostate tissue, tomato sauce consumption increased apoptotic cells in benign prostate hyperplasia (BPH) and in carcinomas. The apoptotic cell death in carcinomas increased significantly with treatment, and apoptotic cell death in BPH showed a tendency towards an increase when comparable morphological areas were counted. The differences in values were not significant in BPH and carcinomas when the values of apoptotic cells in BPH and carcinomas of patients who consumed tomato sauce were compared with corresponding control lesions of the patients who did not consume tomato sauce in resected prostate tissue. This study provides the evidence that tomato sauce consumption may suppress the progression of the disease in a subset of patients with PCa by increasing apoptotic cell death (Kim *et al*. 2003).

Pomegranate

The pomegranate (*Punica granatum* L.) fruit has been used for centuries in ancient cultures for medicinal purposes. The fruits have also been long and widely used in folk and traditional medicine for the treatment of a number of pathologies (Aviram *et al*. 2000). Employing Matrix-assisted laser desorption/ionization time of flight mass spectrometry, pomegranate juice was found to contain six anthocyanins (pelargonidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, pelargonidin 3,5-diglucoside, cyanidin 3,5-diglucoside, and delphinidin 3,5-diglucoside), ellagitannins, and hydrolyzable tannins. The other flavonoids identified included quercetin, kaempferol, and luteolin glycosides (Gil *et al*. 2000).

In vitro studies

We have shown that pomegranate fruit extract (PFE) treatment (10–100 μM) of human PCa PC-3 cells resulted in a dose-dependent inhibition of cell growth/cell viability and induction of apoptosis. PFE treatment of PC-3 cells resulted in induction of Bax and Bak (pro-apoptotic) proteins and downregulation of Bcl-xL and Bcl-2 (anti-apoptotic) proteins (Malik *et al*. 2005). It has been reported that pomegranate extract inhibited NF-κB and cell viability of PCa cell lines in a dose-dependent fashion *in vitro*. Pomegranate extract-induced apoptosis was dependent on NF-kB blockade (Rettig *et al*. 2008). Pomegranate oil (35 μM), fermented juice polyphenols, and pericarp polyphenols each acutely inhibited *in vitro* proliferation of LNCaP, PC-3, and DU145 human PCa cell lines. These effects were mediated by changes in both cell cycle distribution and induction of apoptosis (Albrecht *et al*. 2004). Oral administration of PFE to athymic nude mice implanted with androgen-sensitive CWR22Rυ1 cells resulted in a significant inhibition of tumor growth concomitant with a significant decrease in serum PSA levels (Malik *et al*. 2005).

In vivo studies

In the LAPC4 xenograft model, pomegranate extract delayed the emergence of LAPC4 androgen-independent xenografts in castrated mice through an inhibition of proliferation and induction of apoptosis. On treatment with pomegranate extract, the observed increase in NF-kB activity during the transition from androgen dependence to androgen independence in the LAPC4 xenograft model was abrogated (Rettig *et al*. 2008).

Clinical trials

To determine the effects of pomegranate juice consumption, a phase II clinical trial was conducted in men with rising PSA after surgery or radiotherapy. Mean PSA doubling time significantly increased with treatment from a mean of 15 months at baseline to 54 months post-treatment. There was a decrease in cell proliferation and an increase in apoptosis in *in vitro* assays comparing pretreatment and post-treatment patient serum on the growth of LNCaP cells (Pantuck *et al*. 2006).

Fisetin

Fisetin (3,3′,4′,7-tetrahydroxyflavone) is found in fruits and vegetables, such as strawberry, apple, persimmon, grape, onion, and cucumber (Arai *et al*. 2000). Fisetin exhibits a wide variety of activities including neurotrophic (Maher 2006), anti-oxidant (Hanneken *et al*. 2006), anti-inflammatory (Higa *et al*. 2003), and anti-angiogenic (Fotsis *et al*. 1998) effects.

In vitro studies

We have reported that fisetin treatment (10–60 μM) was found to result in a decrease in the viability of LNCaP, CWR22Rν1, and PC-3 cells but had only minimal effects on normal prostate epithelial cells. Treatment of LNCaP cells with fisetin resulted in induction of apoptosis, PARP cleavage, modulation in the expressions of Bcl-2-family proteins, inhibition of PI3K, and phosphorylation of Akt at Ser^{473} and

Thr[308]. There was also induction of mitochondrial release of cytochrome *c* into cytosol, downregulation of XIAP, and upregulation of Smac/Diablo upon treatment of cells with fisetin. Treatment of cells with fisetin also resulted in significant activation of caspases-3, -8, and -9. Pretreatment of cells with caspase inhibitor (Z-VAD-FMK) blocked fisetin-induced activation of caspases (Khan *et al*. 2008*b*).

Lupeol

Lup-20(29)-en-3h-ol (Lupeol), a triterpene found in fruits such as olive, mango, strawberry, grapes and figs, in many vegetables, and in several medicinal plants, is used in the treatment of various diseases. It possesses strong anti-inflammatory, anti-arthritic, anti-mutagenic, and anti-malarial activity when examined in *in vitro* and *in vivo* systems (Geetha & Varalakshmi 2001).

In vitro studies

Study from our laboratory has shown that lupeol (1–30 μM) induced the cleavage of PARP protein and degradation of acinus protein with a significant increase in the expression of FADD protein and Fas receptor in androgen-sensitive human PCa cells. The small interfering RNA-mediated silencing of the *Fas* gene and inhibition of caspases-6, -8, and -9 by their specific inhibitors confirmed that Lupeol specifically activates the Fas receptor-mediated apoptotic pathway in androgen-sensitive PCa cells. The treatment of cells with a combination of anti-Fas monoclonal antibody and lupeol resulted in higher cell death compared with the additive effect of the two compounds alone, suggesting a synergistic effect. Treatment with lupeol also resulted in a significant inhibition in growth of tumors with concomitant reduction in PSA secretion in athymic nude mice implanted with CWR22Rν1 cells (Saleem *et al*. 2005). It has been shown recently that lupeol (400–600 μM) caused anti-proliferative effect associated with an increase in G_2/M phase arrest in PC-3 cells. There was also induction of apoptosis with upregulation of Bax, caspases-3 and -9, and *APAF-1* genes, and downregulation of anti-apoptotic *Bcl-2* gene in PC-3 cells. The role of caspase-induced apoptosis was confirmed by increase in reactive oxygen species, loss of mitochondrial membrane potential followed by DNA fragmentation (Prasad *et al*. 2008*a*; Table 1).

Table 1 Concentrations of dietary agents reported to cause anti-proliferative and apoptotic effects *in vitro* in prostate cancer cells

Dietary agents	*In vitro* concentrations (μM)	References
EGCG	80	Ahmad *et al.* (1997)
	10–80	Gupta *et al.* (2000)
	1–100	Smith *et al.* (2002)
	40–150	Brusselmans *et al.* (2003)
	20–80	Hastak *et al.* (2003, 2005)
	10–40	Adhami *et al.* (2007)
	20 and 40	Siddiqui *et al.* (2008*a*)
Genistein	75–150	Onozawa *et al.* (1998)
	100	Suzuki *et al.* (2002)
	5–50	Wang *et al.* (2003)
	50–200	Kazi *et al.* (2003)
	10	Bemis *et al.* (2004)
	10–70	Kumi-Diaka *et al.* (2004)
	15–50	Li *et al.* (2005)
	15	Raffoul *et al.* (2006)
	15–20	Yuan-Jing *et al.* (2009)
Curcumin	1–100	Mukhopadhyay *et al.* (2001)
	2–5	Chendil *et al.* (2004)
	25	Kim *et al.* (2006)
	20–40	Shankar *et al.* (2007*a*)
	30	Deeb *et al.* (2007)
Resveratrol	100	Fulda & Debatin (2004)
	1–50	Aziz *et al.* (2006)
	1–150	Benitez *et al.* (2007)
	0–30	Shankar *et al.* (2007*b*)
	50	Gill *et al.* (2007)
	100	Sallman *et al.* (2007)
Lycopene	0.3–3.0	Hantz *et al.* (2005)
	8–32	Tang *et al.* (2005)
	0.1	Ivanov *et al.* (2007)
Pomegranate	35	Albrecht *et al.* (2004)
	10–100	Malik *et al.* (2005)
Fisetin	10–60	Khan *et al.* (2008*b*)
Lupeol	1–30	Saleem *et al.* (2005)
	400–600	Prasad *et al.* (2008*a*)

In vivo studies

In a recent study, lupeol and mango pulp extract supplementation has resulted in a significantly high percentage of apoptotic cells in the hypodiploid region in male Swiss albino mice. The induction of apoptosis in mouse prostate was preceded by the loss of mitochondrial transmembrane potential and DNA laddering. In LNCaP cells, lupeol caused early increase in reactive oxygen species followed by induction of mitochondrial pathway leading to cell death (Prasad *et al*. 2008*b*).

Conclusions and perspectives

Apoptosis is strictly controlled in response to integrity of pro-death signaling and plays critical roles in the development, maintenance of homeostasis, and host defense in multicellular organisms.

In preclinical and clinical settings, various therapeutic approaches to target diseases by regulating apoptosis are being developed. Approaches include the traditional use of small molecules to target specific players in the apoptosis cascade. With the increase in the understanding of apoptosis, additional opportunities will become available for tailor-made therapies that will result in improved therapies. It is of critical importance that the balance between cell death and proliferation is strongly regulated. Consequently, an enormous interest has emerged for devising therapeutic strategies to modulate key molecules involved in apoptosis. Several dietary compounds have been shown to affect the process of apoptosis *in vitro* and *in vivo*. Identifying the key proteins involved in apoptosis represents an attractive way to prevent the development of many diseases including PCa. Understanding how these proteins affect the apoptotic pathways may lead to more effective cancer treatments.

To translate the *in vitro* efficacy of dietary agents in the prevention of cancer to clinical use, attention should be given to physiologically relevant concentrations and chronic exposures to imitate *in vivo* conditions. To optimize the desired physiological response, further consideration should be given to the significant intake of dietary components, their duration, and their validation in suitable animal models.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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