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Mini-review

# Dietary agents for chemoprevention of prostate cancer

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## Abstract

Prostate cancer (CaP) is the leading cause of cancer-related deaths in American men, responsible for over 29,000 deaths in the year 2007. Chemoprevention is a plausible and cost-effective approach to reduce cancer morbidity and mortality through inhibition of precancerous events before the occurrence of clinical disease. Indeed, CaP is an ideal candidate disease for chemopreventive intervention as it is typically diagnosed in the elderly population with a relatively slower rate of growth and progression. The potential of dietary substances to act as chemopreventive agents against CaP is increasingly appreciated. Further, epidemiological studies have identified significant correlations between CaP incidence and dietary habits. It is hoped that, combining the knowledge based on agents with targets, we will be able to build an armamentarium of naturally occurring chemopreventive substances that could prevent or slow down the development and progression of CaP. In this review, we have summarized the findings from clinical and preclinical studies on dietary agents including green tea, pomegranate, lupeol, fisetin, and delphinidin that are currently being investigated in our laboratory for their chemopreventive potential against CaP.

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**Keywords:** Chemoprevention; Prostate cancer; Dietary agents

## 1. Introduction

According to the American Cancer Society, CaP accounts for 29% of all new cancer diagnoses in men and has surpassed heart disease as the top killer of men over the age of 85 years in the United States. The number of new cases projected to be diagnosed in the United States alone in 2007 was estimated at 218,890, with 27,000 deaths expected from the disease [1] suggesting that about 1 in 6 men will be

diagnosed with CaP during his lifetime, out of which 1 in 34 will die of the disease. In addition, similar trends have been observed in most industrialized Western countries.

The process of CaP development is a consequence of genetic and epigenetic alterations that transform normal glandular epithelium to preneoplastic lesions and on to invasive carcinoma. Chemoprevention generally is defined as the use of specific agents to block or delay the process of carcinogenesis, thereby preventing the development of invasive cancer. We define chemoprevention as slowing the process of carcinogenesis. The goal of CaP chemoprevention research is to find agents that

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modulate the progression from normal epithelium to clinically significant and localized cancer, and also prevent the progression from localized cancer to locally advanced, to metastatic, to hormone refractory cancer. It is now recognized that cancer chemoprevention can be achieved by targeting various cellular processes. Blocking the formation of the ultimate carcinogen, detoxification through phase I and phase II metabolic enzymes, inhibition of DNA-carcinogen adduct formation, enhanced DNA repair, and modulation of enzymes are some of the target processes of chemoprevention [2]. In addition, agents may exert their effect through scavenging oxygen radicals, inhibiting polyamine metabolism or regulation of signal transduction pathways, hormones, growth factors, or target receptors present in the cells [2]. Restoration of immune response, induction of apoptosis, inhibition of angiogenesis, preventing basement membrane degradation, and activation of antimetastasis genes are other mechanisms through which chemopreventive agents may act to retard the growth of tumor cells [2].

The molecular pathology of prostate cancer is complex; not only are multiple genes involved in its pathogenesis, but additional environmental factors such as diet and inflammation are also involved. Although epidemiologically CaP can be divided into hereditary and sporadic forms, most CaPs seem to be sporadic with <10% inherited. In addition, it is not possible to distinguish between these two groups at the molecular level. Even though possible inherited CaP susceptibility genes such as the ELAC2, RNASEL, MSR1, NSB1, and CHEK2 genes have been identified in some families, the proportion of cases of hereditary CaP attributable to germline mutations in these loci is small [3]. Racial and ethnic differences in CaP incidence and mortality are well recognized, with African-American men being at the greatest risk for diagnosis, followed by Caucasian and Hispanic men while the Asian Americans seem to be at the lowest risk for CaP. Furthermore, marked geographic variations have been observed in the incidence of clinical CaP, with higher rates in the North America and northern Europe, intermediate in Mediterranean region, and relatively low in many parts of Asia. Asian immigrants, who adopt a Western diet, show an increased incidence in CaP, thought to be related to environmental factors and variations in dietary pattern [4]. In addition, there is evidence that increased consumption of selenium, vitamins E and D, lycopene, soy and isoflavonoids, green tea

and low-fat diet reduces the risk of prostatic cancer. In fact, there is extensive data on the role of dietary agents against CaP in the literature, an area of research we recently review. In this mini-review, we have focused on the potential chemopreventive role of dietary agents that are being investigated in our laboratory (Table 1).

## 2. Green tea

Tea produced from the leaves of the plant *Camellia sinensis* is, next to water, the most widely consumed beverage in the world. Alterations in the manufacturing process result in black, green, and oolong tea, which account for approximately 75%, 23%, and 2% of the global production, respectively. Even though each of these non herbal teas is derived from the same source, different processing techniques render them chemically different from each other [5]. Thus, black tea contains more complex antioxidants called theaflavins and thearubigins, while steamed and parched green tea is rich in the chemically simpler antioxidants called catechins. In fact, green tea has been used in traditional Chinese medicine for centuries to treat and prevent chronic diseases, but it is only recently that it is being recognized as an effective chemopreventive agent against various cancers. [6]. The catechins or the flavonols present in green tea are the epicatechin, epigallocatechin, epicatechin-3-gallate, and epigallocatechin-3-gallate (EGCG), of which the latter has gained the most attention with respect to its chemopreventive and potential anticarcinogenic activity. EGCG constitutes up to 50% of the total catechin content and has a higher antioxidant activity than vitamins C and E. EGCG treatment has been shown to result in the induction of apoptosis in LNCaP, DU145, and PC-3 CaP cells. In addition, EGCG inhibits cellular proliferation primarily through the induction of G1 phase cyclin kinase inhibitors (cki), which inhibits the cyclin-cdk complexes operative in the G0/G1 phase of the cell cycle, thereby causing an arrest, which may be an irreversible process ultimately leading to apoptotic cell death [7]. EGCG can also impede cell-to-cell contact adhesion and inhibit intracellular communication pathways which are required for cell division. Inhibition of proteasome by the ester bond-containing EGCG results in the accumulation of proteasome substrates p27 and I $\kappa$ B $\alpha$ , followed by growth arrest in the G1 phase of the cell cycle, contributing to the cancer-preventive effects of tea.

Table 1  
Multiple effects of dietary agents against prostate cancer

Dietary agent	<i>In vitro</i> studies	<i>In vivo</i> studies	Clinical trials
Green tea	Inhibits cell growth, induces apoptosis, increases p21, p53, and Bax [6], inhibits HIF-1 $\alpha$ degradation, inhibits biomarkers of invasion, angiogenesis and metastasis, inhibits PSA-triggered basement membrane degradation, and MMP-2 activation [4], inhibits 5 $\alpha$ -reductase [15] and ODC activity [19], downregulates CK2 [10], sensitizes CaP cells to TRAIL-mediated apoptosis [11], upregulates eEF1A through laminin receptor [13]	Induces clusterin [14], decreases IGF-I and increases IGFBP-3 levels, inhibits tumor growth and PSA secretion [16], reduces CaP progression with decrease in S100A4 and restoration of E-cadherin [20]	Reduces risk of CaP [23,26] Shows minimal activity against CaP [24,25]
Pomegranate	Exerts antiproliferative, antiinvasive, antimetastatic effects [28,29,31], induces apoptosis through modulation of Bcl-2 proteins [31], increases p21&p27, downregulates cyclin-cdk network [31]	Decreases tumor incidence, tumor growth and serum PSA levels [28,31]	Increases PSA doubling time [34]
Delphinidin	Exerts antiproliferative effects, induces cell cycle arrest, increases p21 and p27, downregulates cyclin-cdk network, modulates Bcl-2 proteins in favor of apoptosis, releases cyt <i>c</i> , activates caspases-3, -6, -8, and -9, downregulates PI3K, $\beta$ -catenin, Notch, and NF- $\kappa$ B signaling [36,37]		
Lupeol	Induces apoptosis, upregulates Fas & FADD, activates caspase-8 with degradation of acinus and PARP [41], downregulates IGF1-R, myc, cyclin D1, ERBB2, Jun, MMPs-2 and -7, upregulates IGFBP6, STEAP1, TIMP3, and KLK10, downregulates $\beta$ -catenin signaling [43]	Inhibits tumor growth and PSA secretion [41], inhibits testosterone-induced generation of ROS, depletes antioxidant enzymes [42]	
Fisetin	Exerts antiproliferative effects, induces cell cycle arrest, increases p21&p27, downregulates cyclin-cdk network, induces apoptosis, modulates Bcl-2 proteins, releases cyt <i>c</i> , downregulates XIAP, upregulates Smac/DIABLO, activates caspases-3, -8, and -9, induces PARP cleavage [44], inhibits 5 $\alpha$ -reductase [47] and uPA [46]		

Another report suggests that inhibition of cell proliferation by EGCG may in part be mediated via modulation of the constitutive activation of PI3K/Akt [8] pathways as well as MEK-independent ERK1/2 activation [9].

Ahmad et al. have observed that EGCG may mediate its cellular activity, at least in part, via targeting the kinase CK2 since down-regulation of CK2 sensitizes CaP cells to EGCG-induced apoptosis [10]. A recent study from our laboratory indicates that EGCG sensitizes TRAIL-resistant LNCaP cells to undergo apoptosis via modulating both intrinsic and extrinsic apoptotic pathways. Furthermore, treatment of cells with combination of EGCG and TRAIL resulted in synergistic inhibition of biomarkers associated with invasion, angio-

genesis and metastasis [11]. There is evidence that EGCG exerts its cancer inhibitory effect through the 67-kDa laminin receptor that allows it to bind to the cell-surface of cancer cells. This receptor is expressed on a variety of tumor cells, and its expression level strongly correlates with the risk of tumor invasion and metastasis [12]. Umeda et al. showed recently that eEF1A, a G protein that delivers amino acyl-tRNA to the elongating ribosome, is up regulated by EGCG through the laminin receptor and is crucial for EGCG-induced cancer prevention [13]. Bettuzzi et al. reported that EGCG treatment to CaP cells, but not normal cells, resulted in the induction of clusterin with cleavage of both procaspase-8 and -3, resulting in apoptosis of cancer cells. Moreover, the chemopreventive

action of catechins in the TRAMP mouse model was also accompanied by over-expression of clusterin [14].

Studies show that EGCG effectively inhibits  $5\alpha$ -reductase in cell-free assays, indicating that it can regulate androgen action in target organs. Replacement of the gallate ester in EGCG with long-chain fatty acids produces potent  $5\alpha$ -reductase inhibitors that are active in both cell-free and whole-cell assay systems [15]. The attenuation of the androgen receptor (AR), AR regulated PSA and hK2 genes by EGCG in cell culture studies has also been reflected in *in vivo* models. It is well known that high level of IGF-1 with concomitant reduction in IGF binding protein (IGFBP)-3 are associated with increased risk for CaP development and its progression. TRAMP mice fed with green tea polyphenols exhibited significant inhibition of IGF-1 and restoration of IGFBP-3 with marked delay in CaP progression [16]. In addition, down-regulation of AR and insulin-like growth factor-1, modulation of inflammation biomarkers, and decrease in the MAPK signaling seen in EGCG treated mice may contribute to the reduction in cell proliferation and induction of apoptosis by EGCG and provide a biochemical basis for suppressing CaP without overt toxicity [17,18]. In addition, testosterone-mediated induction of ornithine decarboxylase, an important contributor of CaP development, is inhibited by green tea polyphenols both under *in vitro* and *in vivo* situations [19].

Recent data suggest that the calcium-binding protein S100A4 represents a promising marker for CaP progression. Saleem et al. showed that with progression of age and CaP growth, an increase in the expression of S100A4 at mRNA and protein level occurred in the dorsolateral prostate of TRAMP, but not in nontransgenic mice. Green tea polyphenol feeding to TRAMP mice resulted in marked inhibition of CaP progression, with reduction of S100A4 and restoration of E-cadherin [20]. This is important as S100A4 is overexpressed during the progression of CaP in humans and is thought to control the invasive potential of human CaP cells through the regulation of MMP-9 and its tissue inhibitor TIMP-1 [21].

Although *in vitro* research of the anticarcinogenic properties of EGCG seems promising, many diverse and unknown factors may influence its *in vivo* activity in animal and human models. Current data indicate that EGCG suppresses early stage, but not late stage CaP in TRAMP mice. In this context, ongoing

studies in our laboratory suggest that the chemopreventive potential of green tea decreases with increasing tumor grade and underscores the need to identify the stage(s) of CaP development most vulnerable to chemopreventive intervention [22].

Moreover, while epidemiological studies suggest that green tea compounds could protect against CaP, existing data are inconsistent, and limitations in study design hinder full interpretation of the published observations. A case-control study, conducted in southeast China during 2001–2002, reported a reduced CaP risk with increasing frequency, duration, and quantity of green tea consumption [23]. In another study, patients with asymptomatic androgen-independent metastatic prostate carcinoma and progressive PSA elevation were evaluated after ingestion of 6 g of green tea per day [24]. Only one patient manifested a decline in serum PSA, and no patient manifested a tumor response on radiographic assessment or physical examination. Thus, a limited antineoplastic effect with a maximum response rate of 2% was seen with green tea [24]. Similar results were observed in another clinical trial involving patients with hormone refractory CaP. Green tea extract capsules, prescribed at a dose level of 250 mg twice daily, showed minimal clinical activity against the disease [25]. Both these studies were conducted in end-stage disease, signifying that green tea may be more effective if used in the early stages of the disease or in patient at high risk. More recently, Bettuzzi et al. [26] have shown that after a year's p.o. administration of green tea catechins, only one man in a group of 32 with high-grade PIN developed CaP compared with 9 of 30 in the control group; a rate of only 3% in men developing the disease versus the expected rate of 30% in men treated with placebo. However, there is a need for large-scale, prospective, randomized trials to test the efficacy of green tea for the prevention and treatment of CaP.

### 3. Pomegranate

Pomegranate, used for centuries for medicinal purposes, is the fruit of a deciduous shrub (*Punica granatum*) widely cultivated in the South Asian and Mediterranean region. The tree/fruit can be divided into several anatomical compartments: seed; juice; peel; leaf; flower; bark; and roots; each of which has substantial pharmacologic activity [27]. A rich source of polyphenolic compounds, including anthocyanins and hydrolyzable tannins,

pomegranate possesses a higher antioxidant activity than green tea and red wine [27]. Its juice, peel and oil, all have been reported to possess anticancer activities, including interference with tumor cell proliferation, invasion and angiogenesis. Albrecht et al. showed that pomegranate extracts exhibited potent growth inhibitory activity against CaP cells along with induction of apoptosis [28]. Ellagic acid, caffeic acid, luteolin, and punicic acid, important components of the aqueous or the oily compartment of pomegranate fruit were reported to inhibit *in vitro* invasion of human PC-3 prostate cancer cells across matrigel membranes [29]. Another study showed that anatomically discrete sections of the pomegranate fruit acting synergistically exerted antiproliferative and antimetastatic effect against CaP cells. Here, invasion across matrigel by PC-3 prostate cancer cells was found to be inhibited after treatment with combinations of fermented pomegranate juice polyphenols, pomegranate peel polyphenols, and pomegranate seed oil, and the decrease in the invasive potential of CaP cells was measured as a function of the expression of phospholipase A2 [30]. Our laboratory aimed to discover the molecular basis of the anticancer activity of the pomegranate fruit. Employing Mass Spectrometry, pomegranate fruit extracted in our laboratory was found to contain six anthocyanins (pelargonidin 3-glucoside, cyanidin 3-glucoside, delphinidin 3-glucoside, pelargonidin 3,5-diglucoside, cyaniding 3,5-diglucoside, and delphinidin 3,5-diglucoside), ellagitannins, and hydrolysable tannins [31]. Using human prostate cancer cells, we next evaluated the antiproliferative and proapoptotic properties of pomegranate fruit extract both *in vitro* and *in vivo*. The dose-dependent inhibition of cell growth and viability was shown to be mediated through the cki-cyclin-cdk network with upregulation of p21 and p27 during G1-phase arrest, independent of p53. This was accompanied with down-modulation of the cyclins D1, D2, and E and cdks-2, -4, and -6, operative in the G1 phase of the cell cycle [31]. Furthermore, oral administration of pomegranate fruit extract to athymic nude mice implanted with androgen-sensitive CWR22Rv1 cells resulted in significant inhibition in tumor growth concomitant with a decrease in serum PSA levels [31]. This data was corroborated by a recent report where significant inhibition of LAPC-4 CaP xenograft growth was observed by Seeram et al. in the SCID mouse model [32].

Ellagitannins, the most abundant polyphenols present in pomegranate juice are thought to contrib-

ute significantly towards its reported biological properties. It was shown that ellagitannin metabolites were concentrated at higher levels in the mouse prostate, colon, and intestinal tissues as compared to other tissues after administration of pomegranate extract [32]. Another report suggests that the anticancer activity of pomegranate may be through the prevention of procarcinogen activation mediated through the inhibition of CYP enzyme activity [33].

A phase II, Simon two-stage clinical trial for men with rising PSA after surgery or radiotherapy was recently conducted. Clinical end points included safety and effect on serum PSA, serum-induced proliferation and apoptosis of LNCaP cells, serum lipid peroxidation, and serum nitric oxide levels. Patients were treated with 8 ounces of pomegranate juice daily until disease progression. The study showed that treatment with pomegranate juice was associated with statistically significant prolongation of PSA doubling time from a mean of 15 months at baseline to 54 months post treatment [34]. *In vitro* assays comparing pretreatment and post-treatment patient serum on the growth of LNCaP cells showed a 12% decrease in cell proliferation, 17% increase in apoptosis, 23% increase in serum nitric oxide, significant reduction in oxidative state and sensitivity to oxidation of serum lipids after versus before pomegranate juice consumption [34]. No serious adverse effects were reported, and the treatment was well tolerated. These results are being further tested in a randomized, double-blind, three-arm, placebo-controlled study, which began in April 2006, and addresses several limitations of the current study, with the inclusion of two treatment arms in a dose-response design, as well as the use of a placebo-control [34].

#### 4. Delphinidin

Delphinidin, an anthocyanidin that gives bright hues to flowers like violas and delphiniums is also present in pomegranate, berries, grapes, beets and eggplant [35]. There is considerable evidence that delphinidin possesses potent antioxidant, anti-inflammatory, and anti-angiogenic properties [35]. Ongoing work in our laboratory hints at the chemopreventive potential of delphinidin against various human cancers. Earlier, we had shown that delphinidin protects human HaCaT keratinocytes and mouse skin against UVB-mediated oxidative stress and apoptosis [35]. As current chemotherapy is lar-

gely ineffective for CaP and has serious toxic side effects, we aimed to identify and develop novel, safe and naturally occurring agents that can target various intracellular signaling pathways deregulated in CaP. Our studies show that delphinidin through modulations in the cki-cyclin-cdk machinery, resulted in inhibition of cell growth followed by apoptosis of highly aggressive human prostate carcinoma PC-3 cells [36,37]. The cell growth inhibition was associated with induction of cyclin kinase inhibitors p21 and p27, down-regulation of cyclin E, D1, and D2 and down-regulation of cdk2, -4, and -6. Moreover, this cell cycle arrest was accompanied by the induction of apoptosis with a downregulation of the anti-apoptotic protein Bcl-2 and increase in proapoptotic protein Bax, thus shifting the Bax:Bcl-2 ratio in favor of apoptosis. In addition, activation of caspases, with significant decreases in the protein expression of procaspase-3, -6, -8, and -9 and release of cytochrome c from the mitochondria to the cytosol indicated an essential role of caspases in delphinidin-mediated apoptosis of PC-3 cells [36].

Inhibition of Wnt/ $\beta$ -catenin signaling pathway is an attractive target for new chemopreventive and chemotherapeutic approaches. The Wnt signaling pathway and its key component  $\beta$ -catenin play critical roles in embryonic development as well as in human diseases, including various malignancies. Since deregulation of  $\beta$ -catenin signaling pathway contributes to CaP progression, we examined the effect of delphinidin on  $\beta$ -catenin signaling pathway in human prostate cancer PC-3 cells. The canonical Wnt pathway consists of a series of events that occur when Wnt proteins bind to cell-surface receptors of the Frizzled family, causing the receptors to activate Dishevelled family proteins ultimately resulting in a change in the amount of  $\beta$ -catenin that reaches the nucleus. Delphinidin treatment resulted in modulation of secreted Frizzled-related protein-3, low density lipoprotein receptor-related protein 6, Dickkopf and Dishevelled proteins [36]. Dishevelled, when activated by Wnt binding inhibits the axin/GSK3/APC complex involved in the proteolytic degradation of the  $\beta$ -catenin molecule. Delphinidin treatment of PC-3 cells resulted in the induction of phosphorylation of GSK3 $\beta$ , and protein expression of APC and axin. In addition, delphinidin-mediated increase in the phosphorylation of  $\beta$ -catenin at serine and threonine residues resulted in increased degradation and decreased nuclear translocation of  $\beta$ -catenin. Stabilization of

$\beta$ -catenin and interaction with TCF/LEF family of transcription factors to promote specific gene expression was further inhibited by delphinidin as evidenced by decreased TCF-DNA binding activity with subsequent decrease in the expression of target genes cyclin D, c-myc, and axin-2 [36]. Our data suggest that delphinidin could be useful as a potent inhibitor of Wnt/ $\beta$ -catenin signaling in human CaP cells.

It is well established that Wnt, Notch, FGF, and Hedgehog signaling pathways network together during embryogenesis, tissue regeneration, and carcinogenesis [38]. Indeed, increased Wnt signaling is known to trigger oncogenic conversion of human breast epithelial cells by a Notch-dependent mechanism [39]. Our studies showed that delphinidin-induced cell growth inhibition and apoptosis of human PC-3 cells is mediated, at least in part, through inhibition of Notch-1 and/or NF- $\kappa$ B/PI3K pathways [37]. We found that delphinidin treatment to PC-3 cells resulted in inhibition of constitutive expression of Notch-1 along with inhibition of its active cleaved form. Overexpression of Hes-1, a downstream target of Notch-1 that promotes cell proliferation by repressing cell cycle inhibitory proteins is observed in various cancer cells including CaP. We observed that delphinidin treatment to PC-3 cells resulted in inhibition of protein expression of Hes-1 suggesting deregulation of cell cycle by delphinidin [37]. The anti-apoptotic effects of Notch-1 proteins are known to be regulated through NF- $\kappa$ B signaling. In addition, studies show that increased NF- $\kappa$ B activity contributes directly to the invasive behavior of PC-3 CaP cells. Delphinidin treatment to PC-3 cells resulted in dose-dependent inhibition of phosphorylation of upstream kinases IKK $\alpha$  and IKK $\gamma$  that regulate NF- $\kappa$ B activity, decrease in NF- $\kappa$ B inhibitory protein I $\kappa$ B $\alpha$ , with decrease in NF- $\kappa$ B/p50 and NF- $\kappa$ B/p65 protein expressions and NF- $\kappa$ B/p50 and NF- $\kappa$ B/p65 DNA binding activities suggesting inhibition of NF- $\kappa$ B at the transcription level [37]. Acquired mutations of the PTEN gene reported in up to 30–60% of prostate cancer tumors results in constitutive activation of the PI3K/Akt pathway which then represents a major target to prevent dysfunctions in cell growth, survival and motility. Delphinidin treatment to PC-3 cells significantly inhibited p110 (catalytic) and p85 (regulatory) subunits of PI3K, an essential downstream regulator of growth signaling pathways [37]. In summary, delphinidin seems to act as a multifunctional anticancer agent in *in vitro* studies.

However, thoughtfully designed clinical trials are necessary to reveal the possible benefits of this agent in preventing tumor development and malignant progression.

## 5. Lupeol

Lup-20(29)-en-3 $\beta$ -ol (Lupeol) is a triterpene found in fruits such as olives, mangoes, strawberries, grapes, and figs, vegetables, and several medicinal plants [40]. It possesses strong anti-inflammatory, antiarthritic, antimutagenic, and antimalarial activity and has been used for the treatment of various diseases [40]. Recent data from our laboratory and others suggest that lupeol possesses potent anti-cancer activities *in vitro* and *in vivo* systems [41]. We have shown that lupeol treatment resulted in significant inhibition of cell viability of LNCaP and CWR22Rv1 androgen-sensitive prostate cancer cells with minimal effect on normal prostate epithelial cells [41]. Lupeol-induced apoptosis in these cells was associated with cleavage of poly (ADP-ribose) polymerase protein and degradation of acinus protein. Moreover, lupeol increases the expression of the death receptor Fas along with upregulation of the adaptor protein FADD, required for the recruitment and activation of procaspase-8 to form the death inducing signaling complex. The small interfering RNA-mediated silencing of the Fas gene and inhibition of caspases-6, -8, and -9 by their specific inhibitors further confirmed that lupeol specifically activates the Fas receptor-mediated apoptotic pathway [41]. Furthermore, 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 that can be exploited against CaP. In mice xenograft model, lupeol treatment significantly inhibited the growth of CWR22Rv1 tumor cells with concomitant reduction in PSA secretion [41].

Prasad et al. have also shown that lupeol exerts protective effect against androgen-induced oxidative stress. Oral treatment of lupeol and mango pulp extract to testosterone injected Swiss albino mice resulted in inhibition of testosterone-induced increase in the levels of reactive oxygen species, depletion of antioxidant enzymes such as catalase and superoxide dismutase, and increase in lipid peroxidation in the murine prostate [42].

We recently investigated the mechanistic basis of the multi-targeted effect of lupeol in CaP cells.

Lupeol treatment was observed to inhibit the proliferative and clonogenic potential of CaP cells with reduced expression level of PCNA protein, a marker of cell proliferation [43]. Microarray studies further showed that lupeol significantly modulated the expression of genes known to be associated with proliferation and survival. Lupeol treatment decreased the mRNA expression levels of insulin-like growth factor-1 receptor (IGF-1R), myc, cyclin D1, ERBB2 and Jun, and caused increase in the mRNA expression levels of IGFBP6, STEAP1, TIMP3, and KLK10. Lupeol-induced modulations in the mRNA expression levels of these genes corroborated well with their protein expressions [43]. Genes modulated by lupeol are known to be associated directly or indirectly with  $\beta$ -catenin signaling, highly aberrant in CaP. Predictably, lupeol reduced the cellular levels of  $\beta$ -catenin with an increase in the GSK3 $\beta$ /axin destruction complex, concomitant with significant reduction in the transcriptional activation of the TCF. Finally, lupeol-mediated down-regulation of matrix MMPs-2 and -7 relates to the ability of the agent to effectively target the IGF-1R/ $\beta$ -catenin axis leading to the inhibition of proliferation of CaP cells [43]. Ongoing animal experiments will shed more light on the chemopreventive potential of the agent against CaP.

## 6. Fisetin

Fisetin, or 3,7,3',4'-tetrahydroxyflavone, belongs to flavonol subgroup of flavonoids together with quercetin, myricetin, and kaempferol. Fisetin can be found in many fruits such as strawberries, apple, persimmon, kiwi fruit, and vegetables including onion and cucumber [44]. Cell culture studies show that fisetin exerts antiproliferative effect on human CaP cells. Data from our laboratory indicate that fisetin selectively decreases the viability of LNCaP, CWR22Rv1 and PC-3 CaP but has only minimal effect on normal prostate epithelial cells [44]. Flow-cytometric analysis indicated that the cell cycle arrest observed in PC-3 cells was in G2/M phase, whereas the LNCaP cells demonstrated a different cell cycle profile [45]. We showed that treatment of LNCaP cells with fisetin induced arrest in the G1 phase of the cell cycle, accompanied with decreased level of cyclins and cdks and concomitant induction of p21 and p27. Furthermore, fisetin mediated apoptosis was associated with the release of mitochondrial cytochrome *c* into the cytosol [44]. Cytochrome *c*, Apaf-1, adenosine triphosphate, and

procaspase-9 are known to 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 the procaspase-3 and generate the active caspase-3 that serves as the central executor of apoptosis. Fisetin treatment resulted in significant activation of caspases-3, -8, and -9 in CaP cells. The caspase pathway is regulated by inhibitors of apoptosis protein (IAP) but during apoptosis, inhibitory effects of IAPs are neutralized by the second mitochondria-derived activator of caspase (Smac), direct IAP-binding protein with low pI (DIABLO) and/or high-temperature requirement protein-A2, which are released from mitochondria. Fisetin treatment resulted in the downregulation of XIAP and upregulation of Smac/DIABLO along with modulation in the expression of Bcl-2 family proteins, critical regulators of the apoptotic pathway [44]. This indicates that fisetin can alter the mitochondrial membrane function of CaP cells thereby inducing apoptosis, an important molecular target for chemoprevention of cancer.

A recent study indicated that inhibition of uPA by fisetin [45] in the advancing capillary vessels surrounding the tumor may be responsible for reducing angiogenesis and consequently tumor growth. In addition, fisetin and other flavonoids that contain a catechol group have been shown to be potent inhibitors of the type 1  $5\alpha$ -reductase [46]. It is thought that since these compounds are consumed as part of the normal diet or in supplements, they have the potential to inhibit  $5\alpha$ -reductase activity, which may be useful for the prevention or treatment of androgen-dependent disorders including cancer. Collectively these data provide the first evidence that fisetin could be developed as an effective agent against CaP.

## 7. Conclusions

The future role of dietary supplements in CaP is of much interest, and preliminary data are noteworthy. Regardless, unresolved issues still linger. For most cancer interventions, the expected time to achieve an effect is much longer, more variable, and far less well understood, and the progression of disease is hard to follow. In addition, the optimal dose and duration needed to test nutritional agents for cancer prevention are largely unidentified, making null findings hard to interpret. Baseline nutritional status can be critical. In addition, particular

nutrients may be effective only in subgroups defined by genotypes or by nutritional status of another nutrient. Therefore, before these agents can be recommended as useful chemopreventive strategies for patients, there is a need to confirm their activity in rigorous well designed clinical trials.

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