Natural Products: Potential for Developing *Phellodendron amurense* Bark Extract for Prostate Cancer Management

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Abstract: Our ability to detect and treat most primary cancers has improved dramatically given the advances in our understanding of cancer biology. However, with increased life expectancy and our inability to treat or cure advanced stages of cancers, the number of cancer-related deaths is expected to double in the next decade. Epidemiological studies suggest that dietary factors are an important aspect that influences cancer risk. Despite a lack of scientific evidence in most cases, cancer patients have whole-heartedly accepted the concept of "alternative medicine" using natural compounds and spent more than $1B a year on herbal supplements. Given the significant toxicity-associated problems with current long-term standard of care, scientifically validated natural supplements can serve as novel and effective alternative strategies for effective cancer management. We will discuss the utility of natural products in modulating critical signaling pathways for effective cancer prevention with special emphasis on prostate cancer and their potential translational benefit.

Keywords: Prostate cancer, complex mixtures, nexrutine, multi-target approach, natural products.

PROSTATE

The prostate is an accessory sex gland that is located at the base of the bladder anterior to the rectum in male mammals. The prostate surrounds the urethra and produces secretions that liquefy seminal fluid. Anatomically human prostate gland is divided into three zones namely peripheral, central and transitional, each with distinct functions. The peripheral zone comprises approximately 65% of the prostate gland and is the region that is more susceptible to malignant neoplastic transformation. The transitional zone of the periurethral region of the prostate comprises approximately 10% of the gland and is involved in the development of non-malignant neoplastic changes called benign prostatic hyperplasia (BPH). The remaining 25% of the gland is the central zone and consists mostly of ejaculatory ducts.

PROSTATE CANCER (PCA)

Prostate cancer is the malignant form of prostate disease that accounts for about 25% of all new cancers diagnosed and is the second leading cause of cancer-related deaths in men [1]. Autopsy studies have demonstrated that approximately 30% of men older than 50 years old have histologic evidence of prostate cancer [2]. In addition, the probability of developing invasive prostate cancer increases from 0.01% in the age group of 0-39 to 13% in men above 70 and older [1]. In other words, approximately 1 in every 10,553 men in the age group of 0-39; 1 in 39 in the age group of 40-59; 1 in 15 in the age group of 60-69 and 1 in 7 in the age group of 70 and older will develop prostate cancer in their lifetime [1]. Although the exact cause of PCA remains unknown, epidemiological studies suggest age, family history, race, and high fat-diet as prominent risk factors for PCA [1]. Further it has also been shown that prostate cancer is frequently accompanied by chronic inflammation [3], and biochemical relapse following radical prostatectomy is more frequent in patients with high-grade inflammation [4]. Cyclooxygenase 2 (Cox-2) has been shown to be upregulated in prostate cancer specimens and Aspirin and other NSAIDS are thought to play a role in chemoprevention by inhibiting the associated inflammation [5-7]. These observations suggest that chronic inflammation and associated Cox-2 overexpression may be an early event in the pathogenesis of "inflammation-related" prostate cancer and may be associated with a more aggressive phenotype.

Interestingly, incidence of PCA is highest in the western population compared with Asian population. Among western population, it is more prevalent in African-Americans than Caucasians. Additionally, migration of the Asian population to Western countries increases the risk of developing PCA, indicating that lifestyle changes including dietary factors contribute to the observed high incidence of cancer in westerns in addition to genetic and epigenetic factors. These data suggest that diet may play a prominent role in the development of PCA. Several recent excellent review articles have discussed how nutrients affect PCA and potential mechanism involved; therefore this is not the subject of this review [8-11].

TREATMENT OPTIONS FOR PCA

PCA is the second leading cause of cancer related deaths in men despite advancements in surgical and therapeutic approaches [12]. Prostate cancer is known to vary significantly in aggressiveness and in its behavior; however, at this time the ability to predict the biological aggressiveness of the tumor is unknown. Serum-based prostate-specific antigen

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(PSA) level and the biopsy-based Gleason score (GS) can be discordant and a tumor with minimally (4-10 ng/ml) to moderately (10.1-20 ng/ml) elevated PSA can harbor highly to very highly aggressive tumors (GS7 and GS 8 to 10). Most prostate tumors are initially androgen-responsive and hence androgen-deprivation therapy (ADT) is a standard therapeutic approach. Although as many as 80% of patients respond initially to androgen ablation therapy, the duration of this response in most patients is transient (only 12-18 months) because recurring tumors grow either in the absence or low concentrations of androgens leading to the development of androgen independent prostate cancer (AIPCA). Emergence of androgen independent disease is fatal since no effective systemic therapy currently exists [12-16].

CHEMOPREVENTION

Chemoprevention is an approach of using natural or synthetic compounds or hormonal modulators of pharmacological inhibitors to prevent initiation, inhibit progression or delay progression of normal appearing prostatic epithelium to high grade prostatic intraepithelial neoplasia (HGPIN) to locally invasive adenocarcinoma to clinically significant metastatic disease [17-19]. Progression through this process takes several decades and involves deregulation of several genes involved in various cellular processes as well as epigenetic events that regulate these genes. Since the progression of PCA is slow, in most cases, taking several decades for diagnosis of clinically significant disease; thus delaying its progression can significantly impact the overall incidence of clinical PCA. Given the long latency involved in the development of clinically significant hormone refractory prostate cancer, tremendous opportunities exist for reducing the risk through life-style changes including dietary agents. Accordingly, evidence is accumulating to implicate complementary and alternative medicine (CAM) therapies as methods of diagnosing, preventing, or treating cancer in a non-traditional manner [20]. Patients utilizing CAM are often seeking to avoid traditional "Western" medical treatments for cancer such as surgery, radiation, and chemotherapy. Instead, CAM therapies provide holistic care for cancer patients with options that support mental and physical healing. CAM includes practices such as herbal remedies, meditation, massage therapy, acupuncture, humor therapy, and a variety of dietary options and supplements. Many cancer patients choose complementary therapies to accompany their mainstream medical treatment to alleviate side effects while others choose alternative therapies to avoid traditional medical treatment altogether.

In a survey conducted by Sparber and colleagues, 63% of cancer patients participating in clinical trials reported using at least one CAM therapy, while the average per patient was two CAM therapies [21]. A study conducted by Bernstein *et al.* reports an astonishing 80% CAM use in cancer patients, with therapies ranging from vitamin use and herbal remedies to relaxation techniques and home therapies [22]. According to Science Daily PC Magazine, 48% of cancer patients receiving chemotherapy and radiation use at least one type of CAM treatment [23]. Additionally, researchers conducting a series of computerized literature searches involving 26 surveys in 13 countries found that the average percentage of adult cancer patients using CAM is 31.4% (range: 7-64%) [24].

A study conducted in Canada in 2003 found that almost one third (29.8%) of men diagnosed with prostate cancer reported using CAM for their prostate cancer care [25]. Singh and colleagues found in a study amongst prostate cancer patients that those preferring CAM saw it as "safe and holistic" as compared with traditional "aggressive" medical treatment. The most common reasons CAM was sought include fear of impotency and incontinence from medical treatment and the belief that CAM would be effective despite lacking evidence [26]. In another study of prostate cancer patients and CAM use, conducted by the Tzu Chi Research group of Vancouver, BC, it was found that more than 33% of participants used some form of CAM [27]. A study conducted by Chan and colleagues reports that within the CaP-SURE (Cancer of the Prostate Strategic Urologic Research Endeavor) program, one third of prostate cancer patients responding to a recent survey reported some form of CAM use [28]. In the following section we will review the clinical application of some of the natural compounds used as CAM in prostate cancer management.

NATURAL PRODUCTS FROM BENCH TO BEDSIDE

Many components derived from dietary or medicinal plants have been found to possess substantial chemopreventive properties [29-32]. Further it has been reported that approximately 60% of the approved anticancer drugs are of natural origin. Moreover over 25% of US Prescriptions dispended in 1973 contained active ingredients derived from plants [31]. In a study of CAM and advanced prostate cancer by Rackley and colleagues, several natural compounds and supplements are reviewed in relation to their ability to prevent the disease [33].

Despite this progress and anecdotal evidence of botanicals for cancer prevention, to the best of our knowledge their clinical utility as anti-cancer agents remains under-investigated. This is partly due to lack of systematic *in vitro*, preclinical and mechanistic studies to evaluate them as a strategy to reduce cancer risk and/or modify tumor behavior. Since it is well established that constitutive activation of multiple signaling pathways that inhibit apoptosis, promote proliferation, invasion, metastasis and angiogenesis are commonly seen in malignant cell, inhibition of one specific signaling pathway may have a minimal effect on the cancer phenotype. Targeting multiple signaling pathways simultaneously may offer a better approach for cancer prevention [34]. This can be achieved by use of complex mixtures due to presence of multiple components that could exert synergistic or additive biological activities. The following section summarizes the work conducted with some complex mixtures against prostate cancer.

COMPLEX MIXTURES AND PROSTATE CANCER

Several natural products have been shown promise in preclinical models including Green Tea Polyphenols (GTP), pomegranate, soy, tomato paste and PC-SPES [35-50]. Consumption of GTP has been shown to be associated with reduced PCA risk that depends on frequency, quantity of tea consumption [35-38]. In another study, patients with asymptomatic androgen independent metastatic prostate carcinoma and progressive PSA elevation were evaluated after ingestion of 6 g of GTP per day. Only one patient manifested a decline in serum PSA and no patient manifested a tumor response on radiographic assessment or physical examination [27]. This study was conducted in end-stage disease that showed minimal clinical activity indicating that GTP may be more effective if used in the early stages of the disease. More recently, Bettuzzi *et al.* have shown that after a year po administration of green tea catechins, only one man in a group of 32 with HGPIN developed PCA compared with 9 of 30 in the control group [35]. However there is a need for large scale prospective randomized trials to test the efficacy of GTP for the prevention and treatment of PCA. Men receiving polyphenon E (supplements with active compounds of green tea) with positive prostate biopsy and scheduled for prostatectomy showed significant decrease in the serum PSA in addition to vascular endothelial growth factor (VEGF) and human growth factor (HGF) [35-38]. Pomegranate has been tested in phase II studies in men with rising PSA after surgery or radiotherapy (RT). Consumption of eight ounces of juice daily was associated with statistically significant prolongation of PSA doubling time from a mean 15 months to 54 months [39-40].

Several studies demonstrated the potential of lycopene alone and in combination with soy products to either stabilize or regress prostate tumor progression as evidenced by serum levels of PSA [41-47]. Thirty-four percent of patients taking lycopene followed by soy with recurrent prostate cancer for 4-8 weeks showed reduced levels of serum PSA and 100% showed decreased serum levels of VEGF. Similarly patients receiving 50 g tomato paste daily for 10 weeks with histological evidence of BPH showed decrease in the serum levels of PSA [47]. Although this is not a placebo controlled prostate cancer prevention study, such encouraging results have impact for prostate cancer management. Table **1** summarizes various compounds that have been used in prostate cancer clinical trials [48-53]. More detailed information regarding various natural compounds and their use in cancer management can be found in recent excellent review articles on this subject [8, 32]. Despite such promising results, some of the limitations of these studies include small sample size, use of different endpoints including PSA levels, PSA doubling time, PSA velocity or molecular markers). Such studies should be conducted in a large population using the same or similar end points in order to assess the efficacy of natural products for translational use.

In addition Curcumin has shown to be well tolerated despite limited bioavailability in patients with advanced pancreatic cancer and pre-malignant lesions [54-55]. To the best of our knowledge no trial has assessed the clinical utility of Curcumin for prostate cancer management. In this regard studies from our laboratory show dietary administration of curcumin to TRAMP mice for 6 weeks inhibits progression of pre-malignant lesions (Kumar *et al.* unpublished observations). In addition the efficacy of tomato paste, broccoli alone and in combination (tomato and broccoli) was compared with lycopene in rodent models of prostate cancer. These studies show statistically significant reduction in the tumor growth of about 7-18% (depending on the dose) with lycopene; 34% with tomato paste; 42% with broccoli and 52% with combination of tomato and broccoli [56]. Such preclinical studies suggest the potential for developing complex mixtures (phytoceuticals) or their combinations for effective prostate cancer management. However such studies should be validated stringently for human use.

PC-SPES

It is noteworthy to mention here that a, herbal mixture of eight different extracts called PC-SPES has been shown to inhibit prostate cancer cell growth *in vitro* and reduce PSA in patients with hormone-refractory prostate cancer [57]. However this product was withdrawn due to concerns regarding the quality of the extract, legitimate concern with developing complex mixtures for cancer management. As discussed above, given the wide spread use of over-the-counter (OTC) herbal extracts and dietary supplements by cancer patients, it is important to validate the use of such extracts available for human use scientifically so that such OTC supplements manufactured under good manufacturing practices and undergo vigorous quality control tests are produced. This is substantiated by a recent report showing that PC-SPES (manufactured under GMP) is effective against hormone refractory prostate cancer patients [58].

In addition bark extract from Pao Pereira, Rauwolfia vomitora extract, an aqueous extract of dried roots of plant Dulcmara grown in Ecuador known as biological immune response modulator (BIRM) have been shown to inhibit growth of prostate cancer cells *in vitro* and prostate tumors *in vivo* [59-61]. Although the precise molecular mechanism how these extracts inhibit tumor development is unknown, these preliminary reports indicate the potential for developing wide variety of plant extracts for prostate cancer management.

NEXRUTINE

Nexrutine, is a commercially available herbal extract from the Chinese plant, *Phellodendron amurense (*Phellodendron is "cork tree" in Greek*)*, which is widely used for the treatment of inflammation, gastroenteritis, abdominal pain and diarrhea in folk-lore medicine [62-65]. This tree is native to Asia and has been reported to contain isoquinoline alkaloids, phenolic compounds and flavone glycosides. Nexrutine is available as a dietary supplement ingredient sold in tablet and capsule form in food, drug and mass market outlets. Nexrutine been shown to *induce* apoptosis in HL60 cells and possess anti-inflammatory activity through inhibition of Cox-2 expression without inhibiting the activity of Cox-1 [65-66].

TOXICITY STUDIES WITH NEXRUTINE

Studies conducted in rats show that 100 mg/kg Nexrutine did not cause any gastric mucosal irritation [67]. Single dose (5000 mg/kg) oral toxicity studies conducted in Sprague-Dawley rats found no significant gross internal toxicity changes at necropsy on study day 14 [67]. Further Nexrutine has been shown to be biologically active in humans. The Living Longer Clinic in Cincinnati, Ohio, conducted a 288-subject open-label, single center study to test Nexrutine^{R} as a potential analgesic. These subjects were given Nexrutine 1-2

Table 1. Summary of Selected Natural Compounds Tested in Clinical Trials

Compound(s)	Trial Description	Result	Reference
Lycopene plus Soy	41 pts / recurrent PCA / lycopene *or* soy alone for 4wks followed by combination of lycopene plus soy for additional 4wks	Decreased PSA in 34% of subjects	[41]
Lycopene plus Soy	71 pts / PCA with rising PSA / 6 months	Stable disease in > 67% of subjects	[42]
Lycopene	20 pts (placebo-controlled) / BPH / 6 months	Decreased serum PSA and prostate enlargement	[46]
Lycopene	41 pts / localized PCA / 1 year	Maintenance of serum PSA velocity	[43]
Lycopene	20 pts / metastatic HRPC / 3 months	Stable disease 50% subjects; CR 5%; PR 30%; DP 15%	[45]
Lycopene	32 pts / localized prostate adenocarcinoma / 3wks prior to surgery	Decreased serum PSA levels	[48]
Lycopene	26 pts / localized PCA / 3wks prior to surgery	Decreased serum PSA levels	[49]
Combination	49 pts / history of PCA with rising PSA / supplement combination of soy, isoflavones, lycopene, silimarin, & antioxidants / 20wks	Delayed serum PSA progression	[44]
Tomato Paste	43 men / BPH / 10 wks	Decreased serum PSA levels	[47]
Green Tea polyphenols	26 men / positive prostate biopsies / polyphenon E / varying # wks until radical prostatectomy	Decreased serum PSA levels	[36]
Green Tea polyphenols	30 pts (placebo-controlled) / HG-PIN / GTC (green tea catechins) / 1yr	No change in serum PSA levels	[35]
Soy	32 pts / PCA prior to radical prostatectomy / dietary soy isoflavones for 31 days	Decreased serum PSA levels	[50] and references therein
Broccoli	22 pts / HGPIN / broccoli-rich diet for 12 months	Men on broccoli rich diet had changes in GSTM1 genotypes, mRNA processing, TGFbeta1, EGF, and insulin signaling (vs.control group on pea-rich diet)	[51]
Mushrooms	62 pts / PCA & 2 consecutive elevated PSA readings / shitake mushroom extract (SME) orally for 3 months	38 pts had stable serum PSA levels	[52]
Pomegranate	46 evaluable pts / rising PSA given 8ounces pomegranate juice daily until disease progression	Mean serum PSA doubling time significantly increased with treatment	[53]

capsules thrice daily. Two hundred fifty-five subjects (88%) reported beneficial effects from Nexrutine, including reduction in pain and/or inflammation, while the remainder reported no improvement. An open-label, home use trial of Nexrutine (1-2 250 mg capsules once or twice a day) reported that it was gentle on the stomach with minimal side effects. A double-blind, placebo controlled clinical trial was conducted at Miami Research Associates to investigate the potential benefits and safety of Nexrutine Capsules (one 250 mg capsule 3 times daily) in 33 patients with osteoarthritis for 6-weeks. There were no substantial changes in safety variables over the course of the study [67]. These studies demonstrate the non-toxic nature of Nexrutine and studies conducted in humans for pain management suggest that (i) it is safe to consume; (ii) it is absorbed and biologically active (Pain reduction).

MOLECULAR TARGETS OF NEXRUTINE

Unlike most of the natural products that have been extensively studied for their utility in various diseases including cancer, scanty information is available to suggest that Nexrutine could be used as a preventive or therapeutic agent for cancer. We initiated studies to investigate the potential use of Nexrutine as an anticancerous agent. These studies demonstrated for the first time that Nexrutine treatment inhibits the proliferation of both androgen-responsive and -independent human prostate cancer cells through induction of apoptosis. Inhibition of Akt activity represents one possible mechanism by which Nexrutine inhibits prostate cancer cell proliferation [68]. Interestingly, we identified transcription factor cyclic-AMP response element binding protein (CREB) as one of the downstream effectors of Akt. Majority of prostate cancer cases have been shown to overexpress these two proteins (Akt and CREB) and overexpression of Akt correlates with progression of human prostate cancer and contributes to resistance to apoptosis. Additionally, we found that Nexrutine can down-regulate Cox-2 and Cyclin D1 through cyclic-AMP response element (CRE) binding sites. We have also shown that inactivation of either Akt (using kinase dead mutant) or CREB (using dominant negative CREB) or inactivation of both Akt and CREB by Nexrutine treatment reduced Cyclin D1 promoter activity in PC-3 cells [68-70]. These data suggest that Nexrutine can down regulate Cox-2 or Cyclin D1, which may contribute to its antiproliferative activity.

NFκB is a nuclear transcription factor that under normal conditions is sequestered in the cytoplasm as a heterodimer composed of Rel proteins p50 and p65 by the inhibitory protein IκBα [71, 72]. In response to cell stimulation, IκBα is rapidly phosphorylated and targeted to be degraded, allowing nuclear translocation of NFκB. It regulates the expression of target genes involved in various processes including apoptosis, cell proliferation, cell invasion, metastasis, angiogenesis and chemoresistance [71, 72]. In addition, NFκB has been shown to be constitutively activated in human prostate tumors, androgen-independent prostate cancer cell lines and human prostate tumor xenografts, implicating a role in prostate tumor progression [73-75]. Akt also signals activation of NFκB through phosphorylation and activation of IKKα or by phosphorylating RelA [76, 77]. Our laboratory has shown that Nexrutine treatment down-regulates the constitutive and TNFα-induced activation of NFκB that is mediated through inactivation of pIκBα in androgen-independent prostate cancer cells [78]. Since Nexrutine treatment also inhibited invasion of prostate cancer cells, it is possible NFκB could be involved in mediating this process [79]. Further since Nexrutine down-regulates both Cox-2 and Cyclin D1 transcriptional activity, it is possible that the observed Nexrutine-induced down-regulation of Cox-2 and Cyclin D1 could also be mediated through NFκB. However, the precise mechanism of Nexrutine-induced NFκB inactivation or its interaction with CREB remains unexplored at the time of writing this review.

IN VIVO STUDIES

Dietary administration of Nexrutine to 8-week-old TRAMP mice with 300 and 600 mg/kg for 20 weeks significant decrease in the volume of prostate seminal vesicle complex (PSVC) as evaluated by magnetic resonance imaging (MRI) and tumor burden as evaluated through at 18 histological analysis of prostate tumor or tissue [69]. In addition recent results using small number of animals suggest that Nexrutine administration also regresses progression of established prostate tumors in the TRAMP model [79]. Interestingly administration of Nexrutine inhibited tumor development was associated with significant decrease in the levels of pAkt, pCREB, NFκB and CREB-DNA binding activity and Cyclin D1 in prostate tissue compared to tumors from control animals. These data collectively indicate that Nexrutine suppresses prostate tumor development in TRAMP mice in part through Akt/CREB mediated activation of Cyclin D1 [69].

PREVENTION OF BONE LOSS

Between 50-80% of all patients diagnosed with prostate cancer are predicted to have metastasis to bone at the time of their death. Further, androgen deprivation therapy (ADT), a standard treatment for metastatic disease, produces skeletal complications including bone loss [80, 81]. Such skeletal complications lead to poor quality of life. Such bone metastatic prostate cancer causes tremendous morbidity including pain, pathological fractures and other problems. Currently there are no effective non- toxic strategies available to prevent bone loss that occurs either as a consequence of treatment or during natural metastatic disease. Although bisphosphonates have been shown to be effective in reducing skeletal complications, its prolonged use is associated with renal toxicity and osteonecrosis [80, 81]. To the best of our knowledge, the activity of natural compounds to inhibit metastatic spread to bone has not been examined. Interestingly, Nexrutine intervention prevents bone loss in transgenic mice that develop spontaneous prostate tumors [79]. However, it is not clear how Nexrutine prevents the observed bone loss. Nonetheless, since ADT is associated with bone loss, use of Nexrutine in conjunction with ADT may decrease/reverse the bone effects. Studies are warranted to test its utility in bone-related diseases including hormone-therapy induced bone loss associated with other cancers such as breast cancer.

ACTIVE COMPONENTS OF NEXRUTINE

Using activity-guided fractionation, we identified a butanol fraction (F3) that recapitulates the antiproliferative activity of Nexrutine. Further, ultra performance liquid chromatography (UPLC) analysis identified berberine or berberine-like compound, palmatine in the F3 fraction. We compared the growth inhibitory activity of these compounds with that of the fractions using cell proliferation assay. Both Berberine and Palmatine inhibited proliferation of prostate cancer cells significantly [78]. Berberine has been shown to induce apoptosis in HeLa, leukemia, colon, melanoma and lung cancer cells. Further, berberine has recently been shown to inhibit proliferation of prostate cancer cells through induction of apoptosis involving deregulation of cell cycle checkpoint proteins [82, 83]. However, there are no reports showing the effect of Palmatine on prostate cancer. Currently, the effect of palmatine on prostate cancer is a topic of intense investigation in our laboratory. Further, additional studies are currently being carried out in our laboratory to identify and characterize the precise molecular pathways involved in its anti-cancerous activity and whether berberine or palmatine alone or their combination can fully recapitulate the biological activities of Nexrutine.

USE OF NEXRUTINE IN OTHER TUMOR TYPES

Although the precise molecular mechanism of Nexrutine induced prostate tumor growth inhibition is not fully understood, given its ability to modulate several critical signaling pathways (Akt, CREB, Cox-2, NFκB and Cyclin D1), it is possible that Nexrutine could have potential use in other types of cancers. A group of investigators at Thomas Jefferson University have found that Nexrutine is also protective against breast cancer. Nexrutine decreased cell survival, which was accompanied by G1 cell cycle arrest, increased autophagy, and caspase-independent cell death in estrogen receptor negative (ER-) breast cancer cells. Nexrutine decreased activities of Cox-2 and peroxisomes proliferators activated receptor gamma (PPAR)-γ: two potential biomarkers of breast cancer. Further feeding HER-2/neu mice a diet supplemented with 2000ppm of Nexrutine for two months reduced the content of PGE_2 in the mammary glands. Studies are underway to investigate the ability of Nexrutine to prevent development of spontaneous mammary tumors in transgenic mouse models of breast cancer (Lanza-Jocoby, Personal communication). Studies from our laboratory show that Nexrutine inhibits proliferation of colon cancer cell lines that differ in the status of Cox-2 (Kumar *et al.* unpublished observations). Similarly, Nexrutine inhibits proliferation of pancreatic cancer cell lines that is associated with induction of apoptosis and inhibition of Cox-2 activity (Gong and Kumar, unpublished observations). In addition recent studies also show that Nexrutine inhibits proliferation of melanoma cells (Ghosh, personal communication). Although these studies are very preliminary, these findings suggest that Nexrutine may be useful for management of several tumor types due to its inherent ability to inhibit multiple signaling pathways. Table **2** summarizes the mode of action of some of these natural compounds and their potential targets [84-110].

CONCLUSIONS

Approximately 69% of cancer patients used at least one complementary and alternative medicine (CAM) therapy as part of their cancer treatment. Given the fact that cancer arises due to deregulation of multiple signaling pathways including Akt, CREB, NFκB, targeting multiple signaling pathways using a combination of agents or complex botanicals such as Nexrutine offers an added advantage of providing a synergistic or additive effect. Further constitutive activation of multiple signaling pathways including Src-MEK-1-2-ERK-1/2-CREB signaling pathway has been shown to be associated with androgen-independent phenotype. As discussed above, Nexrutine has been shown to inhibit prostate cancer cell growth *in vitro* and tumor development *in vivo* that is associated with induction of apoptosis and inhibition of tumor invasion. Further, Nexrutine has a good safety record in humans and has been shown to be biologically active in human test subjects. Given the toxic side-effects associated with chemotherapy or radiotherapy, these non-toxic natural compounds or their active components should be evaluated in combination with existing standard of care for their ability to sensitize cancer cells to chemotherapy or radiotherapy. Such combinations have the potential to (i) increase the sensitivity of tumor cells to lower doses of both radiation and anticancer drugs; (ii) reduce the cytotoxic effects of radiation on normal cells and (iii) improve the therapeutic index of the chemo and radiotherapy. Towards this goal, studies from our laboratory demonstrates that animals receiving *Phellodendron amurense* bark extract for 6 weeks prior to radiation therapy had no overt cancer but exhibited features consistent with HGPIN compared with animals that received radiation therapy alone exhibiting features consistent with well to poorly differentiated adenocarcinoma (Kumar *et al.* unpublished observations). Given the implication of cellular damage induced by oxidative stress in prostate cancer [111], antioxidant ability of Nexrutine should be examined. It is possible that in addition to targeting above mentioned signaling pathways, Nexrutine could exert its preventive benefits through neutralization of toxic and volatile free radicals in the prostate. Special attention should also be given to anti-androgenic activity of Nexrutine. Since AR signaling plays a critical role in the development of hor-

Table 2. Molecular Targets and Mechanism of Action of Select Natural Products

Compound	Mechanism of Action	Targets	Reference
Lycopene	antiproliferation, proapoptosis, cell cycle arrest, antiangiogenesis	Akt, cyclin D1, Bcl-2, Bax, VEGF, IGFBP-3	[48, 84-89]
Soy (Genistein)	antiproliferation, cell cycle arrest, AR regulation, proapoptosis, anti-inflammation	Cox-2, Akt, p21, Prostaglandins, AR, Stat3, NFκB	[90-96]
Green Tea polyphenols	antioxidant, antiproliferation, cell cycle arrest, anti-inflammation, anti-angiogenesis, proapoptosis, epigenetic regulation	Akt, NFκB, HGF, VEGF, HDAC1-3, MDR1, Cox-2, MMPs	[97-100]
Curcumin	antioxidant, antiproliferation, anti-inflammation, proapototic, antiangiogenesis	NFκB, Akt, AR, AP-1, CREB, Stat3	[101-103]
Broccoli (Sulforaphane)	AR regulation, antiproliferation, cell cycle arrest, proapoptosis	E2F1, cyclin D1, Rb, p21	[105-108]
Pomegranate	antioxidant, antiproliferation, proapoptosis, AR regulation	NFκB, AR, Cox-2	[109-110]
Nexrutine	antiproliferation, proapoptosis, anti-inflammation	Akt, NFκB, CREB, cyclin D1	[68-70, 78-79]

mone-refractory prostate cancer, potential of Nexrutine in modulation of AR signaling needs to be evaluated. The results of these and future studies may yield an important addition to the weak therapeutic armamentarium that exists for the treatment of prostate cancer and perhaps other cancers. Such studies offer a hope for developing complex mixtures as anti-prostatic agents and warrant strict, carefully controlled studies to demonstrate clinical evidence for primary or secondary chemoprevention of prostate cancer.

ABBREVIATIONS

ADT = Androgen Deprivation Therapy

AIPCA = Androgen Independent Prostate Cancer

BPH = Benign Prostatic Hyperplasia

CAM = Complementary and Alternate Medicine

CRE = Cyclic-AMP Response Element

CREB = Cyclic-AMP Response Element Binding protein

GS = Gleason Score

HGPIN = High Grade Prostatic Intraepithelial Neoplasia

MRI = Magnetic Resonance Imaging

NSAID = Nonsteroidal Anti-Inflammatory Drugs

PCA = Prostate Cancer

PSA = Prostate Specific Antigen

PSVC = Prostate-Seminal Vesicle Complex

RT = Radiotherapy

TRAMP = Transgenic Adenocarcinoma of Mouse Prostate

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