Chapter

Combinatorial Drug Therapy with Phytochemicals as Adjuvants in Prostate Cancer Management

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Abstract

Prostate cancer is one of the leading cancers in men needs a long period for development from small lesion to become a clinical manifestation. The prostate specific antigen is a prominent tumor marker for prostate cancer. Androgens are involved in the development and progression of prostate cancer regulating the androgen receptor as androgen-dependent or androgen-independent types. The latter occurs in metastatic conditions of prostate cancer developed as hormone resistant prostate cancer (HRPC) that inappropriately activates transcription of other genes involved in molecular pathways inducing cellular proliferation and inhibiting apoptosis. Since prostate cancer is characterized by slow growth and long latency period and thus integration of phytochemicals/compounds in combination with other existing therapies have promising future to manage cancer, thus controlling the disease progression and mortality rate. Therefore, the medicinal plants therapeutic or prophylactic activities on prostate cancer exhibiting anti-androgenic effects, depleting PSA, down-regulating expression of androgen receptor, regulating cell cycle regulators have promising future to be applied as adjuvant drugs in prostate cancer treatment.

Keywords: prostate cancer, androgen receptor, PSA, systemic therapy, phytochemicals, molecular pathways

1. Introduction

Statistical reports revealed that prostate cancer is the third leading cause of cancer deaths in men [1] causing the life of one in every 39 men in United States. Related to prostate cancer rates in India, the rates are more than 20 times higher in US whites, more than 10 times higher in US Asian Indians/Pakistanis, seven times higher among UK South Asians and twice as high among Singapore Indians [2]. Factors escalating the risk of prostate cancer include age above 50 years, cancer incidence in family, ethnicity, modern diet, environmental factor, pollutants, etc. The role of inflammation in prostate diseases is suggested by the presence of inflammatory cells within the prostate of cancer patients [3]. The molecular mechanism responsible for inflammation mediated prostate cancer is not yet clear.

However, prostate cancer needs a long period for development from small lesion to become a clinical manifestation [4]. Tumor progression is revealed with complications in erectile dysfunction, skeletal bone pain, obstruction of lymph vessels,

and veins causing lower body edema. The development of high-grade prostatic intraepithelial neoplasia (HGPIN) is identified as an intermediate stage between benign epithelium and the invasive malignant carcinoma at the outset of prostate cancer. Four main patterns of high-grade PIN (HGPIN) have been described as tufting, micropapillary, cribriform, and flat [5].

Prostate specific antigen (PSA), major constituent in prostatic secretion and is used for screening prostatism. PSA testing is primarily associated with benign prostatic hyperplasia (BPH), however there is only rare connectivity of patients having BPH to develop prostate cancer. With conditions like BPH, inflammation, and disruption of prostate basal membrane increases the permeability and releases PSA into the circulation. Prostate cancer antigen 3 (PCA3) is more specific than PSA which is an important biomarker in personalized medicine. Also analysis of urinary protein markers, such as TMPRSS2-ERG and PCA3 are helpful in early diagnosis of the disease. The other tumors markers reported as prostatic acid phosphatase (PAP), cytoskeletal proteins, and annexin I are downregulated in PIN whereas C-erbB-2 (HER-2/neu) and C-erbB-3 oncoproteins, c-met protooncogene, Bcl-2 oncoprotein, several growth factors, nitric oxide synthase, alpha-methylacyl-CoA racemase, glycoprotein A-80, and apolipoprotein D, are upregulated in PIN. The histological grading system of prostate cancer is obtained from the Gleason score [6] which provides information that score 1–5 indicates low-grade prostate cancer and score 8–10 indicates high-grade prostate cancer. Digital rectal examination (DRE), examination of PSA levels, prostate ultrasound, and prostate biopsy are the current diagnostic methods in examining the primary and metastatic stages for prostate cancer [7, 8]. Prostate cancer stages are generally classified into three categories—T category, N category, and M category. T represents the primary tumor, N represents the cancer that has spread to regional (nearby) lymph nodes and M represents the distant metastasis cancer. The treatment strategies are administered based on the stage of cancer progression.

2. Androgen-dependent and androgen-independent prostate cancer

Human prostate is a walnut shaped, fibromuscular organ located beneath the urinary bladder and is made-up of several glandular and non-glandular components that are tightly fused together within a common capsule [9]. It is an exocrine gland functioning in secretion of complex proteolytic solution into the urethra during ejaculation which is important for sperm motility and nourishment. The growth and function of the prostate are regulated by androgens. The growth and development of normal prostate requires functioning androgen signaling pathway, which is regulated by hypothalamic-pituitary gonadal axis. Androgens (includes testosterone and DHT) are responsible for the male secondary sexual characteristics. Testosterone is synthesized in the testes and released into the circulation in response to specific hormonal signals regulated by GnRH, FSH, and LH. Testosterone is transported by steroid hormone binding globulin (SHBG) to the prostate, where it is converted by 5α-reductase to its active metabolite 5α-dihydrotestosterone (DHT).

In the prostate, androgens mediate their effects *via* high affinity to the androgen receptor (AR), a nuclear transcription factor that controls expression of genes involved in growth, differentiation, homeostatsis, and apoptosis. Receptor for steroid and thyroid hormones are mostly cytoplasmic/nuclear receptors and hormone-receptor complex binding to the promoter regions of responsive genes and stimulate or inhibit transcription from those genes. Nuclear receptors are ligand-inducible transcription factors that mediate the signals of a broad variety of fat-soluble hormones, including the steroid and vitamin D3 hormones, thyroid hormones retinoids.

Upon ligand binding, AR translocates to the nucleus, binds to DNA recognition sequences, and activates transcription of genes involved in cell proliferation, apoptosis, and differentiation [10]. Androgens are involved in the development and progression of prostate cancer [11] regulating the AR. During this phase the tumors are referred to as androgen sensitive or dependent. The most common therapies done with prostate cancer patients are androgen deprivation therapy removing testicles partially or completely in addition to administration of drugs inhibiting LHRH secretion and anti-androgen therapy blocking the androgen receptor interaction.

Prostate cancer can evolve into castration resistant cancers which is independent of androgen levels [12]. The AR signaling pathway continues to be active in hormone resistant prostate cancer (HRPC) and can inappropriately activate transcription [13]. Recent studies show that cancer progression and metastasis lead to alteration in the androgen receptor pathway genes with transition of prostate cancer from the androgen dependent to independent stage [14]. Mutations in the ligand-binding domain alter the specificity of the AR enhancing the binding of estrogens, progesterone, and anti-androgens; and thereby decreasing its dependency on androgens while stimulating cell growth. In addition to AR mutations, a variety of growth factors, including insulin-like growth factor I, epidermal growth factor, and keratinocyte growth factor, can activate androgen-responsive genes *via* the AR, suggesting that androgen independence which occurs due to the over expression of growth factors in the local environment [15]. There are multiple evidences suggesting that estrogens are involved in prostate carcinogenesis. In worldwide the African-Americans have the higher risk of prostate cancer with elevated levels of serum estrone and estradiol levels even in healthy young men [16]. CCDC62/ERAP75 is a new co-activator of ER and this protein is mainly present in the nucleus and widely expressed in many prostate cancer cell lines (PC-3, DU145, LNCaP, 22Rv1) than in the normal prostate epithelial cells (BPH-1) [17].

3. Molecular regulations in prostate cancer

The inappropriate expression of the growth inhibitory factors appears to contribute to prostate cancer progression. Epidermal growth factor (EGF) promotes chemo-migration of metastatic prostate cancer cells to lymph node and medullary bone sites [18]. In insulin signaling pathway, the ligand insulin binds to its receptor followed by tyrosine phosphorylation of insulin receptor substrates (IRS) by the insulin receptor tyrosine kinase. Several studies suggested that alteration in the IGF signaling axis is associated with an increased risk of prostate cancer [19]. Signal transduction proteins interact with IRS including GRB2. GRB2 is a part of the cascade including SOS, RAS, RAF, and MEK that leads to activation of MAPK and mitogenic response in the form of gene transcription.

In AKT signaling pathway, PTEN is a regulator which is down regulated to protect the cell from tumor growth. The phosphatase activity on phosphatidylinositol 3,4,5-triphosphate allows dephosphorylation of PIP3 to PIP2. The PIP2 inhibits the P13K which is the membrane bound domain. When the phosphatase activity is lost, P13K transfers its phosphate group to PDK1 and PDK2 which in turn causes phosphorylation of AKT protein and regulatory amino acids Ser473 and Thr308. This leads to activation of MDM2, P21, CASP9, mTOR genes leading to apoptosis inhibition, tumor growth, etc. Smad3 gene plays a key role in prostate cancer serving as an essential mediator of most Smad-dependent TGF-beta responses, including control of gene expression, cell growth, apoptosis, and tumor suppression. Deregulated/enhanced expression and activation of AR in prostate carcinomas may intercept the tumor suppressor function of TGF-β through transcriptional suppression of Smad3.

TNFRs are activated by TNF in order to exhibit cellular response. TNF triggers the transcription of apoptotic proteins through NF-κB thereby achieving cell survival. Activation of TRAFs, such as TRAF2, TRAF5, and TRAF6 results in transduction of cellular response by TNFRs. In TNFRs pathway, TRAF3 acts as a repressor. APRL, BAFF, BAFF-R, BCMA, and TACI belong to the TNF superfamilies and play vital roles in immunity through B cells and T cells. BAFF binds to BAFF-R, BCMA, and TACI, while APRIL bands to two of them—BCMA and TACI only. In this pathway nuclear factors (NF-κB) are activated in signaling cascades. This activation process can be triggered by canonical and non-canonical pathways. The latter is activated by activation of BCMA, TCAI, and BAFF-R by BAFF. This counter activates TRAF2 and TRAF5 that signals NIK, a mitogen activated protein kinase. Once NIK is activated, phosphorylation of IKK-alpha that restrain processing NK-κB2 into NF-κB2 onsets. Along with RelB, NF-κB2 activates Bcl2 or Bcl-XL or both. Upon this activation cell survival is promoted. BAFF transcription is activated by NF-κB2 resulting in a positive feedback. I-kB is phosphorylated by IKK alpha, beta, and gamma which is activated by TRAF2 by canonical pathway. I-kB is disintegrated and ubiquitylated inside proteosome 26S. This process discharges NF-κB2 and RelA which are swiftly repositioned from cytoplasm to nucleus. Following which anti-apoptotic components, such as Bcl-2, Bcl-XL, and BFL1 are activated by transcription by NF-κB transfactors.

In non-canonical pathway, IKK alpha is activated by its phosphorylation once NIK kinase is promoted by TRAF. NF-κB2 is processed from p100 to p52 by IKK alpha which is adhered to RelB. The dimer (RelB-p52-NF-κB) engages in affecting gene transcription once it shifts to the nucleus. This pathway does not involve IKK beta and gamma. IKK, RelB, and NIK are mediated by TNF-R1 signal or TNF-R2 signal as well. NF-κB promoted cell growth and proliferation in prostate cancer cells by regulating expression of genes, such as c-myc, cyclin D1, and IL-6. Furthermore, NF-κB-mediated expression of genes involved in angiogenesis (IL-8, VEGF), and invasion and metastasis (MMP 9, uPA, and uPA receptor) may further contribute to the progression of androgen depleted prostate cancer [20].

Apoptosis is programmed cell death involving sequential events of elimination of cells without releasing harmful substances into the surrounding area. Apoptosis plays a crucial role in developing and maintaining the health of the body by eliminating old cells, unnecessary cells, and unhealthy cells. The two classes of regulatory molecules play vital role in the cell cycle progression and apoptosis processes are protein kinases, such as cyclin dependent kinases (CDKs), and cyclins. Disturbances in the cell cycle regulation due to uncontrolled cell growth and divisions through and escaping of the cell cycle checkpoints occur in the mutated cells. The cell cycle events are facilitated through the activated cyclin D-CDK4/6 complexes phosphorylating the retinoblastoma protein (pRb) bound with E2F transcriptional factors. This in turn inactivates pRb and weakening its affinity for E2F which then becomes free to enter the nucleus and transcription of cell cycle progression genes. The hypophosphorylated pRb impounds the transcription factor E2F in the cytosol, thus blocking the cell cycle at G1 phase.

Similarly B-cell lymphoma (Bcl-2) family genes are involved in the apoptosis pathway, including prostate, breast, and ovarian cancers. The Bcl-2 family proteins had the anti-apoptotic subgroup, such as, Bcl-extra-large (Bcl-xL), Bcl2-like 2 (Bcl-W), myeloid cell leukemia (Mcl-1) that interacted with another subgroup of proteins called the pro-apoptotic proteins (Bcl2-associated X protein (Bax) and Bcl2 antagonist/killer (Bak)). In the signal transduction cascade of apoptosis the pro-apoptotic proteins began induction of apoptosis *via* mitochondrial outer-membrane permeabilization, followed by the release of cytochrome c, and finally the activation of cysteine aspartyl proteases (caspases). Second is the intrinsic pathway, with the release of cyt-c into the cytosol from mitochondria, a multiprotein

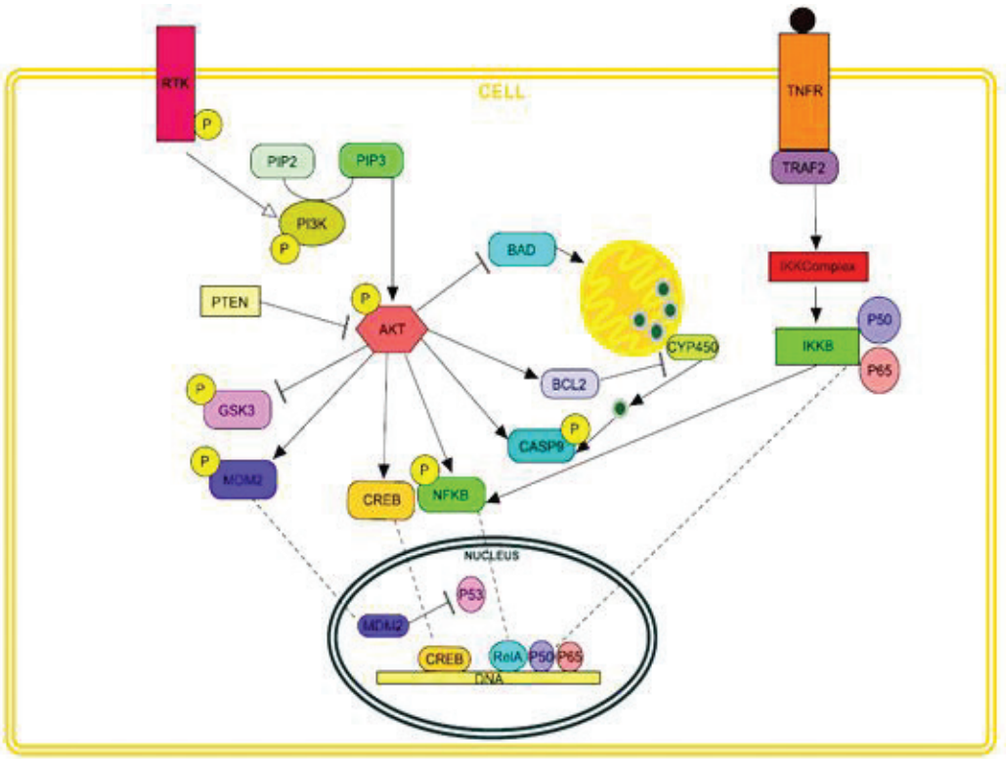

Figure 1.
Molecular pathways involved in prostate cancer progression (developed by pathway construction tool—PathVisio).

caspase-activating complex, called "apoptosome" binds procaspase-9 and cyt-c with its central component Apaf1, and activates apoptosis. Thus there should be a balance in the levels of anti-apoptotic and pro-apoptotic proteins for the proper progression of apoptosis (**Figure 1**).

4. Phytomedicine: promoting synergistic actions in prostate cancer management

The history of ayurvedic and traditional systems of medicine showed that the medicinal plants have global importance in treating human diseases and disorders [21]. The references of ancient literature, a Sumerian clay slab from Nagpur, approximately 5000 years old suggested that people were depended on drugs from the nature [22]. Theophrast (371-287 BC) in his scientific book titled "De Causis Plantarium" has referred and classified nearly 500 medicinal plants. About 700 plant species including pomegranate, castor oil plant, aloe, senna, garlic, onion, fig, willow, coriander, juniper, common centaury, etc. [23] were reported having medicinal properties. The World Health Organization (WHO) estimated that trades of plant-derived pharmaceutical drugs would account for five trillion US dollars by 2050.

The survey reports on these medicinally valued plants and their preparations as tinctures, teas, poultices, powders, and other herbal formulations [24] served as the basis for novel drug discovery wherein a large number of synthetic drugs are developed on the small-molecule natural chemical entities and has been introduced as potential drugs worldwide [25]. Even our natural food intake relays on the medicinal aspects followed in our tradition thus have a strong impact on determining health at different stages/phases of life. In addition many food-based nutrients contribute to the prevention and management of deadly diseases like cancer. Wholesome diet including vegetables, fruit, and vitamins lowers the risk up to 80% of cancers of the large bowel, breast, and prostate.

Based on the studies done at the US National Cancer Institute, selenium, vitamin E, and Omega-3 fatty acid have preventive roles in prostate carcinoma [26]. Plants produce a wide range of chemical compounds as by-products of their metabolic pathways known as secondary metabolites. These compounds have no direct role in growth yet they have significant role in defense mechanisms. The secondary metabolites generally flavonoids, tannins, curcumin, resveratrol, and gallocatechin are reported as effective anti-cancer drugs [27]. Molecular studies have proved that luteolin, quercetin, and kaempferol rich in onions, olives, grapes, tea, pomegranate, broccoli, and cauliflower [28] are effective in suppressing tumor development with low dose-limiting toxicities and negligible side effects [29].

Plants in nature contain many active compounds which elicit the synergistic effects in combination with chemical drugs through its own molecular mechanisms of targeting signaling pathways in cancer cells in order to overcome drug resistant cancers, also strengthens the therapeutic activity of chemical drugs and subsides drug-induced toxicity. The pharmacological efficacies of chemotherapeutic drugs are improvised by alteration in the pharmacokinetics of drug compounds regulating the receptor targets and downstream effector molecules in the cancer cells.

Sporadically, anti-androgens (anti-hormones) are administered for initial stages of prostate cancer which is prone to frequent failures in patients diagnosed with recurrent androgen-independent prostate cancer and metastasis. Radiotherapy and/or androgen deprivation therapy can be considered as an adjunct to surgery, in either the adjuvant or salvage setting, to further control the disease course. These combinatorial therapies are highly recommended in combating primary tumors, but metastatic tumors are more challenging due to loss of remedial activity and disease remission. Also these therapies are reported in exerting complications of damaging the normal cells in vicinity of tumor growth, disturbing the functioning of immune system, triggering autoimmunity [30], damage to adjacent gastro-intestinal tract, and bladder incontinence. Hence, multifaceted therapies producing synergistic effects and long-term outcomes by lowering the local tumor burden and eradicating metastatic disease compared to each individual change are required for cancer management.

Prostate cancer is characterized by slow growth and long latency period and thus integration of phytochemicals/compounds in combination with other existing therapies have promising future to manage cancer, controlling the disease progression and mortality rate. The WHO has estimated that approximately 80% of the world's population depends on traditional plant-based medicines for meeting their primary health care needs [31]. In prostate cancer, there is an imbalance between prostate cell growth and apoptosis. In prostate cancer these proteins are over expressed which lead to progression of metastatic prostate cancer through inhibition of apoptotic cell death. This over expression also causes resistance to heat-shock stress, several chemotherapies, and radiotherapy. Phytochemicals are routinely used as an adjuvant to conventional chemo and radio therapies of cancer in order to manage the drug-resistance mechanisms and resensitize tumor cells.

Many medicinal plants are reported to possess chemopreventive activities on prostate cancer exhibiting significant oestrogenic and anti-androgen effects, such as *Agathosma betulina* [32], *Bidens pilosa* [33], *Prunus Africana*, *Cucurbita pepo L.* [34], *Andrographis paniculata* [35], *Vaccinium macrocarpon* [36], *Linum persicum* and *Euphorbia cheradania* [37], *Panax ginseng* [38], *Scutellaria baicalensis* [39], *Wedelia chinensis* [40], *Urtica membranaceae*, *Artemisia monosperma* and *Origanum dayi* [41], *Vitis vinifera* [42], an eight-herb Chinese formulation, that consists of *Isatis indigotica*, *Glycyrrhiza glabra*, *Glycyrrhiza uralensis*, *Scutellaria baicalensis*, *Ganoderma lucidum*, *Panax ginseng*, *Dendranthema morifolium*, *Rabdosia rubescens* [43]. Combined drugs made of vinca alkaloids, Taxus diterpenes, Podophyllum

lignans, and Camptotheca alkaloids were proved with anticancer effects [44]. Pentacyclic triterpenoids reported in *Hypoxis hemerocallidea* reduced the inflammation and swelling in the prostate. Garlic and green tea catechins act as potential chemopreventive agents against cancer by inhibiting PIN [45]. Phytochemicals like geneistin and quercitin [46], lycopene [47], curcumin, epigallocatechin-gallate, resveratrol [48], brassinosteroid [49], derived from medicinal plants have been reported through *in-vitro* studies with potentials of anti-proliferative effects on prostate cancer cell lines, such as PC-3, DU-145, and LNCaP. Their protective action against cancer may be due to their high concentration of antioxidants that react with free radicals thereby neutralizing them [50].

Quercetin and curcumin, plant-derived flavanoids are well established drugs in cancer treatment exhibiting antioxidant, anti-inflammatory, and anti-proliferative activities [51]. Quercetin has anti-inflammatory role through regulation of NF-κB pathway genes [52] inhibiting the expression of pro-inflammatory cytokines, TNF-α, IL-6, and IL-1β and inflammatory mediators, nitric oxide, and catalase. Combination of quercetin with doxorubicin increases the sensitivity of PC3-resistant cells by inducing apoptosis *via* reduction of mitochondrial membrane potential, activation of the PI3K/AKT pathway, and acceleration of chemo resistance [53]. The chemopreventive and anti-neoplastic activity of the phytochemicals could be achieved through multiple effects including reduction of PSA and AR expression, induction of apoptotic pathways, inhibition of angiogenesis, induction of PKC-α, suppression of TrkE, induction of p53, inhibition of proteasome activity, induction of S-phase and G0/G1 phase and G2/M cell-cycle arrest, suppression of DNA synthesis, up-regulation of protein expression WAF1/p21, KIP1/p27, INK4a/p16, INK4c/p18, down-regulation of protein expression cyclin D1 and D3, cyclin E, cdk2, cdk4, cdk6, inhibition of PI3K/PKB phosphorylation of AKT, Inhibition of COX-2 expression [54]. *Wedelia chinensis* extract possesses the ability in reducing the gene expressions of proinflammatory cytokines and STAT3 activity in tumor-elicited myeloid cells [40]. Altogether, the combination of herbal compounds includes pharmacokinetic and pharmacodynamic synergisms to provide significant therapeutic effects. Baicalein form *Scutellaria baicalensis* [39] reduces the synthesis of eicosanoid that function as important mediators in inflammatory responses through inhibiting enzymatic oxidation of essential fatty acids.

One of our studies reported that, a potential medicinal herb *Gymnema sylvestre* has been proved for its potency to control prostate cancer progression [55]. It is (family: Asclepiadaceae) described as miracle fruit, is native to central and western India that has been used in the traditional health care system, for several centuries [56]. The leaves are rich in triterpene classes of oleanane saponins (gymnemic acids and gymnemasaponins) and dammarene saponins (gymnemasides). The phytocompound dihydroxy gymnemic triacetate (DGT) isolated from acetone extract of *G. sylvestre* leaves were effective in inhibiting prostate cancer cell growth, inducing apoptosis, modulation of cell cycle, and downregulation of the protein expressions. Also the depletion of PSA observed in the PC-3 cell line caused by DGT was observed. Since PSA gene is positively regulated *via* binding of AR to the androgen responsive elements in the promoter of PSA, decline of PSA levels proves down-regulation of AR [57].

5. Conclusion

Thus multimodal approach of treating prostate cancer with the integration of phytochemicals as chemopreventive agents in down-regulation of cell proliferation and induction of apoptosis has taken forefront in development of potential therapeutic drugs in cancer management.

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