1. Introduction

Hormone imbalance associated diseases such as prostate cancer (PCa) can originate either purely as a disorder of a gland or as a consequent of changing hormonal status of an organ due to factors such as age and environmental influences. PCa accounts for 9% mortality burden globally and is placed second after lung cancer (Cancer Facts & Figures 2012). Despite advances in attainment of early detection of PCa, we are unable to keep a check on the mortality figures of PCa. Even today metastatic PCa remains an incurable disease and therapy can only delay progression. Androgen deprivation therapy (ADT) blocks testosterone-driven proliferation of PCa cells and causes apoptosis in majority of the PCa cells (Pienta and Bradley 2006). ADT can only delay progression of PCa, but the benefit to harm ratio in prolonged ADT is still debatable. More work is needed to have a better understanding of the disease onset by and large.

Benign nodular enlargement of the prostate, commonly referred to as benign prostatic hyperplasia (BPH), is the most common proliferative disease of the prostate of elderly men. More than two thirds of men older than 50 years have histologic evidence of BPH; after age 70, the proportion increases to 80% or more (Isaacs 1994). The histopathology of BPH strongly implicates local paracrine and autocrine growth factors and inflammatory cytokines in its pathogenesis (Rick et al., 2010). A complex milieu of growth-regulatory proteins includes members of the fibroblast, insulin-like, and transforming growth factor families (Rick et al., 2010).

The etiology of both pathologies (BPH and PCa) is not well defined; however it is irrefutable that variations in the hormonal status of the prostate are involved. Both of these diseases are extremely common in ageing males; almost 90% of the men develop either BPH or PCa between their fourth and ninth decades of life. Despite their high prevalence, current medical care is unable to eradicate or completely cure BPH and PCa. With the unprecedented ageing population, there is a demand for more novel forms of treatment strategy or perhaps a shift to preventive medicine.
Plants are an essential source of therapeutic agents now a day. They are being used to isolate bioactive compounds for direct use of drugs (e.g. digoxin, morphine, taxol) and for producing bioactive compounds of novel or known structures as lead compounds (e.g. metformin and verapamil are based on galegine and khellin respectively) (Fabricant et al., 2001). Furthermore, since phytotherapy is becoming more popular amongst patients, plant-based medicine may have better patient compliance compared to synthetic drugs. The search for bioactive components in tropical plants that may offer potential remedy, in one way or the other, to BPH and PCa will be the centre of interest in the present work.

Life style and dietary habits have been identified as major risk factors in PCa growth and progression (Abdulla and Gruber 2000, Agarwal and Rao 2000). Epidemiological data indicate that vegetables and fruits containing chemopreventive agents have shown protective effects against various types of cancers. The initiation to the appearance of early diagnostic symptoms of PCa is a very long period (appx. 30 years). Due to its long latency period of PCa it provides an ideal therapeutic window for chemoprevention.

Many researchers have reported the chemopreventive role of Lycopene, Genstein and Diadzein, phytoestrogen of Soya products, some metals like selenium against PCa. Some investigators have reported the roles of SERMS (Selective estrogen receptor modulators) in inhibiting the PCa growth but the results are not conclusive and much more research is needed in this area (Thompson 2007, Ellem et al., 2007).

There is a need to identify such compounds, which can be used as prognostic markers that distinguish indolent versus aggressive forms of PCa. More attention should be given to understand the molecular cause that underlies normal prostate growth and development or cancer initiation and progression. Here we intend to investigate the role of two natural chemopreventive agents’ luteolin and diadzein, elucidate the pathway so that these agents in future can be used as prophylactic and therapeutic tools in the treatment of PCa.
2. Review of literature

Cancer is a disease in which abnormal cells divide without control and are able to invade other tissues. Cancer is not just one disease but many diseases. The basic characteristic of cancer is the transmissible abnormality of cells that is manifested by reduced control over growth and function leading to serious adverse effects on the host through invasive growth and metastasis. Cancer cells are characterized by a lack of controlled growth, programmed cell death and acquired immortality. Cancer has always been with us, but not always in the same way. Its care and management have differed over time, of course, but so, too, have its identity, visibility, and meanings.

Cancer remains a global killer with a shifting burden from the developed to the developing countries. It develops along a multistage process that is defined by distinct histological and pathophysiological phases. The body has many controls to prevent cells from doing this; most of the cancer cells have the ability to overcome these controls. Cancer may also be called malignancy, a malignant tumor, or a neoplasm (new growth). Each type of cancer is characterized by the uncontrolled growth of cells (Mizejewski 1999). Under normal conditions, the body carefully controls cell reproduction. However, malfunctioning of these controls, results in abnormal cell growth, development of a lump, mass, or tumor. Some cancers involving the blood and blood-forming organs do not form tumors but circulate through other tissues and there they grow.

A tumor may be benign (non-cancerous) or malignant (cancerous). Cells from cancerous tumors can spread throughout the body. This process, called metastasis, occurs when cancer cells break away from the original tumor and travel in the circulatory or lymphatic system until they have a small capillary network in another area of the body (Raubenheimer and Noffke 2006). The induction of cancer (carcinogenesis) depends on inherited and acquired susceptibility factors, on exposure to initiation factors (exogenous and endogenous carcinogens), and on promotion and progression factors (Rosman-Urbach 2006, Kikuchi 2004, Peltomaki 2001).
2.1. Carcinogenesis

Whatever the cause of cancer, its development is a multi-stage process involving damage to the genetic material of cells (deoxyribonucleic acid, or DNA). This damage occurs in genes regulating normal cell growth and division. Because several stages or several mutations are required for cancer to develop, there is usually a long latent period before cancer appears.

The distinction between initiation and promotion was recognized through studies involving both viruses and chemical carcinogens (Reddy 1983). In the initiation stage, physical, chemical or biological carcinogenic agents cause irreversible damage to the DNA strand. In the promotion stage, genes are altered and cell differentiation and cellular growth controlled by these genes can become distorted.

By damaging critical structures, such as DNA, proteins and lipids, ROS can take part in both initiation and promotion (Kim 2006). The progressive stage is the final stage when the cells become neoplastic and begin their invasive expansion. The causative agents in carcinogenesis are related to increased age, significant exposure to tobacco, high fat consumption and exposure to genotoxic chemicals (carcinogens that cause damage to the base of the DNA). This type of damage may be the result of chronic infections or recreational, occupational, environmental or sexual activities (Steindorf 2005). All of these causative agents expose the molecules of the body to extreme levels of oxidizing toxins that cause DNA damage. Organisms come into contact with numerous endogenous and exogenous hydrophobic xenobiotic agents that must be made hydrophilic by drug metabolizing enzymes. Reactions involved in drug metabolism are often
classified as phase I (activation) and phase II (detoxification) reactions. Enzymes catalyzing phase I reactions include cytochrome P450 enzymes (Kalinina 1991, Yang 2012).

Enzymes catalyzing phase II reactions include the conjugation enzymes UDP-glucuronosyltransferases (UGT), glutathione S-transferases (GST) as well as other enzymes that protect the cell from toxic damage due to oxidative stress, NAD(P)H quinone oxidoreductase (QOR) (Cuendet 2006). Phase I and phase II enzymes act in concert, convert hydrophobic compounds to more hydrophilic compounds that can be readily eliminated in bile or urine. Compounds that specifically induce phase II metabolism, such as antioxidants, may have the potential to protect against chemical carcinogenesis since the mutagenic effects of carcinogens are often mediated through and excess of cytochrome P450 generated reactive intermediates. Studies are being carried out to determine the chemopreventive potential of novel plants and pure compounds by the selective induction of phase II enzymes (Kaur 2006). (Figure 1 and 2)

![Figure 2: Schematic diagram of multistage carcinogenesis](image-url)

*Acquired (environmental) DNA damaging agents: Chemicals, Radiation, Viruses*
i. Initiation of cancer

Initiation involves mutation of cellular DNA resulting in the activation of oncogenes and the inactivation of tumor suppressor genes. Initiation is thought to be irreversible and consist of a single gene mutation that is caused in most cases by environmental genotoxic agents such as chemicals, radiations, and viruses. DNA adduct formation that causes either the activation of a proto-oncogene or the inactivation of a tumor-suppressor gene can be categorized as a tumor-initiating event. Oncogenes can also be activated by chromosomal translocations and gene amplifications. Studies in the human indicate that the carcinogenic process involves multiple genetic alterations in a staged fashion (Emdad 2005).

ii. Promotion of cancer

Promotion follows initiation and involves the process of gene activation, such that the latent phenotype of the initiated cell becomes expressed through cellular selection and clonal expansion. During promotion, the mutated cell is stimulated to grow and divide faster and becomes a population of cells. Eventually a benign tumor becomes evident. In human cancers, hormones, cigarette smoke, or bile acids are substances that are involved in promotion. This can occur through a variety of mechanisms, including toxicity, terminal differentiation or mitoinhibition of the non-initiated cells, and mitogenesis of the initiated cells (Tsutsumi 2006). While promotion occurs over a long period of time, it is reversible in its early stages. Various studies prove that promotion is reversible in humans is supported by the observation that the rate of lung cancer induction in individuals who quit smoking approaches that of nonsmokers (IARC 1986). The available data, as well as the multistage nature of tumor promotion, suggests that this process, occurs in most tissues in which cancer can be induced and occurs spontaneously, may involve the interaction of a number of endogenous factors as well as environmental factors such as chemicals, radiation, viruses, bacteria, and diet and nutrition, thus unifying all current areas of cancer research (Di-Giovanni 1995). Cell to cell communication via gap junctions is essential in the maintenance of the homeostatic balance of multicellular organisms. Aberrant intercellular gap junctional communication (GJIC) has been implicated in tumor promotion, neuropathy and teratogenesis (Nakamura 2005). Most, if not all, tumor promoting agents reversibly inhibit GJIC. Even genotoxic mutagens, as well as nongenotoxic
cytotoxicants or surgery, can induce the blockage of GJIC by killing or removing cells within a tissue, causing compensatory hyperplasia of the surviving cells. The modulation can both down-regulate GJIC and lead to tumor promotion or it can up-regulate GJIC and lead to suppression of the initiated cells. Both phase I and phase II enzymes are subject to induction by a variety of chemical agents (Wada 2006). Regulations of phase I and phase II enzymes are important because toxicity through oxidative stress can result when the balance between activation and detoxification is shifted and may lead to tumor promotion (Umachandran 2006). In human cancer, smoking, environmental factors such as asbestos, hydrocarbons, radiation, hormones, alcoholic beverages, diet and nutrition are now thought to have more of a promotional influence on the multistage carcinogenesis process (Bruce 1998).

iii. Progression of cancer

The last step leading to cancer is called progression. Progression involves genetic damage that results in the conversion of benign tumors into malignant neoplasms capable of invading adjacent tissues and metastasis to distant sites. The additional genetic alterations thought to be required for neoplastic progression often occurs faster than expected from the statistics of accidental genotoxic insults due to so-called genetic instability. The concept of genetic instability implies that while environmental genotoxic agents generally cause cancer initiation, the additional mutations required for neoplastic progression may be attributed to endogenous reactions and factors such as detoxification and removal of damaged cells by programmed cell death. Genetic instability may happen due to the errors in DNA replication, spontaneous hydrolytic alterations of DNA such as depurination and deamination in combination with an impaired ability of premalignant cells to repair DNA damage or due to oxidative DNA damage (Loft 1996). Modified DNA bases, especially 8-hydroxy-2'-deoxyguanosine, produced by oxygen-free radicals have been implicated in the genesis of cancer (Pilger 2006). The importance of free radicals in radiation carcinogenesis and oxygen-free radicals and electrophiles in chemical carcinogenesis is also well recognized (Malins 1993, Collins 1999). During progression, there is further growth and expansion of the tumor cells over normal cells. The genetic material of the tumor is more fragile and prone to additional mutations. These mutations occur in genes that regulate growth and cell function such as oncogenes, tumor suppressor genes, and DNA mismatch-repair genes.
These changes contribute to tumor growth until conversion occurs, when the growing tumor becomes malignant and possibly metastatic.

iv. Metastasis

Cancer has ability to spread, or metastasize, which is its most deadly aspect (Ostuni 1975). Cancer cells initially group together to form a primary tumor, once the tumor is formed, cells may begin to break off from this tumor and move to other parts of the body, this process is termed as metastasis. These cancer cells that travel through the body are capable of establishing new tumors in locations remote from that of the original site. Metastasis is a very complicated process and yet not completely understood. A cancer cell invades either in the circulatory or lymphatic system, to a new location, and establishes itself at the new site. Common sites for metastasis are the bones, lungs, liver and central nervous system. The type of cancer refers to the organ or area of the body where the cancer originally occurred. Cancer that has metastasized to other areas of the body is named for the part of the body where it is originated. For example, if breast cancer has spread to the bones, it is called "metastatic breast cancer" not bone cancer. It is an inheritable disorder of somatic cells; environment and heredity both contribute to the origin of cancer (Singh 2006).

3. Prostate

3.1. Anatomy, Structure and physiology

The prostate is a male accessory sex gland that plays a role in reproductive function. It lies immediately below the bladder surrounding the proximal portion of the urethra. In adult rat it weighs approximately 450 mg (Cunha et al., 1987, Rittmaster et al., 1999). The prostate ductal system of the rat can be divided into three regions based on proximity to the urethra: proximal, intermediate, and distal (Verhagen et al., 1988, Prins et al., 1992, Prins and
Birch 1993, Nemeth and Lee 1996, Banerjee et al., 1998). The secretions of the prostate constitute bulk of the ejaculation volume with high concentrations of prostaglandins, spermine, fructose, citric acid, zinc, immunoglobulins, and various enzymes such as proteases, esterases and phosphatases.

The functional unit of the prostate composes of epithelium and stroma components. The epithelium consists mainly of secretory columnar epithelial cells, which arrange into a single cell layer, lining the epithelium. They synthesize proteins such as prostate specific antigens (PSA), prostate specific phosphatase and secrete them into the ductal lumen mucin. Notably, majority of PCa arises from aberrantly functioning secretory epithelial cells. The prostate epithelium also composes of basal epithelial cells, neuroendocrine cells, non-epithelial fixed macrophages and intra-acinar lymphocytes (Chatterjee 2003). The epithelium is physically separated from the stroma by a basement membrane. The composition of the stroma includes fibroblasts, smooth muscle cells, endothelial cells, nerve cells and infiltrating mast cells and lymphocytes. The basement membrane on which the epithelial cells rest acts as an interface to the stromal compartment, it is a complex structure of collagen types IV and V, polysaccharides and glycolipids.

Figure 4: Immunofluorescence staining of prostate cells (adopted from Kelly and Yin 2008)
Immunofluorescent double-labeling of basal, luminal, and neuroendocrine populations in the mouse dorsal prostate gland. Left and right panels: Luminal and neuroendocrine cells or basal and neuroendocrine cells, respectively, are labeled within a background of all cells stained with DAPI, a general nuclear stain. Cytokeratin 8 (CK8): luminal, Cytokeratin 5 (CK5): basal, Synaptophysin (syn): neuroendocrine, DAPI: general nuclear stain. Colored arrowheads indicate the following: green – a luminal cell, white – a basal cell, red – a neuroendocrine cell. Images were kindly provided by Dr Zongxiang Zhou and Alexander Nikitin, Cornell University, Ithaca, New York.
The prostatic epithelium and stroma interact with each other via various hormones and growth factors. The fibroblasts are stimulated by androgens to produce and secrete various growth factors such as epidermal growth factor (EGF), insulin growth factor (IGF) and keratinocyte growth factor (KGF), which could, in a paracrine fashion, induce epithelial cell growth and glandular development (Carpenter et al., 1976, Niu et al., 2001).

The secretory epithelial cells express androgen receptors (AR) and they require continuous direct androgenic stimulation to maintain structural and functional viability. When the androgen level declines below a threshold, in the case of surgical or chemical castration, the secretory cells undergo apoptosis, causing glandular involution. Animal studies have also indicated that there was a ~90% loss of prostatic secretory epithelial cells through apoptosis after physical castration (Kyprianou and Isaacs 1988). The basal cells remain after castration as most of them do not possess AR. On the other hand, a subset of basal cells is speculated to represent stem cells and although they do not depend on androgens for survival, they require androgens for proliferation and differentiation into secretory cells (Isaacs and Coffey 1989).

Under normal physiological conditions, these stem cells are stimulated by androgens to undergo proliferation and differentiation. Cells with accumulated damage are removed by apoptosis and a steady state balance is maintained between cell proliferation and apoptosis. However certain pathological assaults may trigger the hyper stimulation of androgen and/or growth factors, thus affecting the delicate balance of prostatic cell growth and death. Consequently, a subset of epithelial cells may evade the normal checkpoint control of cell cycle progression and proliferate aberrantly (Chatterjee 2003). (Figure 3 and 4)

3.2. Role of hormones in the prostate gland

The prostate maintains its size and secretory function through the continued presence of serum testosterone that acts as a prohormone and is converted in the prostate to dihydrotestosterone (DHT) for hormonal action by 5α-reductase. Testosterone is synthesized from progesterone through reversible reactions; however, the formation of DHT or estradiol from testosterone is irreversible. Androgen signaling initiates in the hypothalamic-pituitary-gonadal (H-P-G) axis. Pitutary gland stimulates the adrenal glands and gonadal organs to produce androgens. In
gonadal cells, the major biosynthetic steps in the conversion of cholesterol to testosterone are illustrated. In the serum, up to 95% of the testosterone is bound to steroid hormone binding globulin (SHBG). Testosterone in prostate cells is rapidly converted by steroid 5α-reductase to the more bioactive androgen, DHT. The contribution to androgen signaling in the prostate of enzymes that catabolize DHT is still being elucidated. The vast majority of androgen signaling in the normal prostate is then mediated by the AR in response to increasing levels of DHT. (Figure 5)

Figure 5: Schematic diagram of Androgen signaling axis (adopted from Chen et al., Lancet Oncol. 2009) The androgen-signalling axis and Testicular androgen synthesis is regulated by the gonadotropin-releasing hormone–luteinising hormone (GnRH–LH) axis, whereas adrenal androgen synthesis is regulated by the corticotrophin-releasing hormone (CRH)-adrenocorticotropic hormone (ACTH) axis. DHEA=dehydroepiandrosterone. DHEA-S=dehydroepiandrosterone sulphate. DHT=dihydrotestosterone. AR=androgen receptor. ARE=androgen-response element.
4. Androgen receptor (AR)

AR is a ligand dependent transcription factor and it belongs to the Type I steroid hormone receptors, which is one of the three functionally distinct subfamilies of the nuclear hormone gene super family. AR was first described in 1969 (Fang et al., 1969) and cloned in 1988 (Chang et al., 1988). The gene is located on the X-chromosome at Xq11–12, contains 8 exons, and spans a length of approximately 90 kb of DNA (Lubahn et al., 1988). Similar to other steroid receptor proteins, the full-length AR contains 4 domains: the amino terminus regulatory domain, a highly conserved DNA-binding domain, a hinge region, and the ligand-binding domain (GS 2000). (Figure 6)

![A structural and functional map of a typical AR](image)

**Figure 6: A structural and functional map of a typical AR.** It has approximately 900 amino acids and a molecular mass of ~110 kDa. The amino-terminal consists of a constitutively active activation function (AF-1) and a ligand dependent activation function (AF-2) arises in the LBD (Gronemeyer 1995). The DBD has 2 zinc fingers which that dictate the specific binding to the ARE.

4.1. Activation of Androgen Receptor

Activation of androgen receptors occurs by two methods. It activated by ligands (testosterone and DHT) and ligand independent manner by surface proteins kinases etc.

a. Ligand dependent Activation

Unliganded ARs are sequestered in the cytoplasm as a multi-protein complex. They are associated with immunophillins and heat shock proteins (HSPs) 90, 70, and 56, which stabilize their tertiary structure and prevent them from constitutive activation (Jenster 1991). When bound to a ligand, AR is phosphorylated, undergoes a conformation change and dissociates from HSPs. The activated AR forms a homodimer with another AR. This consequently exposes a nuclear localization signal within the dimer, where importins bind and facilitate the translocation of the ligand bound AR to the nucleus (Arruzazabala et al., 2004). Once within the nucleus, they bind to canonical ARE on various androgen target genes. This can turn on or off
transcription of the particular DNA. Co-regulatory proteins (co-activators/co-repressors) are recruited to form a mega-protein complex, which is poised to interact with other transcriptional mediators, cofactors and basal transcriptional machinery to modulate target gene transcription (GS 2000).

b. Ligand independent activation

Nuclear receptors are regulated by reversible phosphorylation and thus may also be activated by signaling pathways that originated at the cell surface. AR possesses a consensus phosphorylation site which indicates that it could be a substrate for protein kinase A & C (PKA & PKC), mitogen activated kinase and casein kinase II. This hypothesis is supported by the observation that PKA and PKC could enhance AR transactivation (Zoran 2004). A number of other AR associated proteins (ARA) such as ARA 54, 55 and 70 also enhances AR transactivation.

4.2. Effects of AR activation

Testosterone and DHT bind with different affinities to the AR. This difference in binding affinity results in different levels of AR activation and therefore distinctive effects (Keller et al., 1996) (Table 1). Androgens modulate the synthesis of growth factors and their receptor availability.

Table 1: The different effects of androgens mediated by AR.

<table>
<thead>
<tr>
<th>Effects of Testosterone</th>
<th>Effects of DHT</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Development of the internal accessory sexual organs</td>
<td>• Development of the external sex organs</td>
</tr>
<tr>
<td>• Regulation of Follicle stimulating hormone synthesis</td>
<td>• Increase DNA replication, cell growth</td>
</tr>
<tr>
<td>• Regulation of growth factor receptors</td>
<td>• Induce SHBG and PSA production</td>
</tr>
<tr>
<td>• Maintenance of epithelium, microvilli, golgi secretory activity</td>
<td>• Induce mesenchymal cells to secrete KGF and FGF</td>
</tr>
<tr>
<td></td>
<td>• Downregulates TGF-β</td>
</tr>
<tr>
<td></td>
<td>• Increasing angiogenesis due to upregulation of EGF and vascular endothelial growth factor</td>
</tr>
<tr>
<td></td>
<td>• Inhibits apoptosis in LNCaP cells (Rokhlin et al., 2005).</td>
</tr>
<tr>
<td></td>
<td>• Antiproliferative and PSA induction effects of 1α-25-dihydroxyvitamin D3 on LNCaP are DHT dependent (Zhao et al., 1997).</td>
</tr>
</tbody>
</table>
One possible explanation to account for these differences is that testosterone dissociates 3 times faster than DHT and is less effective in stabilizing the AR. The differences in dissociation rate of the two ligands to AR could be directly related to their different abilities in stimulating androgen responsive genes (Keller et al., 1996).

4.3. Regulation of AR

AR expression is regulated at several levels: AR mRNA translation, transcription, post-transcription, protein, half-life and degradation (Table 2). AR is the main instrumental tool in eliciting the effects of androgens. However androgens, in turn, play an immense role in regulating the action and levels of AR.

Table 2: Briefly describes the different possibilities to regulate the levels of AR

<table>
<thead>
<tr>
<th>Levels</th>
<th>Regulation mode</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR mRNA transcription</td>
<td>Androgens: Results are controversial. Androgens decrease AR mRNA LNCaP cells and in rat ventral prostate (Quarmby et al., 1990, Shan et al., 1990). However other groups have shown an up-regulation of AR mRNA in rat and mouse prostate (Takeda et al., 1991), genital skin fibroblasts (Kaufman et al., 1981). FSH: Increases AR mRNA in Sertoli cells. GH, Prolactin, and EGF: Increase AR mRNA in prostatic cells.</td>
</tr>
<tr>
<td>AR protein expression</td>
<td>Androgens: Reported to modulate both stability and translation efficiency of AR mRNA (Mizokami et al., 1992)</td>
</tr>
<tr>
<td>AR nuclear import</td>
<td>Androgens: AR transfer is more efficient when bound to DHT then anti-androgens</td>
</tr>
<tr>
<td>AR protein degradation</td>
<td>Androgens: Half-life of AR in LNCaP cells is ~3 hours but it longer than 10 hours in the presence of 10 nM of DHT (Gregory et al., 2001).</td>
</tr>
</tbody>
</table>

4.4. Degradation of AR

Steroid hormone receptors have relatively short half-lives and they undergo systematic protein degradation. This is important in regulating the amount and duration of steroid receptor ligand effect. A study using green fluorescent protein technology demonstrated that AR migrated to the sub-nuclear compartment in the presence of the androgen within 15-60 mins. AR migrated rapidly back to the cytoplasm upon ligand dissociation and maintained its ability to re-enter the
nucleus for at least four rounds of AR recycling after initial androgen treatment before degradation (Tyagi et al., 200). AR may be degraded by two independent pathways, Akt-proteasome and phosphatase and tensin homolog (PTEN), caspase-3 pathways (Lee and Chang 2003).

5. Problems associated with prostate

There are three general conditions that cause prostate problems:

5.1. Prostatitis

Prostatitis is an inflammation of the prostate gland, often resulting in swelling or pain. Prostatitis can result in four significant symptoms: pain, urination problems, sexual dysfunction, and general health problems, such as feeling tired and depressed. According to the classification and definition of USA NIH (National Institute of Health) in 1995, Prostatitis is classified into four categories and is defined as follows: Category I is a bacterial infection of the prostate gland that requires urgent medical treatment (Acute bacterial infection Prostatitis); Category II is chronic bacterial prostatitis that usually presents as intermittent urinary tract infections (Chronic bacterial prostatitis); Category III is also known as bacterial prostatitis but unknown (Chronic prostatitis/chronic pelvic pain syndrome) and Category IV has no subjective symptoms but discovers prostatitis by chance (Asymptomatic inflammatory prostatitis). Generally prostatitis means the category III. Category III is divided into 3A (Inflammation type: a white corpuscle exists in prostatic fluid) and 3B (Non-inflammation type: a white corpuscle does not exist in prostatic fluid). Colon bacillus, Streptococcus faecalis, Gram-positive bacterium are the principle etiologic agents of prostatitis. Nonbacterial prostatitis is the name of diagnosis rendered when an etiologic agent is not detected. (Figure 7)
5.2. Benign Prostatic hyperplasia (BPH)

Benign prostatic hyperplasia is nonmalignant (noncancerous) enlargement of the prostate gland, a common occurrence in older men. In 1649, Riolan described for the first time the enlargement of the prostate and its most common clinical manifestation, the obstruction of the bladder outlet (Riolan et al., 1649). Since then, much research has been focused on the etiology and the clinical management of BPH. It is defined as a progressive hyperplasia of glandular and stromal tissues around the urethra (Rohr et al., 1980). BPH is an age-related disease and is present in 20% of 40 yr old men and in 70% of 60 yr old men (Isaacs 1994). Currently, there is no completely effective treatment for BPH. Men castrated before puberty do not develop BPH (Schroder 1994) and, in men with genetic disorders that inhibit androgen production or androgen action, prostatic growth is impaired (Bartsch et al., 2002). Thus, androgen is thought to play a permissive role in BPH and, although levels of DHT are not elevated in BPH (Krieg et al., 1979, Walsh et al., 1983). Medical therapies consist of α-adrenergic blockers, which lower adrenergic tone, and 5α-reductase inhibitors, which decrease levels of DHT (Bartsch et al., 2002). At present there is no completely effective treatment for BPH. The mainstay of therapies is the combination of 5α-reductase inhibitors, which regulate the levels of DHT, and alpha adrenergic-blockers, which decrease adrenergic tone. However, in some patients surgery, transurethral resection of the prostate (TURP), is the only effective intervention (Tiwari et al., 2005, Roehrborn 2005). Whether abnormal growth in BPH is due to embryonic reawakening (McNeal et al., 1978), stem cell defects (Lin et al., 2007), chronic inflammation (Kramer et al., 2006, Feder-Mengus et al., 2008), imbalance between androgen/estrogen signaling (Kozak et al., 1982), increased TGF-signaling (Huang et al., 2003), tissue remodeling in the aging prostate (Untergasser et al., 2005), chronic inflammation (Kramer et al., 2006), stem cell defects (Lin et al., 2007), over expression

Figure 8: Histological features of BPH (H&E staining) (adopted from http://www.pathologyoutlines.com/topic/prostatenodhyper.html). BPH lesions show numerous irregular shaped glands, each with a distinctive basal cell (black arrow). Note the uniform round nuclei without prominent nucleoli (green arrow).
of stromal and epithelial growth factors (Lucia et al., 2008), hypoxia (Berger et al., 2003), epithelial mesenchymal transition (Alonso-Magdalena et al., 2009), and other obscure factors or to other so far undefined factors, is an area of intense investigation. With aging, changes in the ratio of local concentrations of androgens and estrogens occur with a decrease in DHT levels, leading to an overall increase in the relative levels of estradiol (Shibata et al., 2000). In addition, estrogens can be produced locally in the prostate gland via conversion of testosterone to 17-estradiol by aromatase expressed within the stroma (Tsugaya 1996). With an increased estrogen : androgen ratio, epithelial cells secrete TGF-α pleiotropic factor that induces smooth muscle differentiation and increases the extracellular matrix (ECM) in the surrounding stromal cells (Huang and Lee 2003, Wu 2008).

A model of BPH in male rats can be produced by repeated injections of testosterone (Maggi et al., 1989). This model has been adapted for several studies (Scolnik et al., 1994, Liu et al., 2009, Rick et al., 2010). Because the mechanism of prostate growth is complex and heterogeneous in different species, and the testosterone-induced models of BPH show an epithelial hyperplasia (Scolnik et al., 1994), the androgen-induced models of BPH have limitations. Alonso Magdalena (2009) proposed that BPH is not a proliferative disease of the stroma but rather is an accumulation of mesenchymal-like cells derived from the prostatic epithelium and the endothelium. Alonso-Magdalena’s description of human BPH as predominantly of epithelial origin supports the use of a testosterone-induced model of BPH with predominant epithelial hyperplasia (Alonso-Magdalena et al., 2009).

A study done in 2004 identified certain risk factors for BPH and results have shown than Asian Americans have the lowest risk of clinical BPH. Alcohol and possibly cigarettes are related to a lower risk for BPH (Kang et al., 2004). Other epidemiological studies have indicated that several risk factors associated with cardiovascular diseases apply for BPH as well. These include obesity, hypertension and diabetes type II (Soygur et al., 1996, Hammarsten and Hogstedt 2001). (Figure 8)
5.3. Prostate Cancer

PCa is the most common male cancer in Western countries, including Europe, North America and parts of Africa (Cancer Facts & Figures 2012). Due to rapid westernization in dietary habits PCa has become more prevalent in India. PCa cells are apparent in prostate gland of almost half of all men over 50 yrs of age (Kelloff et al., 1992).

PCa has a natural course that is different from many other human tumors. Most early-stage PCa are latent and only approximately 25% of them will become aggressive and life-threatening (Cuzick et al., 2006). However, currently, it is difficult to differentiate between low- and high-risk localized PCa (Cuzick et al., 2006, Bangma et al., 2007). Following the application of the Prostate specific antigen (PSA) test for early detection of PCa, there is a big issue in managing these early stage cancers. It is a dilemma to treat early stage localized cancers. The current methods commonly used in the US and many other countries may over treat the majority of early stage PCa patients who will not develop metastases. However, conservative management, such as watchful waiting and active surveillance, which is used in certain European countries, may miss the opportunity to cure the small proportion of aggressive disease at an early stage (Bangma et al., 2007). Once aggressive cancer has progressed to the metastatic stage, strategy for metastatic PCa is androgen deprivation. ADT is the first step in treatment of metastatic disease by block testosterone driven proliferation of PCa cells or surgical castration. Recent studies have suggested that ADT do not cause a complete depletion of androgens (Scher & Sawyers 2005). In such conditions, even nanomolar concentrations of testosterone and DHT, most likely produced by adrenal glands or tumor itself, are sufficient for the transactivation of AR to support PCa cell proliferation (Scher & Sawyers 2005) furthermore ADT causes adverse effects like hypogonadism which leads to increase in body mass index (BMI), declines in lean body mass, muscle strength, bone density, sexual function and quality of life (Higano 2008). Although this treatment works efficiently in majority of patients, most cancers usually relapse after two years (Knudsen and Penning et al., 2010, Schrijvers et al., 2010). When the disease becomes androgen resistant, very limited options are left (Knudsen and Penning et al., 2010). Chemotherapy for PCa is generally unsuccessful, although recently new developments have been achieved.
(Schrijvers et al., 2010). Therefore, currently, advanced disease is still incurable and it is difficult to predict the progression of early stage cancer (Knudsen and Penning et al., 2010, Schrijvers et al., 2010). Hence it presents a unique challenge because of its long latency, high prevalence, screening complexity and significant mortality and morbidity making it an important target for chemoprevention (Kelloff et al., 1999). The conquest of PCa will require the integrated efforts in the area of chemoprevention, early diagnosis, and therapeutics.

5.3.1. Epidemiology of Prostate Cancer

Epidemiologic and laboratory evidence support the hypothesis that PCa risk is the result of combinatorial impacts of crucial environmental exposures, inherited susceptibility, and modifying influences of diet and lifestyle factors. In U.S., PCa is the most common cancer in men and is the second leading cause of cancer death in men. Only 3% of men with PCa die from the disease. It is estimated that 241,740 new cases of PCa will be diagnosed in 2012, while 28,170 patients will die from this disease. The estimated lifetime risk of being diagnosed with the disease is 17.6% for Caucasians, 20.6% for African Americans and 2.8% and 4.7% lifetime risk of death respectively. (Cancer Facts & Figures 2012)

5.3.2. Prostate Cancer Causes

The specific cause of PCa remains unknown. Hormonal, genetic, environmental, and dietary factors are thought to play roles. Yet, the only well-established risk factors for PCa are age, ethnicity, and heredity.

- **Age:** 80% of PCa are diagnosed in men older than 65 yrs of age. Autopsy records reveal that 70% of men older than 90 yrs of age have at least one region of cancer in their prostate.
- **Ethnic origin:** African American men are 1.6 times more likely than Caucasian men to develop PCa. They are also 2.4 times more likely to die from their disease as compared to Caucasian men of a similar age. Asian Americans, on the other hand, have a much lower chance of getting PCa as compared to Caucasians or African Americans.
Introduction & Review of Literature

Chapter I

Jamia Hamdard, Department of Medical Elementology and Toxicology

- **Family history:** Men who have a history of PCa in their family. If one first-degree relative has PCa, the risk is at least doubled. If two or more first-degree relatives are affected, the risk increases by 5-11 fold.

- **Diet:** Dietary factors may influence the risk of developing PCa. Specifically, total energy intake (as reflected by body mass index) and dietary fat have been incriminated. In addition, there is some evidence that suggests that obesity leads to an increased risk of having a more aggressive, larger PCa, which results in a poorer outcome after treatment.

- **Infection:** People who have had sexually transmitted infections are reported as having 1.4 times greater chance of developing the disease compared to the general population.

### 5.3.3. Symptoms

Most men with PCa have no symptoms. This is particularly true of early PCa. Symptoms usually appear when the tumor causes some degree of urinary blockage at the bladder neck or the urethra causing difficulty in urinating, erectile dysfunction, bone pain and/or fractures.

### 5.3.4. Prostate Cancer Screening

- **Prostate-specific antigen (PSA):** PSA is a 33 KDa glycoprotein produced by the prostatic epithelial cells and secreted into the seminal plasma (Lilja et al., 1993). PSA belongs to the family of serine proteases and member of the tissue kallikrein family (Yousef et al., 2001). Serum PSA is a clinically important marker used to monitor diagnosis, treatment response, prognosis and progression in patients with PCa (Lieberman et al., 2004). Determining the ratio of free/Total PSA in serum is the clinical usefulness of PSA testing in PCa screening (Catalona et al., 1998). In the pathogenesis of PCa, PSA has the capacity to cleave ECM glycoprotein such as fibronectin and laminin (Balk et al., 2003). The higher a man’s PSA level, the more likely it is that he has PCa. However, there are additional reasons for having an elevated PSA level, and some men who have PCa do not have elevated PSA. Another term, the PSA velocity (PSAV) refers to the serial evaluation of serum PSA concentration over time. This PSAV cutoff was mainly useful in patients with a PSA level between 4 and 10 ng/ml, yielding specificity greater than 90%. American Cancer Society, the American Urological Association, and
the National Comprehensive Cancer Network (NCCN) suggested screening guidelines PCa screening. (Figure 9)

- **Digital rectal exam (DRE):** DRE is part of a thorough regular health examination. A suspicious prostate exam prompts the physician to request for a prostate biopsy to confirm or rule out the presence of PCa. When the findings of the physical exam, DRE, and PSA level, suggest that a cancer might be present in the prostate, the diagnosis is succeeded by biopsy to confirm the presence of cancer.

- **Gleason score:** The Gleason grading system, based on prospective study of more than 4000 patients between 1960 and 1975, is the standard method of grading PCa throughout the world. The Gleason grading system is based on the degree of architectural differentiation. A primary pattern is assigned for the dominant grade and a secondary pattern for the non-dominant grade; the Gleason score is obtained by adding these two values. (Figure 10)

- **Urine test:** Prostate cancer antigen 3 (PCA3) is a gene was discovered in 1999 in PCa patients, on the basis of differential expression between cancer and noncancerous prostate tissues. PCA3 is new test that may help to discriminate between cancer-related versus nonspecific PSA elevations. There is not enough data to determine if PCA3 is useful for PCa screening, but it may help to determine the need for biopsy. Although the sensitivity of the PCA3 test was less than that of serum PSA, its specificity appeared to be much better, particularly in patients with a previous negative biopsy. Recent studies also have suggested that this test could be used to predict cancer prognosis. (Vlaeminck-Guillem et al., 2008)
Introduction & Review of Literature

Chapter I

Figure 9: Guidelines for Prostate Cancer screening

Figure 10: Histological grading of prostate Cancer by Gleason score

<table>
<thead>
<tr>
<th>Gleason’s Pattern</th>
<th>Gleason’s Score</th>
<th>Histologic Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td>2, 3, 4</td>
<td>I. Well-differentiated</td>
</tr>
<tr>
<td>3</td>
<td>5, 6</td>
<td>II. Moderately</td>
</tr>
<tr>
<td></td>
<td></td>
<td>differentiated</td>
</tr>
<tr>
<td>4, 5</td>
<td>7, 8, 9, 10</td>
<td>III. Poorly</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Differentiated</td>
</tr>
</tbody>
</table>

Grade 1 (G1): Well-differentiated
Slight anaplasia

Grade 2 (G2): Moderately differentiated
Moderate anaplasia

Grade 3-4 (G3-4): Poorly differentiated or
Undifferentiated anaplastic
Marked anaplasia
5.3.5. Prostate Cancer Treatment

Different treatment options are indicated depending on the severity of the disease. Treatment for localized PCa includes:

- **Active surveillance**: Active surveillance is an appropriate management for selected patients with localized disease. It is most often used for men who have very early-stage cancers and for men who are not candidates for surgery and other aggressive therapies.

- **Radical prostatectomy**: Radical prostatectomy is the surgical removal of the entire prostate. Radical prostatectomy is used to treat men with clinically localized PCa who have a life expectancy of at least 5 years.

- **Radiation therapy**: The goal of radiotherapy for men with localized PCa is to deliver enough radiation to the tumor while minimizing radiation to adjacent normal tissues. External beam radiation therapy and brachytherapy, both are used to treat PCa that has not spread outside the prostate. In terms of survival, radiotherapy appears to achieve similar results as those obtained with radical prostatectomy. Brachytherapy (internal or implanted radiation) is a variation of radiation therapy in which a small radioactive pellet is implanted into the prostate. This provides radiation to a smaller area than external-beam radiation and minimizes exposure of surrounding normal tissue.

- **Androgen deprivation therapy**: This therapy is likely to be used in cases where cancer has spread to distant regions. Therefore, currently it is not among the standard options for men with localized prostate disease.

- **Surgical removal of both testicles** (Castration/Orchiectomy) is the best way to stop hormonal stimulation of the tumor. Men usually prefer medical castration to surgical castration. A variety of agents have been used to suppress androgen levels acting at different levels of hormonal production and release.

- **Gonadotropin releasing hormone (GnRH) agonists** are the most widely used drugs. They induce medical castration by suppressing luteinizing hormone production and, therefore, the synthesis of testicular androgens e.g. leuprolide, goserelin, buserelin, and triptorelin. GnRH antagonists (degarelix) may be beneficial in cases when immediate decrease in testosterone levels is required.
Antiandrogen monotherapy: In this only single drug is given. Antiandrogens bind to androgen receptors and competitively inhibit their interaction with male hormones (testosterone and dihydrotestosterone). eg: Cyproterone and Flutamide. Cyproterone is an aromatic steroidal antiandrogen. Flutamide is a nonsteroidal anti-androgen. We have used both these compounds in our study.

Medical castration: Medical castration does not decrease luteinizing hormone (LH) levels and androgen production. Rather, testosterone levels remain normal or increased. Thus, men treated with antiandrogen monotherapy do not have the full spectrum of side effects attributable to low levels of testosterone, and many maintain some degree of potency. These agents are usually used in combination with a GnRH agonist either continuously or for 2 to 4 weeks during the initiation of treatment with a GnRH agonist. This is also known as "complete androgen blockade." The most common agents are flutamide (Eulexin) and bicalutamide (Casodex).

Chemotherapy: The utility of chemotherapy in the management of metastatic prostate and hormone resistant cancer has not been thoroughly defined. Newer chemotherapy medicines, such as docetaxel (Taxotere), have shown some promise in prolonging the survival of some patients with extensive PCa.

Side effects: The side effects of these medications vary; orchiectomy and luteinizing hormone-releasing hormone (LHRH) agonists may cause impotence, hot flashes, and loss of sexual desire, osteoporosis, and bone fractures. Antiandrogens may cause nausea, vomiting, diarrhea, and breast enlargement or tenderness. Any of these therapies can weaken bones.

6. Mechanism of benign prostate hyperplasia and prostate cancer

6.1. Role of androgen receptor in benign prostate hyperplasia and prostate cancer

BPH: AR regulates the expression of genes necessary for the growth and development of both normal and malignant prostate tissue. DHT stimulates glandular epithelium growth in the prostate and it is the major cause of rapid prostate enlargement that occurs
between puberty and young adulthood. A study in 1974 observed that men deficient in 5α-reductase had hypoplastic prostates (Walsh et al., 1974) and the relative success of Finasteride, a 5α-reductase type II blocker, in retarding prostatic growth by reducing DHT production both substantiate the role of DHT in BPH. It is well documented that as men age, their testosterone levels decline. Some researchers have indicated that despite an overall decline in testosterone levels, the prostate is still able to synthesise similar quantities of DHT. It is therefore hypothesized that the changes in the equilibrium between testosterone and DHT may lead to an increase in prostatic growth (Lee 2004).

**PCa:** Since the prostate is an androgen-dependent organ, it is rational to presume that prostate malignancy develops under abnormal androgen signaling. This hypothesis is, to some extent, supported by observations that eunuchs do not develop PCa and that a higher incidence of PCa is found in men who used androgens as anabolic agents or therapeutics (Taplin et al., 2001). Although patients show positive response initially to ADT, continuous treatment often results in PCa progressing to androgen independent states within 18-24 months (Feldman and Feldman 2001). There are several postulated theories explaining this development of resistance. Some of which, involve the AR or the development of alternative signaling pathways that bypass the function of AR (Chen et al., 2004). Somatic mutations of AR often bestow the receptor with hypersensitivity and promiscuous usage of ligands. The mutated receptor could be trans-activated by lower concentrations of androgens, by anti-androgens, and by non-androgenic ligands (Fenton et al., 1997). About 50% of the mutations reported in ligand binding domain have been found to be associated with androgen independent PCa. The replacement of threonine 877 with alanine in AR LBD domain (T887A), which is found in LNCaP cells, allows it to be activated by other steroids and even by anti-androgens (Veldscholte et al., 1994). In addition, the mutation changed codon 726 in exon E from arginine to leucine (R726L) AR mutant is known to be activated by estradiol. PCa may consist of clones with a range of different types of AR mutations (Elo et al., 1995). AR amplification is rarely found in androgen sensitive cancer but is common in recurrent therapy-resistant cancer.
AR amplification emerges during androgen deprivation therapy by facilitating tumour cell growth in low androgen concentrations (Koivisto and Helin 1999, Visakorpi et al., 1995).

6.2. Role of estrogen in benign prostate hyperplasia and prostate cancer

➢ **BPH:** As men age, the intraprostatic estradiol concentration increases or remains constant while the androgen concentration decreases. There is a strong correlation between the increasing estradiol : DHT ratio and stromal hypertrophy (Koch 2001). Takase (2006) have detected estrogen receptors and enzymes involved in estrogen metabolism in human prostate (Takase et al., 2006). Although the role and mechanism of estrogens in the prostate is still unclear, there is growing evidence that estrogen could modify prostate growth and differentiation. An estrogen dominant environment is speculated to increase the production of AR and thus encouraging prostatic growth by sensitizing the prostate to androgen (Mobbs et al., 1983). The precise roles of estrogen receptor (ER) [α and β] in the pathogenesis of BPH are not fully understood. It is likely that the two ER subtypes mediate diverse functions through interactions with different ligands, changes in the balance of classical versus non-classical signaling, and interactions with different co-repressors and co-activators (Ho 2004).

➢ **PCa:** Evidence that estrogens are involved in the genesis and progression of PCa came mainly from experiments with organ cultures of normal rat, human prostate or human PCa samples. In these studies, estrogens were found to stimulate DNA synthesis and induce metaplastic epithelial morphology in both human (Nevalainen et al., 1993) and rat prostate (Nevalainen et al., 1991). High doses of testosterone given together with estradiol, but not alone, stimulated carcinogenesis in adult male rats (Prins 1992). Aromatase knockout mice, which are estrogen deficient, did not develop PCa (McPherson et al., 2001). Epidemiological data also showed that men with high serum levels of estrogens have a greater risk of PCa (Hill et al., 1984, Rohrmann et al., 2007).
On the other hand, dietary estrogens, which include phytoestrogens, lignans and flavonoids, have been promoted to reduce and prevent prostate diseases (Morton et al., 1999, Griffiths et al., 1998). Epidemiological studies have suggested a link between increased consumption of phytoestrogens to a lower incidence of PCa. This is particularly true when comparing men living in Asia with men in the West, where dietary estrogens intake is lower and PCa incidence is higher (Morton et al., 1997). These conflicting data indicate the diverse roles of estrogens.

These differing actions of estrogens are mediated by two ER-α and ER-β. ER-α is associated with aberrant proliferation, inflammation and the development of malignancy, whereas ER-β is associated with anti-proliferation, differentiation and apoptosis (Ellem and Risbridger 2007). Estrogens induce proliferation as mentioned earlier and the multilayering of the prostatic epithelial cells. In general, ER-α stimulation in the prostate results in hyperplasia, inflammation and dysplasia (Ellem and Risbridger 2009). Induction of squamous metaplasia of the prostate depends on ER-α, not ER-β (Cunha et al., 2001). In general, ER-β inhibits proliferation in the prostate. ER-β knockout (ERβKO) mice can develop prostatic hyperplasia with age, which is not observed in wild-type or ER-α knockout mice (Krege et al., 1998). The prostatic hyperplasia observed in some strains of ERβKO mice is generally attributed to the unopposed action of ER-α (Sugiyama et al., 2010).

6.3. **Role of apoptosis in benign prostate hyperplasia and prostate cancer**

- **BPH**: The interactions of androgens with the prostate epithelium, stroma, and other hormones growth factors form a complex system, which regulates prostate growth. In normal tissues, homeostasis is maintained by a balance between cell proliferation and apoptosis. Apoptosis, also known as programmed cell death, is a regulated process, consisting of a series of molecular events that lead to cell death. BPH may result from an over activity of cell proliferative processes induced by hormones or from a reduced rate of apoptosis. Several in vitro studies suggested that a reduction of apoptosis might occur in BPH. Kyprianou (1996) reported that basal and luminal epithelial cells in BPH over
express Bcl-2, compared to healthy prostate tissue (Kyprianou et al., 1996). The enhanced expression of Bcl-2 involves the deregulation of the normal apoptotic cell death mechanisms in the human prostate, resulting in a growth imbalance in favor of cell proliferation, which might ultimately promote prostatic hyperplasia. Similar data were proposed by Colombel (1998) who found that Bcl-2 was strongly expressed in the basal cells within mature glandular nodules and in most cells of young small nodules (Colombel et al., 1998).

**PCa**: The intrinsic pathway of caspase activation can be initiated in response to DNA damage, growth factor withdrawal, loss of contact with the ECM, or glucocorticoids. These conditions promote signaling pathways that lead to a loss in the integrity of the mitochondrial membrane and caspase activation. The Bcl-2 family comprises a group of structurally related proteins that play a fundamental role in the regulation of the intrinsic pathway by controlling mitochondrial membrane permeability and the release of the pro-apoptotic factor, cytochrome c. The balance between pro- and anti-apoptotic family members determines whether or not a cell will undergo apoptosis. Bcl-2 and Bcl-xL inhibit apoptosis by binding to the pro-apoptotic BAX and BAK proteins. In healthy cells, BAD is phosphorylated and sequestered in the cytoplasm by the adapter protein, when cytoplasmic levels of free BAD increase, BCL-2 and BCL-xL bind to BAD and release BAX and BAK. BAX and BAK, or processed forms of these proteins, can then insert into the mitochondrial membrane and cause the release of cytochrome c. Cleavage of BID as a result of caspase-8, or caspase-10 activation following death receptor ligation also promotes BAX/BAK-mediated release of cytochrome c.

Bcl-2 is an NF-kB regulated gene that functions by blocking the apoptosis pathway, thus immortalizing cancer cells. Bcl-2 over expression results in the up regulation of VEGF expression with increased neoangiogenesis in human cancer xenografts. Bcl-2 is an anti-apoptotic protein, and it's over expression has been associated with resistance to androgen deprivation and poor outcome in some PCa patients treated with radiotherapy (Khor et al., 2007). Furthermore, Zhou (2007) have recently shown that prostatic epithelium-specific deficiency for TP53 and Rb tumour suppressors, which are pro-
apoptotic proteins, leads to metastatic cancer in mice (Zhou et al., 2007). The phosphorylated Akt (p-Akt), a phosphoinositide-3-OH-kinase (PI3K) activated protein kinase, is highly expressed in prostate tumors. p-Akt can indirectly hinder p53-dependent growth suppression and apoptosis by phosphorylating MDM2. (Hanet al., 2009). MDM2 family proteins are crucial regulators of the Oncosuppressor gene p53. Alterations of their gene status, mainly amplification events, have been frequently observed in human tumors (Mancini et al., 2009). In response to stress, cells activate a complex pathway involving tumor suppressor p53 that is responsible for cell cycle arrest, DNA repair, and apoptosis as protection from the deleterious effects of mutation (Pietsch et al., 2006). MDM2 is a key negative regulator of tumor suppressor of p53, by targeting p53 for proteasomal degradation (Haupt et al., 1997, Kubbutat et al., 1997, Bond et al., 2005). MDM2 overexpression was significantly associated with advanced stage PCa (Leite et al., 2001, Khor et al., 2005). Recent studies have also shown that inhibiting MDM2 expression enhances the effects of radiation and chemotherapy on PCa cells (Bianco et al., 2004, Zhang et al., 2003).

6.4. Role of inflammation benign prostate hyperplasia and prostate cancer

- **BPH:** Prostatic inflammation is very common in BPH patients. Histological studies of BPH tissues have detected inflammatory cell infiltrates of varying densities in 30%-50% of the cases (Nickel et al., 1999). Inflammatory infiltrate such as macrophages and lymphocytes are known to produce growth factors such as bFGF, cytokines IL-1 and IL-6. In situ studies have indicated that there is an elevated expression of pro-inflammatory cytokines in BPH. It is speculated that IL-6, IL-8, IL-17 may perpetuate chronic immune response and induce fibromuscular growth by an autocrine or paracrine loop or via induction of COX-2 expression (Kramer et al., 2006). COX-2 is a major enzyme that converts arachidonic acid to prostaglandins. Prostaglandins have various roles in mediating and moderating inflammation and are associated with the progression of BPH (Kramer et al., 2006). Moreover, aromatase gene (CYP19) is regulated by a promoter (PII), which is responsive to inflammatory cytokines (Irahara et al., 2006). An increase in
aromatase expression increases local estrogen levels that may lead to an increase in prostatic proliferation. A recent study has indicated that consumption of non-steroidal anti-inflammatory drugs (NSAIDs) is linked with lower risk of developing BPH and LUTS (St. Sauver et al., 2006). It is unclear if inflammation is the cause or result of BPH but its involvement indicates that anti-inflammatory drugs may help to retard development and worsening of the disease.

- **PCa:** Cytokines are released in response to a diverse of cellular stresses including carcinogen-induced injury, infection and inflammation (Dranoff, 2004). Chronic or recurrent inflammation is responsible for the development of many human cancers including PCa (De Marzo et al., 2004). Epithelial cells have the capacity to secrete a wide range of cytokines which can regulate cell growth and immune or inflammatory responses. The prostate epithelial cells have also shown to produce proinflammatory cytokine in androgen-dependant and androgen-independent prostate cells and influence the growth and differentiation of normal and prostate cancer cells (Ricote et al., 2004). Signaling pathways activated by cytokines IL-6 has been shown to activate the AR and stimulate PSA expression (Lee et al., 2003). Elevated serum levels of pro-inflammatory cytokines in association with increased PSA have been previously described in PCa and PCa cell lines (Mizokami et al., 2000). Higher Gleason score correlated with high levels of conditioned medium derived IL-6. Moreover, cell signaling analysis of periprostatic adipose tissue identified activated signaling molecules, including STAT3 that correlated with Gleason score. Since STAT3 is IL-6 regulated, these findings suggest that periprostatic adipose tissue may have a role in modulating PCa aggressiveness by serving as a source of IL-6. High circulating levels of leptin, IL-6, and VEGF are associated with increased prostate cancer risk and increased aggressiveness. (Mistry et al., 2007). Recent evidence suggests that androgens, leptin, IL-6, VEGF, insulin and IGF-1 may play a role in prostate cancer progression, while adiponectin and IGFBP-3 may act as "anti-PCa" adipokines. (Lopez et al., 2004). One mechanism of suppression of cytokine signaling is through inhibition NF-kB. (Pan et al., 2002). COX-2 has been reported to be constitutively over expressed in a variety of malignancies and is frequently constitutively
elevated in prostate carcinoma. Several studies have suggested that COX-2 is commonly over expressed in PCa (Katkoori et al., 2012, Shao et al., 2012, Yang et al., 2012). The combination therapy of COX-2 and 5-LOX inhibitor, and PPAR-γ ligand may be a benefical new treatment of human PCa. (Matsuyama and Yoshimura 2008).

6.5. Proliferation Ki-67 in benign prostate hyperplasia and prostate cancer

- **BPH:** Benign prostatic hyperplasia is associated with the proliferation of prostate tissue. Kusljic (2010) showed an increase in Ki-67-positive nuclei in the stromal and epithelial cells of the ventral prostatic lobes in estrogen-treated rats (Kusljic et al., 2010). The expression of Ki-67 and Bcl-2 was significantly higher in prostatitis when compared with BPH patients and down-regulate the expression of caspase-3 in BPH patients (Wang et al., 2008). Shariat showed that Survivin and Bcl-2 expression increased incrementally from normal prostate to epithelial BPH to stromal BPH. Caspase-3 expression was higher in BPH epithelium than in BPH stroma, which in turn was higher than that in normal prostate. Ki-67 was significantly over expressed in BPH stroma and epithelium (Shariat et al., 2005).

- **PCa:** The expression of Ki-67 is associated with cell proliferation. Ki-67 was up-regulated in PCa and PIN and was associated with Gleason grades (Mirtti et al., 2001). High Ki-67 expression was a predictor of poor prognosis after radical prostatectomy (Halvorsen et al., 2001). The expression of the human protein Ki-67 is associated with cell proliferation. During interphase, the antigen can only be detected within the nucleus, whereas in mitosis, the majority of the protein is translocated to the surface of the chromosomes. The fact that Ki-67 protein is present during all the active phases of the cell cycle (G1, S, G2 and mitosis), and absent from the G0 phase, has made it an excellent marker for determining the growth fraction of a determined cell population (normal or tumoral) (Scholzen and Gerdes 2000). Furthermore, the fraction of tumor cells positive for Ki-67 (labeling index) has been correlated with the clinical course,
recurrences and survival in PCa (Aaltomaa et al., 1997, Borre et al., 1998, Bettencourt et al., 1996; Bubendorf et al., 1996, Moul et al., 1996, Keshgegian et al., 1998, Bai et al., 1999, Brown et al., 1996, Cher et al., 1996, Hepburn et al., 1995, Stapleton et al., 1998), suggesting that Ki-67 can be used as a prognostic factor in the monitoring of these patients.

7. Models of prostate cancer

Given the fact that cancer development can be roughly divided into three stages of initiation, promotion and progression, control over this disease may only be achieved if molecular changes underlying each stage can be well-characterized and recapitulated in animal models of carcinogenesis. Hence animal models are critically important and play a central role in expediting the development of new chemopreventive approaches and therapies for cancer. Several new animal models of PCa are being developed that have unique characteristics which may be relevant to specific aspects of the carcinogenic process or to subtypes of PCa exhibiting specific molecular defects and/or biologic properties (Lucia et al., 1998, Bosland 1999, Bosland 1992).

The Lobund-Wistar rat is a very well-characterized model of PCa (Pollard 1998). Derived from the 57th generation of inbred germ-free Wistar rats, the Lobund-Wistar strain develops large tumors in the dorsolateral and anterior lobes of the prostate, as well as the seminal vesicles. The Fischer F344 rat model is another chemically induced PCa model. It is developed by administration of combination of 3, 20-dimethyl-4-aminobiphenyl and testosterone propionate, Fischer F344 rats commonly develop noninvasive cancers in the dorsolateral and anterior lobes of the prostate, as well as seminal vesicles (Shirai et al., 1993). Although distant site metastases have been observed in this model, they are rare and mostly confined to the abdominal cavity. Fischer rats have historically been used to study induction of PCa by carcinogens. Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP) is a genetically modified mouse model that is commonly used to study PCa chemoprevention and has received considerable attention over several past years. (Greenberg et al., 1994, Greenberg et al., 1995). These mutant mice represent an excellent model to study prostate cancer initiation.
It has been difficult to study the natural history of PCa at the molecular level and rapidly develop effective treatment strategies, in part, because there are so few animal models that accurately mimic the heterogeneity, androgen independent (AI) growth and metastatic spread of clinical disease. To circumvent this problem, researchers have historically exploited immortalized human PCa cell lines, such as LNCaP (androgen dependent) and DU-145, PC-3 (androgen independent), to elucidate the biology of tumor progression, AI disease, and metastasis. (Culig et al., 1994, Murillo et al., 2001, Wu HC et al., 1994, Jungwirth et al., 1997) However, clonal cell lines in culture limit our ability to study the dynamic interactions between the various cellular compartments that comprise the intact prostate gland, such as the stromal, endothelial and luminal basal and neuroendocrine (NE) epithelial cells (Poste and Fidler 1980). We performed our studies on chemically induced two stage prostate cancer model using NMU-testosterone.

7.1. Chemically induced two-stage prostate carcinogenesis

N-methyl-N-nitrosourea (NMU)–androgen-induced rat model of PCa developed by Bosland (Bosland 1999, Bosland 1992) and used in several recent chemoprevention studies (Liao et al., 2002, Rao et al., 1999). Whereas many rodent PCa models result in cancers that predominantly affect the seminal vesicle and ventral prostate (Pollard 1998, Tamano et al., 1996), the NMU–androgen-induced model causes tumors of the dorsolateral and anterior prostate (McCormick et al., 1998). These lobes of the rat prostate are generally considered homologous to the areas of the human prostate that are most susceptible to cancer (Lucia et al., 1998, Bosland 1999, Bosland 1992, McCormick et al., 1998). NMU–androgen-induced rat model is an anatomically and physiologically relevant system for the preclinical evaluation of substances that are hypothesized to inhibit or enhance human prostate carcinogenesis (McCormick et al., 1998, McCormick et al., 1999, Rao et al., 1999). In this study, we assessed the individual and interactive effects of precisely controlled dietary interventions on the survival of rats treated with NMU androgens to stimulate prostate carcinogenesis.

A well-known phenomenon in chemical carcinogenesis is the enhancing effect of cell proliferation in the target tissue during treatment with carcinogens leading to fixation of promutagenic DNA lesions (Craddock 1975, Snow et al., 1984). In the rodent ventral prostate,
but also in other accessory sex glands in males, cell proliferation can be induced by daily administration of androgens to animals that are castrated 7-30 days previously (Coffey et al., 1968, Tuohimaa et al., 1980). In this system, the rate of cell proliferation usually reaches a peak on the fourth day of androgen administration (Coffey et al., 1968). We applied temporary chemical castration (cyproterone propionate) rather than surgical castration and gave a single carcinogen administration after three daily of testosterone injections following cessation of the chemical castration. This ensured an intact status of the animals during the promotion and progression stages of prostatic carcinogenesis. In addition, the effects of this treatment on rats that were prepubertal at the start of the CA treatment and that had juvenile prostates were compared with those on young adult rats with actively secreting prostates. The carcinogens arbitrarily selected for this study are known pluripotent carcinogens in rats, belonging to three categories of chemical carcinogens: MNU, DMBA, and DMABP. DMABP has also been claimed to induce in situ adenocarcinomas of the ventral prostate in F344 rats (Katayama et al., 1982).

We have followed Liao (2002) with slight modifications to study early dietary interventions in initial PCa. Wistar rats were chemically castrated by used of androgen blocker cyproterone acetate followed by a high dose of testosterone propionate. Immediately after high dose of testosterone propionate a single dose of NMU was given. Here in this model NMU acts as cancer initiator, a single dose of NMU causes initiation of the proliferating cells (secretory cells) caused by a high dose of testosterone propionate. Later on Testosterone propionate i.p injections for a long duration act as promoter leading to cancer development.

8. Chemoprevention of prostate cancer

Cancer chemoprevention is defined as the use of natural or synthetic agents to prevent, inhibit or reverse the carcinogenesis process before malignancy. Dr. Michael Sporn is widely credited with launching the modern era of cancer chemoprevention research (Sporn et al., 1976). In 1976, he first pointed out that the target of clinical efforts should be the process of carcinogenesis rather than the state of cancer. He advocated the treatment of precancerous conditions and
coined the term “chemoprevention”. Chemoprevention may be conducted at variety of time points in this process to reduce occurrence of *in situ* or *invasive cancers* (primary intervention at earlier stages in the process) or cancer morbidity and/or mortality (secondary intervention at later stages in the process).

The development of cancer occurs over years and involves multiple genetic and phenotypic alterations that lead to invasive cancer. Chemoprevention is based on the fact that intervention is possible during the development and progression of pre-cancerous cells through use of non-cytotoxic nutrients and/or pharmacological agents during the time period between tumor initiation and malignancy (Pezzuto 1997).

There are three sequential levels of disease prevention depending on whether the interventionist addressed to healthy individuals and there is prevention of occurrence of the disease (primary prevention) or patients in preclinical or premalignant stage (secondary prevention). At later stage, attempts can be made to prevent local recurrences as well as invasion and metastasis of malignant cells (tertiary prevention). In particular, primary prevention means preventing the occurrence of diseases. Secondary prevention means early detection and intervention, preferably before the condition is clinically apparent and has the aim of reversing, halting or at least retarding the progress of a condition. Tertiary prevention means minimizing the effect of diseases by preventing complications and premature deteriorations. Chemoprevention of cancer is a young discipline that is progressively emerging from its pioneer stage. It is reassuring that many drugs and food constitutes, some of which are widely consumed by the population, potentially possess cancer preventive mechanisms and are effective in preclinical models. Chemoprevention aims to directly modulate specific steps in the carcinogenic process, block mutagenic carcinogens, prevent DNA damage by free radicals, suppress epithelial cell hyperproliferatin, and/or modulate epithelial cell differentiation and apoptosis. An increasing number of nutrient and noncurrent compounds present in fruits, vegetables and cereal grains have been found to interfere with the process of cancer development in laboratory research (Sultana et al. 2000, Block 1992, Bresnick et al., 1990). Epidemiologists have found that population that consume large quantities of plant-derived foods have lower incidence rates of various types of cancer.
8.1. Classes of chemopreventive agents

Cancer is a multistep process that occurs over an extended time frame, therefore, there are number of possible stage at which the process could be halted, slowed downer even reversed. Wattenberg (1985) developed a scheme to classify the chemopreventive agents. According to it, there are three major types of chemopreventive agents.

i. Metabolic inhibitors

This includes a group of compounds that prevent the formation of carcinogens from precursors. The best-studied inhibitors in this category include ascorbic acid, caffeic acid, ferulic acid, gallic acid, N-acetylcysteine, proline, and thioproline (Stoner et al., 1997). Although these compounds were suggested to “act predominantly to prevent the formation of nitrosamines from secondary amines and nitrite in an acidic environment”, their ability to prevent the formation of heterocyclic amines is now being recognized. Thus, antioxidants (catechins, flavonoids, caffeic acid) and organosulphur compounds (diallyl sulphide and dipropyl disulphide), have been reported to limit heterocyclic amine formation under various conditions (Kucuk 2002, Mehta et al., 2010).

ii. Blocking agents

These are typically those compounds that can inhibit initiation either by inhibiting the formation of carcinogens from precursor molecules or reactive metabolites from the parent carcinogens, or by preventing the ultimate electrophilic and carcinogenic species from interacting with critical cellular target molecules, such as DNA, RNA, and proteins (Wattenberg 1985). Initiation of carcinogenesis, involving damage to the DNA can be prevented or reduced by blocking agents that are particularly effective if administered before the carcinogen. Blocking mechanisms include alterations to the profile of both phase I and II drug metabolizing enzymes altered rates of DNA repair and scavenging of reactive oxygen and other free radical species (Manson et al., 2000). Even if DNA has been damaged, they can still be effective at limiting further adduct formation. They prevent carcinogens from modifying DNA and causing mutations. This is usually achieved by increasing the expression of detoxification and antioxidant enzymes in target
tissues, though alterations in the pharmacokinetics of xenobiotics may also serve to protect against tumorigenesis (Wattenberg 1985). Such responses are thought to represent a form of cellular adaptation to chemical and oxidative stress. They prevent cancer-producing compounds from reaching or reacting with critical target sites in the tissues. They prevent carcinogens activation, enhance detoxification of carcinogenic agents, or trap cancer-producing compounds before they reach or react with target sites in tissues (Greenwald et al., 2002, Wattenberg 1985).

Example of blocking agents includes: Ellagic acid, caffeic acid, ferulic acid, p-hydroxyl Cinnamic acid, indole-3-acetonitrile, indole-3-carbinol, benzyl isothiocyanates, phenyl isothiocyanates, couramin, quercetin and B-carotene.

iii. Suppressing agents

These agents act in either promotion or progression stage of the carcinogenic process to inhibit malignant expression of initiated cells. Because carcinogenesis is a multistep process that often progresses slowly in the early stages and, hence, there is great potential for suppressing its development. Suppressing agents can be classified as compounds that inhibit polyamine metabolism; induce terminal cell differentiation; modulate signal transduction; modulate hormonal/growth factor activity; inhibit oncogene activity; promote intercellular communication; restore immune response; induce apoptosis; correct DNA methylation imbalances; inhibit basement membrane degradation; and inhibit arachidonic acid metabolism (Hail et al. 2008, Stoner et al., 1997). They reduce the consequences of altered gene expression by reducing proliferation of initiated cells or restoring apoptosis to normal levels, thus preventing the accumulation of damaged cells (Surh 2003, Manson et al., 2000, Wattenberg 1985).

Suppressing agents prevent the evolution of the neoplastic process in cells already altered by carcinogenic stimuli (Wattenberg 1985) Some act by producing differentiation; others specifically counteract the consequences of genotoxic events, in particular, oncogene activation, and still other specifically counteract the consequences of genotoxic events, in particular, oncogene activation, and still others selectively inhibit the proliferation of neoplastic cells (Moon et al., 1992, Reddy et al., 1993). Post-initiation events generally are less well understood than those
that occur during the initiation phase, and for this reason the classification of suppressing agents is more difficult. Suppressing agents alter signaling pathways that control apoptosis or cell proliferation. They also protect during the initiation phase of heterocyclic amine-induced carcinogenesis. These include various polyphenols, retinoids, carotenoids, and vitamins (Wattenberg 1993). Example of Suppressing agents includes: Soya bean protease inhibitor, benzyl-isothiocyanate, B-sitosterol, caffeine, fumaric acid and selenium.

Indole-3-carbinol, a breakdown product of glucobrassicin vegetables, and curcumin, a major component of the spice turmeric, exhibit both blocking and suppressing mechanisms of action (Chinni et al., 2001, Hudson et al., 2003).

8.2. Types of prevention

![Diagram of chemoprevention]

Figure 11: Schematic diagram of chemoprevention
- **Primary prevention:** Prevention has come to light as one approach to reduce the public health impact of PCa various types of prevention approaches may be applied in PCa including both primary and secondary prevention methods. Primary prevention (preventing the disease before it occurs), although the desired preventive strategy for chronic diseases with clearly defined and modifiable risk factors, such as lung cancer, is a less suitable approach in PCa where the etiology is multi-factorial and risk factors are less well defined. Further research into the mechanism of initiation of prostate carcinogenesis is required in order to identify the specific molecular events and risk factors involved in PCa transformation before primary prevention approaches can be appropriately applied.

- **Secondary prevention:** The secondary prevention refers to interventions that prevent or minimize the progression of a disease at an early stage, thereby limiting disability once the disease is diagnosed. Therefore, while the underlying prevalence of the disease may not be altered by secondary prevention measures, progression to clinically apparent disease may be reduced. The utility of this approach in PCa is supported by epidemiologic observations. Studies of ‘latent’ PCa, that is cancer detected at post-mortem examination, have shown that the incidence of small latent cancers does not vary with geographic region (Breslow et al., 1977, Shiraishi et al., 1994, Yatani et al., 1988) (Figure 11). In contrast, population studies of worldwide PCa incidence and mortality rates suggest that certain regions impart a favorable risk to the development of PCa. The incidence in China and Japan, for example is 80 times less than the incidence in high risk regions such as North America and Northern Europe (Parkin et al., 2005). While differences in incidence have to be interpreted with caution since the widespread use of PCa screening in North America undeniably accounts for the dramatically higher incidence, other indices such as mortality rate demonstrate the same overall pattern. The PCa mortality rate, which is a more accurate reflection of PCa risk, is 15 times lower in China and Japan compared to North America (Parkin et al., 2005), although under-reporting in Asian countries could be a confounding factor in this difference. Since we know from autopsy studies that the prevalence of PCa is the same worldwide,
the observation of reduced PCa mortality in low risk countries implies that a secondary prevention type of effect appears to be occurring, such that select environmental pressures appear to be affecting progression of the disease into clinically aggressive cancer.

Certain biological features of PCa further define it as a suitable target for secondary prevention. PCa is a slowly progressing disease that arises decades prior to diagnosis. The time between the onset of microscopic evidence of PCa and clinically perceptible disease is believed to be 20 years or more in many cases. Autopsy studies have shown that 29% of men in their thirties harbor microfoci of latent PCa (Sakr et al., 1995), however, PCa is rarely clinically apparent before the age of 40 and incidence reaches a peak between 50-70 yrs. The gradual progression of PCa over many years provides an ideal window of opportunity for lifestyle modifications or for the use of chemopreventive agents (Klein et al., 2005, Neill and Fleshner 2006).

Prevention of PCa has become increasingly attractive owing to the significant limitations of current management options once PCa has clinically progressed. Prevention is particularly favorable since most PCa deaths occur after the sixth decade, so that even modest delay in the natural progression of the disease could impact significantly on mortality (Thompson 2007). Epidemiologic evidence suggests that although the underlying rates of initiation of PCa in high and low risk populations are the same, clinical progression of the disease is much greater in high risk populations, and this may in large part be attributable to environmental factors. These observations support the rationale for secondary prevention of PCa by demonstrating that under favorable conditions, PCa progression can be inhibited or perhaps arrested. PCa is a disease that gradually progresses over 20-30 years, and in this regard is ideally suited to prevention strategies with lifestyle modification or targeted chemical agents. Finasteride was effective at reducing PCa prevalence; the side effects of 5-alpha reductase inhibitors and lingering uncertainty about the increase of grade associated with finasteride administration highlight the necessity for alternative chemoprevention agents.
9. Flavonoids in prostate cancer prevention

Flavonoids are a special class of polyphenolic plant secondary metabolites. Each group of flavonoids possesses unique chemical properties and has a particular distribution in plants. Anthocyanins (glycosylated anthocyanidins) and proanthocyanidins (polymers that produce anthocyanidins when hydrolyzed) primarily provide color to flowering plants and fruit, and are therefore found in high concentrations in the skin of red grapes, red wine and berries. Flavan-3-ols, such as catechin, epicatechin gallate are colorless and are found in high concentrations in green tea. Isoflavones are only found in legumes (e.g. soy) and are therefore consumed in high quantities in regions of the world with high soy consumption (Beecher et al., 2003, Harnly et al., 2006).

9.1. The epidemiology of flavonoids in relation to prostate cancer

A substantial epidemiologic literature supports the beneficial health effects of flavonoid consumption. The main areas of interest have been the anticancer and cardioprotective effects of flavonoids. PCa has gained special attention in this regard owing to observations of dramatic differences in mortality of PCa between populations consuming high (China) and low (North America/Europe) levels of flavonoids. Epidemiological studies, including several case control and cohort studies have broadly supported this hypothesis. The chemopreventive effect of flavonoids in PCa has been further supported by a number of large prospective cohort studies that have demonstrated an inverse association of cancer risk with flavonoid intake. In these studies, soy, isoflavone and green tea consumption was significantly correlated to lower PCa risk (Kurahashi et al., 2008, Kurahashi et al., 2007, Jacobsen et al., 1998).

A number of studies have examined the association of non-flavonoid phytoestrogens such as lignans (e.g. enterolactone) with PCa risk and have generally highlighted a protective effect of lignans. Since lignans are found in high levels in soy, positive epidemiologic associations of the soy flavonoids (genistein and daidzein) with cancer may indeed be a reflection of the confounding effect of lignans, or other as yet unidentified phytochemicals (Hedelin et al., 2006, Piller et al., 2006).
9.2. The mechanisms of action of flavonoids

i. Flavonoids as antioxidants

Reactive oxygen species (ROS) are highly reactive molecules with both physiologic and pathologic roles. ROS can occur in the form of molecules with highly reactive unpaired electrons known as free radicals (e.g. superoxide, O$_2^-$), or as non-radicals that are highly liable to form free radicals (e.g. hydrogen peroxide, H$_2$O$_2$). They can exist in the body as a result of deliberate synthesis (e.g. production by macrophages for bacterial killing), or as a result of accidental production by metabolic processes such cellular respiration in mitochondria, or via exogenous insults such as smoking (Halliwell et al., 1994). ROS are highly damaging as they can attack lipids in cell membranes, proteins, carbohydrates and DNA. The resulting oxidative damage may play a role in aging and chronic and degenerative diseases including cancer (Muller et al., 2007, Valko et al., 2004, Nagy 2001).

The human body relies on both endogenous as well as exogenous (dietary) anti-oxidant systems to buffer the effect of the ROS constantly produced by metabolic processes. Despite these many levels of protection against ROS damage, endogenous antioxidant systems are incompletely efficient in elimination of all ROS, particularly with the added insult of various environmental ROS from smoking, air pollution etc. Exogenous antioxidant supplementation, from dietary sources therefore has a critical role in the prevention of oxidative stress in human physiology (Halliwell 1994, Seifried et al., 2007).

Direct scavenging of free radicals is one of the major mechanisms of antioxidant activity by flavonoids. The resulting aroxy radical (Flavonoid-O·) is more stable than other ROS and gains further stability on reacting with a second radical to form a stable quinone structure (Pietta 2000). Several other mechanisms of antioxidant activity of flavonoids have been proposed which includes scavenging of transition metal ions (Mira et al., 2002), and inhibition of enzymes responsible for antioxidant production. In terms of the latter property, flavonoids have been shown to inhibit several pro-oxidant enzymes including xanthine oxidase (Van Hoorn et al 2002), glutathione S-transferase (van Zanden et al., 2004), nitric oxide synthase (Raso et al., 2001), and NADH oxidase (Morre et al., 2000) amongst others.
ii. Hormonal properties of flavonoids

The estrogenic properties of flavonoids first came to light in 1950’s when sheep grazing on red clover pastures had reduced breeding rates (Bennets et al., 1946). Red clover contained several isoflavones and the estrogen-like properties of isoflavones were shown to account for fertility disturbances in animals feeding on them. Hence, isoflavones are also classed as phytoestrogens.

Isoflavones (genistein and formononetin) have been shown to displace radiolabeled estradiol from the ER (Martin et al., 1978). Flavonoids from the flavone, flavonols, flavonone and chalcone classes are considerably weaker phytoestrogens than the isoflavones as determined by competitive binding assays (Kuiper et al., 1998). Of the two estrogen receptor isoforms, genistein has a 7-fold greater binding affinity to ERβ than ERα, although binding affinity is 20 and 3.7 fold less than 17-β estradiol (Kuiper et al., 1997). Three-dimensional structure analysis confirmed that genistein bound to the ligand-binding site of ERβ is similar to the natural ligand estradiol (Pike et al., 1999). Furthermore, the recruitment of ER co-activators was modified by genistein differentially with the ERβ-genistein complex binding ER co-activators to a much greater degree than ERα-genistein (Routledge et al., 2000).

The differential binding of phytoestrogens to ER isoforms is of importance, since each of the ER isoforms has been shown to have distinct functions in proliferation. At the promoters of certain proliferation genes, ERα and ERβ have opposite actions, with ERα being pro-proliferative and ERβ being anti-proliferative (Heldring et al., 2007). The data suggest from various researchers suggests that at low doses, flavonoids may be pro-proliferative in an ERα and possibly ERβ dependent fashion, while neither ERα nor ERβ are critical for the anti-proliferative effect of flavonoids at higher doses. The precise physiologic effects of flavonoids mediated by their binding to ERα and ERβ are yet to be fully determined. This is an important area of future research because phytoestrogenic flavonoids are consumed in large amounts in the human diet. If the estrogenic effects of these compounds are predominantly pro-proliferative at low concentrations, flavonoids could potentially pose a health risk in terms of promoting hormone dependent cancers. (van der Woude et al., 2005, Wang et al., 1996, Maggiolini et al., 2001) (Pettersson et al., 2000).
Flavonoids may also exert anti-estrogenic effects by various enzymatic mechanisms. Blocking the synthesis of estrogens by inhibiting aromatase is an established strategy in the treatment of breast cancer. Flavonoids have been shown to bind the active site of aromatase, and inhibit its function, with flavones and flavanones, rather than isoflavones having the greatest effect (Hackett et al., 2005, Kao et al., 1998).

The similarity in structure of flavonoids to all steroid hormones raises the possibility of ligand binding of flavonoids to other members of the nuclear steroid receptor family. Flavonoids have been shown to bind and activate a number of nuclear receptors including androgen (Beck et al., 2003, Fang et al., 2003), progesterone (Beck et al., 2003), thyroid (Ricketts et al., 2005), and peroxisome proliferator-activated receptor (PPARγ) (Ricketts et al., 2005). Genistein and quercetin were shown to induce AR activation, however, it is evident from these studies that the effect of flavonoids, like that of DHT is biphasic, with activation of AR occurring at low doses and AR inhibition at higher flavonoid doses (Maggiolini et al., 2002, Gao et al., 2004, Morris et al., 2006, Ren et al., 2000).

Overall, the interactions of flavonoids with steroid hormone pathways are highly complex. However, the effect of flavonoids on these pathways has been shown to ultimately cause alterations resulting in beneficial effects such as the negative regulation of proliferative stimuli. As with much of flavonoid research, the in vivo effects of flavonoids on hormonal signaling require further study. This is highlighted in a recent review by Hamilton-Reeves (2007) where the majority of intervention studies reviewed did not find a difference in circulating sex steroid hormone levels (Hamilton-Reeves et al., 2007).

iii. Flavonoids and apoptosis

Flavonoids have been shown to induce apoptosis in a variety of cell types. Apoptosis occurs through two well-characterized pathways: the external pathway which is initiated by ligand binding to cell membrane death receptors, and the intrinsic pathway triggered by changes in internal cellular signals. Both pathways ultimately result in activation of the caspase cascade. Caspases are cysteine dependent proteases that initiate the sequence of events culminating in the apoptotic phenotype. In the intrinsic pathway, apoptogenic stimuli cause cytochrome c release.
from the mitochondria, an event inhibited by bcl-2 and promoted by bax (Cory et al., 2002). The ratio of bcl-2 to bax is an important factor in apoptosis progression. Once in the cytoplasm, cytochrome c binds to Apaf-1 which recruits ATP and caspase 9 to form the apoptosome. Caspase 9 recruits pro-caspase 3 to the apoptosome and activated caspase 3 mediates cell death (Budihardjo et al., 1999).

Apigenin was shown to activate the mitochondrial apoptotic pathway as evidenced by loss of mitochondrial Bcl-2 expression, mitochondrial permeability, cytochrome C release, and the cleavage of caspase 3 and 9 (Gupta et al. 2002). Quercetin potentiated TRAIL induced apoptosis via the extrinsic pathway in DU145 and LNCaP PCa cell lines, and induced apoptosis in a p53 independent fashion in PC3 cells, associated with an increase in Bax protein expression and a decrease in Bcl-x(L) and Bcl-2 protein (Kim et al., 2007, Vijayababu et al., 2006). EGCG on the other hand has been shown to cause apoptosis by a p53-dependent mechanism. EGCG mediated apoptosis in PC3 cells was attenuated by inhibition of p21 and bax by siRNA (Hastak et al., 2005). Inhibition of transcription factor NF-kB by EGCG leading to increased apoptosis has also been demonstrated (Hastak et al., 2003). Other flavonoids shown to induce apoptosis via similar mechanisms in PCa cells include genistein, isoliquiritigenin, silibinin, baicalin, amongst others (Agarwal et al., 2007, Chan et al., 2000, Davis et al., 1998, Jung et al., 2006). A novel relationship between flavonoids and apoptosis is the association of degree of apoptosis induced by flavonoids (EGCG) and their ability to inhibit fatty acid synthase (FAS) activity. FAS are a key lipogenic enzyme over-expressed in cancer cells (Brusselmans et al., 2005).

iv. Flavonoids and angiogenesis

Liu (2005) demonstrated the effect of apigenin on VEGF expression in lung cancer cells. Apigenin was shown to inhibit transcription of VEGF through the hypoxia inducible factor (HIF) binding site and by reduction of total HIF1alpha levels (Liu et al., 2005). Suppression of VEGF transcription and activity was also shown for EGCG in gastric cancer cells (Liu et al., 2005). Genistein was shown to be a potent inhibitor of angiogenesis in a xenograft model. It was shown to inhibit expression of VEGF, platelet derived growth factor, and up-regulated anti-angiogenic factors such as endostatin and angiostatin (Su et al., 2005). Thus by inhibition of VEGF and MMPs, and activation of anti-angiogenic factors, flavonoids may have an important
role as anti-angiogenic agents in cancer. Not all studies, however, have demonstrated an anti-angiogenic effect of flavonoids. EGCG, for example, was shown to demonstrate strong activation HIF1α in human breast cancer cells. A similar up-regulation of HIF-1α was also noted for quercetin (Park et al., 2007). The precise cause of the disparity in HIF-1α and VEGF expression between flavonoids is undetermined.

v. Flavonoids and inflammation

Inflammation is an established etiological factor for several cancer types including PCa. Flavonoids have been shown to possess anti-inflammatory properties. Flavonoids have been shown to inhibit phospholipase A2, cyclooxygenases, and lipoxygenases resulting in reduction of levels of pro-inflammatory mediators. These alterations have partly been accounted for by inhibition of transcription of pro-inflammatory genes. Nitric oxide, an inflammatory mediator is induced by inducible nitric oxide synthase iNOS, which has been shown to be inhibited by flavonoids such as quercetin and luteolin. The transcription factor NF-kB has important functions in several cellular processes. Activation of NF-kB has been shown to inhibit apoptosis. Gong (2003) has demonstrated the effect of genistein on abrogating NF-kB DNA-binding activity. The effects of genistein were shown to be partly mediated by inhibition of the cell survival Oncoprotein Akt (Gong et al., 2003). Similar findings were also shown for the tea flavonoid EGCG (Sen et al., 2006). Although genistein inhibits NF-kB activity, it appears to increase NF-kB gene transcription as evidenced by increased level of the p50 NF-kB subunit in nuclear extracts of genistein treated cells (Borras et al., 2006).

vi. Flavonoids and matrix metalloproteinase’s

Proteases secreted by cancer cells include urokinase plasminogen activator (uPA) and matrix metalloproteinases (MMPs). These proteases are believed to lead to the facilitation of metastasis and promotion of angiogenesis by degradation of the ECM. Ho (2007) demonstrated the inhibition of invasion and migration of oral cancer cells in vitro by EGCG. This was associated with a reduction in levels of MMP2 & 9. Similar findings were shown for quercetin in PC3 PCa cells (Vijayababu et al., 2006). The membrane-type 1 MMP which activates pro-MMP2 was shown to be inhibited by tea polyphenols including EGCG (Oku et al., 2003).
The epidemiologic evidence supporting the role of environment on the rate of progression of PCa has paved the way for concerted research efforts to identify specific environmental, dietary and lifestyle factors that could account for the preventive effect described in epidemiologic studies. In summary, the modulation of a large number of signaling pathways is a distinctive property of flavonoids. The multi-targeting of several pathways at once by a single compound is likely to be more effective at suppression of cancer cell proliferation than single target inhibition. The key to the suitability of flavonoids as chemopreventive agents lies in the translational potential of these in vitro findings to the human situation. Bridging the gap to the clinical application of flavonoids requires the use of suitable animal models to study the effects and safety of flavonoids in vivo. In the present work elucidates the mechanism of chemoprevention and studied the role of dietary flavonoids (daidzein and luteolin) on early stage progression of PCa in Wistar rats. We used two compounds, daidzein an Isoflavone and luteolin a flavones, to study their role in ECM homeostasis in androgen deprived rats ventral prostate, on testosterone propionate induced BPH and finally studied their chemopreventive efficacy in NMU-testosterone induced early stage PCa in wistar rats.

10. Modulators used in the study

High intake of fruit and vegetables is believed to be beneficial to human health. Fruit, vegetables and some beverages, such as tea and coffee, are particularly rich in dietary polyphenols. A substantial epidemiologic literature supports the beneficial health effects of flavonoid consumption. Various studies have suggested that dietary polyphenols may protect against cancer, cardiovascular diseases and neurodegenerative diseases. The main areas of interest have been the anti-cancer agents. Dietary polyphenols may exert their anticancer effects through several possible mechanisms, such as removal of carcinogenic agents, modulation of cancer cell signaling and antioxidant enzymatic activities, and induction of apoptosis as well as cell cycle arrest. We selected two compounds for our study daidzein and luteolin.
10.1. Daidzein

Daidzein 3', 4', 5, 7-tetrahydroxyflavone, is a flavonoid belongs to a group flavonoids and categorized as Isoflavones (Figure 12). Isoflavones are naturally occurring plant chemicals belonging to the “phytoestrogen” class (Murkies et al., 1998, Price and Fenwick 1985). Daidzein is naturally derived flavonoid found widely in soya. The main sources for isoflavones are soy products, beans, peas, nuts, grain products, coffee, tea and certain herbs such as red clover. It is metabolized in the colon by bacteria to equol, another isoflavone. Soybeans and soy products such as soy flour, tofu, and soy milk are major components of Asian diets. It is widely consumed in Asian subcontinent. Many researchers has reported the decreased incidences of hormonal related cancers (Prostate and Breast cancer) in Asian countries compared with its American counterparts to more consumption of soy isoflavones. We showed reduced oxidative stress, pro-inflammatory cytokines level, decreased activation of NF-kB, expression of COX-2 and ki-67 in TPA induced in mouse skin by soy administration (Khan et al., 2012). Daidzein inhibited the production of NO and IL-6, suppressed NF-kB transcriptional activity via regulation of the nuclear translocation and DNA-binding activity of NF-kB p50 subunit and blocked STAT1 phosphorylation in Prevotella intermedia ipopolysaccharide-treated RAW264.7 cells (Choi wt al., 2012). Daidzein is known for its antioxidant, anti-inflammatory and chemopreventive properties. Laboratory studies have also shown daidzein to have anti-tumor effects against pancreas, colon, cervix, prostate, and breast cancer. (Masilamani et al., 2012, Messina 2010, Mishra et al., 2009, Wijeratne and Cuppett 2007).
10.2. Luteolin

Luteolin, 3', 4', 5, 7-tetrahydroxyflavone, belongs to a group of flavonoids and categorized as flavones. (Figure 13) Evidence from cell culture, animal, and human population studies has suggested that flavonoids are also beneficial to human and animal health. Because of their abundance in foods, e.g., vegetables, fruits, and medicinal herbs, flavonoids are common nutrients that are antioxidants, estrogenic regulators, and antimicrobial agents (Birt et al., 2001). Cancer preventive (Neuhouser 2004). May block progression of carcinogenesis at several points, including cell transformation, invasion, metastasis, and angiogenesis, through inhibiting kinases, reducing transcription factors, regulating cell cycle, and inducing apoptotic cell death (Birt et al., 2001). Vegetables and fruits such as celery, parsley, broccoli, onion leaves, carrots, peppers, cabbages, apple skins, and chrysanthemum flowers are rich sources of luteolin (Neuhouser 2004, Gates et al., 2007, Sun et al., 2007, Mencherini et al., 2007). Plants rich in luteolin have been used as Chinese traditional medicine for hypertension, inflammatory diseases, and cancer (Harborne and Williams 2000). The anti-inflammatory effect of luteolin also may be linked to its anticancer function. The anticancer property of luteolin is associated with inducing apoptosis, which involves redox regulation, DNA damage, and protein kinases in inhibiting proliferation of cancer cells and suppressing metastasis and angiogenesis. Furthermore, luteolin sensitizes a variety of cancer cells to therapeutically induced cytotoxicity through suppressing cell survival pathways and stimulating apoptosis pathways (Wruck et al., 2007).

Figure 13: Sources of Luteolin and structure