CHAPTER- I: REVIEW OF LITERATURE
1. REVIEW OF LITERATURE

Interest in cancer research has grown during the past century as infectious diseases have increasingly been controlled as the result of improved sanitation, vaccination and antibiotics. Although this interest is relatively recent, Prostate Cancer (PC) is not a new disease. Autopsies of ancient Egyptian mummies have shown the presence of bone tumours and possibly other neoplasia (Brothwell, 1967). Symptoms of what can be assumed to be malignant diseases were also described in Chinese and Arabic medical writings. By the time of Hippocrates in the 4th century BC, many types of tumours were clinically recognized and described. Hippocrates introduced the term carcinoma from the Greek word *karkinos*, for crab: he saw cancer as crab like spread through the body (Long, 1928). Some 600 years later, Galen distinguished three types of tumour: ‘tumours according to nature’, which included all normal physiological swellings, such as enlargement of the breast with normal female maturation; ‘tumours exceeding nature’, which included the productive process following injury, such as the proliferation of bone that occurs during the reuniting of a fracture; and ‘tumours contrary to nature’, which included what we now define as neoplastic growths, as well as many inflammatory lesions (Long, 1928).

However, it was not until the end of the 18th century that cancer began to be studied systematically and intensively. Bichat (1771–1802) described the pathology of many neoplasms in humans and suggested that cancer was an ‘accidental formation’ of tissue built up in the same manner as any other part of the organism. Some decades later, Muller (1801-1858) and Virchow (1821-1902) extended Bichat’s findings, using the microscope to show that cancerous tissue was made up of cells (Long, 1928). Ever since, pathologists and clinicians have considered cancers in the various organs of the body as being in many respects completely
different diseases with distinct morphologies, clinical manifestations and prognosis. But only during the past few decades has it emerged that their causes also differ enormously. As a discipline, epidemiology and molecular genetics has been crucially important in defining the causes of different cancers and in evaluating preventive measures.

1.1. Cellular and Molecular Basis of Cancer

The term cancer is synonymous with neoplasm and tumour. Human cancer is not a single disease rather a myriad collection of diseases with as many different manifestations as there are tissues and cell types in the body, involving numerous internal and/or external causative agents, and various disease mechanisms (Coleman & Tsongalis, 2002). Willis (1967) defined a neoplasm as a mass, the growth of which is in coordinate with the surrounding normal tissues and that persists in the absence of the inciting stimulus (McKinnell et al., 2006). At cellular level, neoplasm is characterized by unregulated cell growth, impaired differentiation, invasiveness (increased cell mobility and loss of contact inhibition) and metastatic potential (ability to spread to distant organs). These biological traits of neoplastic cells enable them to exert nutritional, patial, signal and mobility pressures on normal neighboring cells. The neoplastic cells also, in many cases form structures and patterns that morphologically distinguish them from normal cells.

1.1.1. Neoplasms can be Benign or Malignant. Depending on cell and tissue of origin, the main classes (nomenclature) of neoplasm are:

i. Carcinoma, which is the malignant form of cancer arising from the skin and epithelial lining of internal organs and surfaces. The benign tumors of epithelial origin have various names (depending on basic cell type) such as papilloma (from skin and urinary bladder), adenoma
(solid epithelial organs, epithelia of gonads and bronchial epithelium), melanoma (skin pigments) and teratoma (germ cells).

ii. Sarcoma, which is malignant cancer of the mesenchyme (bone, fibrous tissue, fat, cartilage, muscle, blood vessels and lymph vessels) and shades of names for the benign forms (Osteoma, Fibroma, Lipoma, Chondroma, Leiomyoma and Haemangioma).

iii. Blastoma for the malignant cancers of the nervous system (neuroblastoma for nerve cells, Astrocytoma for astrocytes and Oligodendrocytoma for oligodendrocytes) while benign forms for meningeal cells are called Meningioma.

iv. Leukemia for white blood cells, Erythro-leukemia for red blood cells, Lymphoma for the lymph nodes and reticulo-endothelial system. Cancer of the embryonic type tissues are known as terato carcinoma (Franks & Teich, 1997).

1.2. Cancer Progression

Cancer development is a multi-step process through which cells acquire increasingly abnormal proliferative and invasive behaviors. The main trigger for tumourigenesis is mutation. It is now known that neoplastic cells also acquire multiple somatic mutations, which result in genetic variations and/or abnormal gene expressions that enhance their ability to invade (migrate to) neighboring normal tissues. This involves a clonal expansion of transformed neoplastic cells in a process of natural selection (Isaacs, 1997). Neoplastic cells often have a growth advantage that allows them to proliferate, loose contact inhibition and increase mobility; and invade adjacent tissues. In some organs, cancer progression is predictive, for example, in the colon, the transition from benign to malignant occurs in discernible stages: benign adenoma, carcinoma in-situ, invasive carcinoma; local and distant metastasis. Such progression is
characterized by accumulation of multiple genetic alterations in the affected cells. However, in many others, the progression is unpredictable, for example, in the prostate gland, the development of pre-malignant lesions, collectively known as prostatic intraepithelial neoplasia (PIN) does not, in all cases, progress predictably to malignant lesions (Bostwick, 1996; Isaacs, 1997). Invasiveness describes the ability of cancer cells to penetrate basement membrane of underlying tissue stroma. This is caused by increased mobility of cancer cells. This is different from metastasis, which describes the spread of cancer cells to near and distant organs via blood and lymph vessels and neural networks. Both characteristics are indicators of aggressiveness of cancer cells. Invasive cancer cells require supply of nutrients, which often result from formation of new blood vessels (angiogenesis) (Fearon & Vogelstein, 1990). Due to the multi-step nature of prostatic carcinogenesis, cells that have undergone some but not all of the transformation steps are present in pockets within the prostates of aging men, and the clonal expansion of these partially transformed cells produces morphologically detectable premalignant lesions in the gland (Bostwick, 1996; Isaac, 1997). Molecularly, there is a prominent clustering of changes in expression for many biomarkers between benign epithelium and high grade PIN, indicating that this is an important threshold for carcinogenesis.

1.3. The Prostate

The term ‘prostate’ was originally derived from the Greek word ‘prohistani’ meaning to stand in front of and has been attributed to Herophilus of Alexandria who used the term in 335 B.C. to describe the organ located in front of the Urinary bladder; detailed anatomical depiction did not appear until the Renaissance. However, while the existence of the prostate has been recognized for over 2300 years. Accurate description of the gland’s internal structure, physiology and pathology has occurred only relatively recently. The early descriptions
suggested that the human prostate followed a lobar pattern of development similar to that of other mammals (Lowsley, 1912).

1.3.1. Anatomy, Morphology and Function

The human prostate (from the Greek word, prostates, meaning "one who stands before," "protector," or "guardian") is a walnut-sized tissue. In a young adult, the prostate weighs approximately 20 g and measures 3 cm in length. Its main function is to store and secrete a slightly alkaline fluid and proteins that are supposed to provide nutritional support to the seminal fluid. Prostate compounds constitute approximately 25% of the volume of semen, together with spermatozoa and seminal vesicle fluid. The alkalinity of semen helps to neutralize the acidity of the vaginal tract, prolonging the lifespan of sperm. Prostatic fluid is expelled in the first ejaculate fraction together with spermatozoa and the fluid of the seminal vesicle. In addition, prostatic fluid improves the motility of spermatozoa, promotes their longer survival and provides better protection to the DNA. The prostate is located in the pelvis, under the urinary bladder and in front of the rectum. The prostate surrounds part of the urethra, the tube that carries urine from the bladder during urination and semen during ejaculation (Figure 1.1). Because of its location, prostate diseases mainly affect the processes of urination and ejaculation, though rarely defecation.
Figure 1.1. The Human Prostate Gland
1.3.2. Formation and Morphogenesis of the Prostate

The development of the prostate gland is largely controlled by sex hormones and is initiated in the uterus at week 12 of fetal development. Prostate formation occurs through epithelial budding from urogenital sinus (endodermal origin). During mid-gestation, the primitive urogenital sinus is separated from the terminal region of the hindgut through the division of the cloaca by the uro-rectal septum. The frontal region of the urogenital sinus forms the urinary bladders, whereas the most caudal region forms the urethra. The prostate gland originates from the intermediate region pelvic part or urogenital sinus (Abate-Shen & Shen, 2000).

1.3.3. The prostate is divided into zone classifications:

The zone classification is used more often in pathology and was first proposed by Mc Neal in 1968 (Mc Neal, 1968). According to this classification, the prostate can be divided into 4 zones: the peripheral zone (PZ) and the central zone (CZ), which together comprise 90% of the prostate mass in the prostate of a normal man, the periurethral transition zone (TZ), and finally, the anterior fibro muscular zone (AFZ) or stroma and the periurethral glandular zone (De marzo et al., 2007) (Figure 1.2.).
Figure 1.2. Zonal anatomy of the prostate showing Central zone (CZ), Peripheral zone (PZ) Transition zone (TZ) and anterior fibro muscular zone (AFZ).
The PZ zone constitutes the bulk of the apical, posterior, and lateral prostatic tissue and accounts for most of the glandular tissue (70%). 70% of prostate cancers emerge in the zone. The TZ accounts for 5-10% of the glandular tissue of the prostate. Cellular proliferation in the TZ results in benign prostatic hyperplasia (BPH). In addition, 20% of the cases of PC arise in this zone. The CZ surrounds the ejaculatory ducts. Only 2.5% of the reported cases of PC appear in this zone, but these cancers tend to be more aggressive and more likely to invade the seminal vesicles (Myers, 2000).

1.3.4. Prostate Disorders

1.3.4.1. Prostatitis or Chronic Inflammation

The term, prostatitis, or chronic inflammation refers to the histological inflammation of the tissue of the prostate gland that may be associated with a large, indefinite number of lower urinary tract symptoms (LUTS) and sexual discomfort and dysfunction. This condition affects 5-10% of the male population and is the most common urologic diagnosis in men younger than 50 years of age. It can be associated with an appropriate response of the body to an infection, but it can also occur in the absence of infection. Emerging evidence suggests that among the many risk factors for developing PC and its progression to metastasis, inflammation represents a major risk (De marzo et al., 2007). Chronic inflammation of long-standing duration has been linked to the development of carcinoma in several organ systems. The proposed mechanism of carcinogenesis involves repeated tissue damage and regeneration in the presence of highly reactive oxygen and nitrogen species. These reactive molecules are released from the inflammatory cells and can interact with DNA in the proliferating epithelium to produce
permanent genomic alterations such as point mutations, deletions, and rearrangements (Weitzman et al., 1990).

1.3.4.2. Benign Prostatic Hyperplasia

BPH is one of the most common age-related disorders affecting men, representing the most frequent proliferative abnormality of the human prostate. The development of BPH takes place in the TZ (Mc Neal, 1990). BPH produces a progressive obstruction of the urethra that leads to urinary retention, bladder function impairment and eventually renal failure (Thomas & Abrams, 2000), and it affects 80% of men by age 65. This implies that one in three of these patients will require treatment to alleviate the obstructive symptoms caused by the disease. Regardless of the obvious importance of BPH as a major health issue, this significantly affects the quality of life in aging men (De marzo et al., 1999). The cellular and molecular events that contribute to BPH are not well characterized, though recent data from various studies support a shift in the balance between cellular growth and apoptosis and senescence (Lee & Peehl, 2004; Gallardo-Arrieta et al., 2010).

1.3.4.3. Prostate Intraepithelial Neoplasia

Prostate Intraepithelial Neoplasia is defined histologically by the presence of nuclear and cytoplasmic features, which are similar to those of PC in glands with normal architecture (Haggman et al., 1997). However, unlike cancer, PIN retains the cell layer. As this process is confined to the epithelium, it is, therefore, termed intraepithelial (Montironi et al., 2007). In addition, PIN lesions generally display a marked elevation of cellular proliferation markers (Bostwick et al., 1998) within the pre-existing secretory epithelium, ducts and acini. Notable cytological changes include prominent nucleoli in at least 5% of the cells, nuclear enlargement,
nuclear crowding, an increased density of the cytoplasm and a variation in nuclear size (Joshua et al., 2008). This prostate disorder is classified into a twin-tier classification, based on the cytological characteristics of the secretory cells: low grade PIN (LGPIN) and high-grade PIN (HGPIN) (Drago, 1992) (Figure 1.3.).

Figure 1.3. (A) Low grade adenocarcinoma where the majority of the glands are relatively uniform in size. (B) Intermediate grade adenocarcinoma shows abundant amphophilic cytoplasm, enlarged nuclei with prominent nucleoli. (C) High grade adenocarcinoma shows fused glands, no intervening stroma and disruption of the basal cell layer.

HGPIN is considered most likely to represent a forerunner to PC, based on several lines of evidence: The incidence and extent of HGPIN in the prostate increase with advancing age (Montironi et al., 2000; Sakr et al., 1996). HGPIN lesions are found in the PZ, where most prostate tumors occur (Curado et al., 2007).
1. The frequency, severity and extent of HGPIN increases in the presence of PC.

2. The appearance of HGPIN lesions generally precedes the appearance of carcinoma by at least 10 years, which is consistent with the idea of cancer progression.

3. Rates of cell proliferation and deaths are elevated in HGPIN prostate cancer when compared to the rates for normal prostates.

4. Chromosomal abnormalities and allelic imbalance analyses have shown that HGPIN lesions are multifocal, as is the case with carcinomas (Qian et al., 1997).

5. The architectural and cytological features of HGPIN closely resemble those of invasive carcinoma, including a disruption of the basal layer and the presence of prominent nucleoli.

6. Differentiation markers that are commonly altered in early invasive carcinoma are also altered in HGPIN lesions (Joshua et al., 2008).

1.4 Prostate Cancer

1.4.1. Census of India 2011-The Highlights

The Census of India released on July 15, 2011 (http://www.censusindia.gov.in/2011census/dchb/KarnatakaA.html) revealed various interesting statistics relevant to the pattern of prevalence and other disease characteristics of prostate cancer. India has a population of 121.0 crores, out of which 68.84% reside in rural areas and 31.16% in urban areas. More than 50% of the population is below the age of 25 years and more than 65% is below the age of 35 years. It is expected that in 2020, the average age of an Indian will be 29 years compared to 37 years for a Chinese and 48 years for Japanese.
9.8, crore males are over 50 years of age and the average life expectancy has increased to 70 years. All these demographic data will have a bearing on the changing incidence and pattern of prostate cancer in the ensuing decade.

1.4.2. Prostate Cancer Demography in India

There is an increasing trend in the burden of non-communicable diseases like cardiovascular disease, diabetes mellitus, and cancers as evidenced by the various demographic and epidemiological studies conducted in India. Oral and esophageal cancers have the highest incidence, whereas rectal, prostate, and lung cancers have the lowest (Sinha et al., 2003). It has been reported that although the cancer rates in India are lower than those seen in western countries, increase in life expectancy and changes in lifestyles increase the rates of cancers in this country, particularly prostate cancer. (Lalitha et al., 2012), analyzed the time trends in the incidence of prostate cancer for different age groups of the Indian population reported in Indian cancer registries, using relative difference and regression approaches covering the following areas: Ahmedabad, Bangalore, Chennai, Delhi, Mumbai, Nagpur, Pune, and Thiruvananthapuram. The estimated age-adjusted incidence rates (AARs) of prostate cancer in India as a whole was 3.7/105 persons during the year 2008. However, the regional variation of AAR was remarkable. It was found to be 0.8 in the state of Manipur (excluding Imphal) while in Delhi, the rate was 10.9/105 person-years. The mean annual percentage change (MAPC) in the crude incidence rates ranged from 0.14 in Ahmedabad to 8.6 in Chennai. Peak incidence was observed in the age group above 65 years, indicating that prostate cancer was a cancer of the elderly. The estimated annual percentage change (EAPC) in the AAR ranged 0.8–5.8 in the various registries. Increase in the trend was seen in men aged 55-64 years in Bangalore,
Chennai, and Mumbai during 1983-2002 and in the 35-44 year’s age group in metropolitan cities such as Bangalore, Delhi and Mumbai. This revealed an increasing trend in the incidence of prostate cancer and the annual percentage change ranged 0.14-8.6. Projected cases of prostate cancer all over India for the periods 2010, 2015, and 2020 were estimated as 26,120, 28,079, and 30,185, respectively (National Cancer Registry Programme, 2009). In 2002, Sen et al., had reported a very low incidence (4.2% of all malignancies) of prostate cancer in Kolkata during the period of 1998-1999 from a population-based cancer registry. However, (Chatterjee, 2012), analyzed prostate cancer profile in the population of West Bengal from 2003 to 2010 and showed that the frequency of this cancer was increasing with the overall 5.71% incidence and this rise was moderate during 2003-2006 but it rose drastically from 2007 (17.76%) and reached the maximum peak (28.97%) in 2010. She also reported a higher prevalence of prostate cancer in persons with blood group A followed by blood group B and blood group O.

In 2010, Tyagi et al., have reported their observation on the incidence and risk factors of prostate cancer on patients registered by the Delhi population-based cancer registry during the period from January 1998 to December 2000. The mean age of patients with prostate cancer was 69.7 years. They reported that over the years, prostate cancer had become the fifth most common cancer in Delhi. These authors also observed that the incidence of prostate cancer was higher among North Indians compared to South Indians. In 2011, Swaminathan et al., in their study observed that the average annual age-standardized rate for prostate cancer had significantly increased by 47% during the period of 2002-2006 in Chennai compared to the previous years. They had observed that prostate cancer had become the ninth most common
cancer in Tamil Nadu. In 2006, Herbert et al., compared data available from various cancer registries and observed that the average annual cancer incidence rate for prostate cancer in India ranged 5.0-9.1 per 100,000/year, whereas the comparative rate in the United States were 110.4 for whites and 180.9 for blacks. Of all prostate cancers, 85% were detected late (stages III and IV) in India in contrast to the United States where only 15% were diagnosed in the late stages. These results indicate that there exist significant differences in the incidence of prostate cancer in the rural and urban areas in India. This again could be either due to the lack of awareness about this disease in the society or due to the poor reporting and documentation of cases in the rural populations. Based on the literature reviewed so far the data from North Karnataka was not reported till date. Thus, the study was aimed to determine the incidence of prostate cancer patients at a Single Tertiary Care Centre in North Karnataka.

1.5. Risk factors for Prostate cancer

Scientists have investigated a wide variety of factors in an effort to determine whether they might increase or decrease the risk of prostate cancer. The evidence pertaining to most of these factors is not conclusive. (Table 1.1.) Lists some of the factors currently under investigation, with rankings of the strength of the scientific evidence for each. Factors with a ranking of “3” have been solidly established as risk factors for prostate cancer. The one factor ranked “2+” has nearly achieved the status of fully established, but it is not completely understood. Factors ranked “2,” “2−”, or “1+” have some degree of scientific support, but the evidence in their favor is tentative or conflicting. Factors ranked “1” are not supported by the current scientific evidence (Meister, 2002).
Table 1.1. Risk factors associated with prostate cancer.

All characteristics listed are risk factors unless they are explicitly identified as protective factors. Key:

3: Established (supported by the scientific evidence)
2+: Nearly established, but not fully accepted or fully understood
2: Reasonable scientific hypothesis, but lacking solid scientific support
2- : Speculated, conflicting, or limited scientific support
1+: Weak scientific support
1: Not supported by the scientific evidence

(Source: American Council on Science and Health (Meister, 2002)).
1.5.1. Risk Factors for Prostate Cancer: The Indian Scenario

India is a land of diversity. The religions, cultures, environment, literacy rates, and food habits of the society vary from one region to another. These variations can have a significant bearing on the incidence of prostate cancer in various regions across the country. There are several risk factors implicated in the causation of prostate cancer, namely, positive family history (Cerhan et al., 1999), history of diabetes mellitus (Giovannucci et al., 1998), height, weight and obesity (Giovannucci et al., 1997), smoking habit, physical activity (Cerhan et al., 1997), body mass index (BMI) and vasectomy (Platz et al., 1997). However, in India the studies on the actual role of these risk factors in the causation of prostate cancer are limited. In 2011, Ganesh et al., reviewed prostate cancer cases registered in Mumbai and found that the average ages for the cases and controls were 64 years and 46 years, respectively. Literacy rate was similar in both the groups. An equal proportion of cases and controls (13.8%) had a family history of prostate cancer. History of diabetes mellitus was fourfold among the cases and history of hypertension was threefold among the cases as compared to the controls. Those with BMI <24.9 had twice the enhanced risk for prostate cancer when compared to those with BMI >25. The number of children and vasectomy did not contribute to any significant risk in this study. Consumers of betel leaf with or without tobacco and pan masala, gutka chewers, smokers, and alcoholics did not show any significant increased risk. Similarly, consumption of raw vegetables, meat, fish, tea, coffee, etc., also did not result in any additional risk of prostate cancer. This is contrary to the studies conducted in 2010 by (Huncharek et al., 2010) which showed an increased risk of prostate cancer for chronic smokers. Interestingly, another study conducted earlier by (Terry et al., 2001) had indicated a reduced risk of prostate cancer for fish eaters. The association of
family history of cancer and prostate cancer risk seen in a previous study (Cerhan et al., 1999) was also not observed in these studies.

In 2010, Tyagi et al., also observed that there was no statistically significant association between family history of cancer and prostate cancer. However, past smoking habit and current alcohol consumption, especially consumption of whisky, significantly increased the risk of prostate cancer. The risk of prostate cancer reduced with the increasing dietary consumption of tea, citrus fruits, melons, eggs, fish, and sunflower oil. Though an increased risk of prostate cancer was evident among vasectomized men, the association was not statistically significant. Production of carcinogenic heterocyclic amines during cooking of red meat and pyrolysates during cooking of meat over charcoal/smoke had been attributed as the reason for increased prostate carcinogenesis in the non-vegetarians (Ali et al., 2011). A2 allele of the CYP17 polymorphism has also been reported to be associated with an increased risk of prostate cancer in smokers and non-vegetarians (Sobti et al., 2009). Singh et al., in 2013 studied the relationship of lifestyle, age, and BMI with PSA levels in BPH and prostate cancer in the North Indian population. They found that the mean age of prostate cancer patients (67.56 ± 5.72 years) was significantly higher than that of BPH patients (63.56 ± 7.92 years). The prevalence of hypertension, smoking, use of tobacco, and alcohol consumption was similar in both the groups. However, there was no significant effect of BMI on the risk of prostate cancer that is in contrast to the findings of (Amling et al., 2004; Freedland et al., 2004) who had earlier studied the positive correlation of obesity to prostate cancer. A large part of the Indian population is involved in agriculture and associated industries. Therefore, these people are potentially exposed (occupationally or environmentally) to some types of pesticides, either directly or indirectly. In the majority of instances, there exist only poor safety measures during
the application and handling of these carcinogenic compounds. This could lead to widespread dispersion of these harmful and carcinogenic compounds, causing toxicity to human beings (Abhilash & Singh, 2009). Studies conducted by (Banerjee, 2011) reported that some of these pesticides, mainly organ chlorine pesticides (OCPs), possessed estrogenic properties and could be called xenoestrogenic pesticides. OCPs such as 1, 1, 1-trichlorocyclohexane (HCH), dieldrin, and endosulfan were found to be the most commonly used xenoestrogenic OCPs in India. He further reported that since prostate cancer was an estrogen-dependent cancer, these pesticides might increase the risk of prostate cancer incidence in the population exposed to these carcinogenic agents.

### 1.6. Characteristic Features of Prostate Cancer

The term, carcinogenesis, describes a concept whereby clinically hidden, multifocal pre-neoplastic foci emerge within the epithelium of an anatomic region exposed to the same carcinogen. Normal cells can progressively evolve to a neoplastic state. In order to do that, those cells need to acquire a succession of capabilities. These capabilities are known as the hallmarks of cancer and include the ability to sustain proliferative signaling, to evade growth suppressors, to resist cell death, to enable replicative immortality (including angiogenesis), and to activate invasion and metastasis (Hanahan & Weinberg, 2000). This multistep process towards human tumor pathogenesis may be understood by examining the necessity for incipient cancer cells to acquire those qualities that enable them to become tumorigenic and finally malignant (Hanahan & Weinberg, 2000). Tumors are more than masses of proliferating cells. Rather, they are complex tissues composed of multiple, distinct cell types that participate in heterotypic interactions with one another. The biology of tumors can no longer be understood by simply characterizing the traits of cancer cells; their descriptions must
encompass the contributions of the tumor microenvironment to the process of tumourigenesis (Hanahan & Weinberg, 2011). This is the reason that, recently, two new hallmarks have been included in the biology of cancer: the constitution and the signaling interactions of the tumor microenvironment (Hanahan & Weinberg, 2011). Prostate Cancer is generally regarded as multifocal, since its primary tumors often contain multiple, independent histological foci cancers that are often described as genetically distinct, even to those in close proximity (Bostwick et al., 1998). Notably, about 80% of all radical prostatectomy specimens show more than one neoplastic focus, as well as 70% of all HGPIN cases (Joshua et al., 2008). These findings suggest that multiple neoplastic foci may emerge and evolve independently, which has significant implications for the molecular mechanisms of disease progression.

The heterogeneity of PC is potentially relevant to understanding the distinction between latent and clinical disease, as well as the strong correlation between PC progression and aging (Shen & Abate-Shen, 2010). Although PC is a disease of older men, studies on healthy specimens obtained from healthy men in their 20s to 40s show the frequent presence of the histological foci of PC (Sakr et al., 1993), suggesting that cancer initiation has already taken place at a relatively early age. Combined with the evidence that PC is multifocal, it appears that this organ may be the site of multiple, neoplastic transformation events, many of which give rise only to latent PC, which does not progress to clinically detectable disease. It is thought that clinical PC follows a different pathogenic program than that of latent PC. Alternatively, most latent PC foci may not undergo the critical activating events that lead to clinical disease, or it is possible that many will remain under active suppression (Shen & Abate-Shen, 2010). These lesions may not be apparent at histological examination, though molecular techniques have found evidence of such changes in a variety of epithelial neoplasms, which are suggestive of
carcinogenic changes (e.g., p53 loss, loss of heterozygosis (LOH) and microsatellite instability). In contrast, despite the phenotypic heterogeneity of metastatic PC (Shah et al., 2004), molecular and cytogenetic analyses show that multiple metastases in the same patient are clonally related, which would indicate that advanced PC is monoclonal (Liu et al., 2009).

Most prostate tumors are adenocarcinomas shown in (Figure 1.3.) which represents a typical luminal phenotype (Shen & Abate-Shen, 2010) that shares numerous common features with other prevalent epithelial cancers, such as breast and colon cancers.

1.6.1. Prostate Cancer Initiation and Progression

The molecular pathways that contribute to the genesis of subclinical, microscopic PC precursor lesions, their progression to invasive cancer, and their androgen-independence remain largely unknown, although certain molecular candidates have been implicated in the overall process of disease progression. For instance, in some prostate carcinomas aberrations in specific signaling molecules have been indicated, such as extracellular growth factors, protein tyrosine kinase cell surface receptors, intracellular anti-apoptotic or transcription factors, nuclear receptors and their ligands, growth suppressors, cell cycle regulators and others (Roy-Burman et al., 2004).

PC lesions can develop in the complete disarrangement of both luminal and basal cells with concomitant loss of basal cell lamina. In its initial stages, when confined to the prostatic capsule, PC is essentially curable by surgical intervention and/or radiation therapy. In fact, most cases of prostate carcinoma are relatively indolent, and the majority of men diagnosed with PC will die of other causes instead. However, if not detected early or in the more aggressive forms of the disease, PC can advance to stages that are characterized by a local
invasion of the seminal vesicles, followed by metastasis, primarily to the bone, which is usually lethal (Figure 1.4.).

Figure 1.4. Pathway for human Prostate Cancer Progression.

Consequently, a major clinical challenge is posed by the current inability to readily distinguish between indolent and aggressive tumors in PC (Rossi et al., 2007). As a starting point for the PC progression pathway, it is important to consider chromosomal abnormalities as indicators for the first stages of PC shown in (Figure 1.4.). Presumably, patterns of consistent allelic loss reflect the reduction or loss-of-function of putative tumor suppressor genes in PC. Despite the significance of allelic loss to prostate carcinogenesis, no single candidate tumor suppressor gene has been definitively assigned a role in PC progression (Shah et al., 2004).

1.6.2. Process that Promote Prostate Carcinogenesis

The hallmarks of cancer comprise eight biological capabilities acquired during the multistep development of human tumors. They include sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis, reprogramming of energy metabolism and
evading immune destruction (Hanahan & Weinberg, 2000). Underlying these hallmarks is genome instability, which generates the genetic diversity that expedites their acquisition, and inflammation, which fosters multiple hallmark functions. PC as well as other types of cancer, occurs when the rate of cell division is higher than the rate of cell death, leading to uncontrolled tumor growth. The causes of these events are still unknown, although some processes, which promote this carcinogenesis, are known.

1.7. Prostate Cancer Diagnosis

1.7.1. Prostate Cancer Screening and Diagnosis

In order to cure patients with PC successfully, it is important to detect the disease at an early stage, as well as monitor its progress accurately. PC is typically a slow growing tumor that affects older men. Despite its slow growth, PC is still a lethal disease. Early PC usually has no symptoms. With more advanced disease, patients may present symptoms related to urethral obstruction (urinary frequency, hematuria, difficulty in initiating urination, or dysuria). Nevertheless, these symptoms can occur as the result of non-cancerous conditions, such as BPH. Normally, most cases of PC are diagnosed before these symptoms develop. Due to the long latency period of this cancer and its potential curability, this disease is an excellent candidate for screening strategies that attempt to identify the disease in its early, curative state (Loeb et al., 2009). The diagnostic tools for detecting PC can be separated into those that screen for the disease, such as PSA and DRE, and the decisive diagnosis set of Transrectal ultrasound guided prostate biopsies (TRUS) (Lilja et al., 2008).

PSA screening is widely utilized and considered an effective detection method for PC. One of the limitations of serum PSA as a tumour marker is its lack of specificity (around 30%), which results in a high rate of negative biopsies. Elevated PSA levels can also be attributed to other
factors such as BPH, prostatitis, prostate irritation, and recent ejaculation (Pannek & Partin, 1997; Catalona et al., 1991). As a consequence of the current screening parameters, around 2/3 of the approximately 1,300,000 biopsies made yearly in the United States and 390,000 in Europe are unnecessary (Makinen et al., 2004; Cervera et al., 2004). In contrast, the false positive rate of a biopsy is about zero, although the false negative rate in the first biopsy may oscillate between 12% and 32% (Cervera et al., 2004). Because of their persistently elevated PSA levels, but negative biopsy results, these men undergo repeated biopsies to rule out PC. This situation is called the “PSA dilemma” (Pannek & Partin, 1997).

1.7.2. Prostate Serum Antigen

Human kallikrein-related peptidase 3 (KLK3) is commonly referred to as PSA. The first report on the detection of PSA in serum was made by (Papsidero et al., 1980). PSA is used as a serum biomarker to monitor and screen for PC since 1986 and 1994, respectively (Lilja et al., 2008). Use of PSA-based screening has become widespread as a cancer marker. PSA has led to increased PC detection and has served as an alert to stage migration with a decreased number of metastatic or locally advanced cases of cancer at diagnosis (Ung et al., 2002). The elevation of serum PSA signals an abnormality in the prostate, whether it is a benign enlargement, an inflammation or a PC. This ability to find early-stage cases of PC has made PSA an interesting biomarker for use in PC screening (Thompson et al., 2004). The PSA levels are continuous parameters: the higher the value, the more likely the existence of PC (Table 1.2.). PSA testing cannot be expected to resolve all ambiguity with respect to PC; instead, it may be best considered as an indicator of risk to be weighed in combination with other factors. Normally, annual PSA screening is recommended for all men over 50 years old; however, in patients with familiar PC a screening should start at age of 40.
Table 1.2. Risk of prostate cancer in relation with PSA values

PSA strongly discriminates between the different cancer stages. It is higher in men with localized disease than in cancer-free controls, is associated with stage and grade in localized disease and is higher in patients with metastatic compared to localized disease. Moreover, men with high PSA levels at the initial time of therapy have an increased risk of recurrence. PSA is a sensitive indicator of recurrence after radical prostatectomy (RP), but it is far less sensitive as an indicator of recurrence after radiation therapy (D'Amico et al., 2004).

1.7.3. The Role of PSA in the Prostate

PSA (or KLK3) is a glycoprotein belonging to the family of kallikrein-related serine proteases that are produced in normal prostate secretion. Its physiological role is believed to be the liquefaction of seminal fluid. The transcription of PSA is governed by androgens, which restrict its high-level production to the prostate epithelium. PSA is synthesized in healthy prostate tissue, in BPH and in PC at all grades and stages (Herrala et al., 2001). For this reason, PSA is defined as being organ-specific, but not cancer-specific. The normal prostate architecture keeps PSA confined to the organ, so that only a minimal amount leaks into the

<table>
<thead>
<tr>
<th>PSA level (ng/mL)</th>
<th>Risk of developing prostate cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 0.5</td>
<td>6.6 %</td>
</tr>
<tr>
<td>0.6 – 1</td>
<td>10.1 %</td>
</tr>
<tr>
<td>1.1 – 2</td>
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<td>2.1 – 3</td>
<td>23.9 %</td>
</tr>
<tr>
<td>3.1 – 4</td>
<td>26.9 %</td>
</tr>
<tr>
<td>4 – 10</td>
<td>20 – 35 %</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>50 – 80 %</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>95%</td>
</tr>
</tbody>
</table>
circulatory system. Increased blood levels of PSA in men with cancer or with other prostate disease conditions cannot be explained by increased PSA expression. In fact, during the development and progression of PC, PSA expression may actually decrease slightly (Qiu et al., 1990). So, the increased blood PSA levels must be caused, instead, by an increased release of PSA into the blood. Even so, there are no experimental data or mechanisms of increased release that are believed to result from the disruption of the prostate architecture seen in prostate tumors, such as the disordering of the basement membrane and a loss of the basal cell layer, ductal lumen architecture and epithelial cell polarity (Lilja et al., 2008).

1.7.4. PSA Levels in Prostate Cancer in India

PSA has been widely used as a diagnostics tool in the screening for prostate cancer. Though PSA level is known to vary with age and various age-adjusted nomograms of PSA values are available, it is not clear whether these reference values for PSA could be applicable in the Asian population, particularly the Indian population. This is because the PSA levels can vary in various ethnic groups. A study by Sin-Eng (Chia et al., 2007) showed that the median PSA level in the Chinese, Malay, and Indian ethnic groups in Singapore were not different. They further observed that the PSA values positively correlated with age and the mean values were lower than the PSA levels noted in the white population in the United States. However, the predictive values of PSA in detecting prostate cancer was found to be quite similar to those in western countries in another study by (Mochtar & Andika, 2010). In 2004, Malati and Kumari suggested a reference value for PSA in healthy males aged 20-89 years belonging to a subpopulation of Andhra Pradesh in South India. The results revealed lowest concentration of 95-percentile serum PSA in Indian males compared to other populations globally. Ganpule et
*al.* in 2007 also reported their observations on age-specific PSA and PSA density values in a community-based Indian population in Gujarat. They observed that the mean PSA values increased from 2.1 ng/mL in the age group of 40-49 years to 5.0 ng/mL in the age group of >70 years. Similarly, the mean PSA density also increased from 0.15 to 0.2 ng/mL in the same age group of patients. Though PSA has been regarded as a useful tool for the early detection of prostate cancer, has been doubted the utility of this tool for screening prostate cancer in Indian males due to the low incidence of this particular disease in this country (Dubey, 2009). They also suggested the need for local guidelines and recommendations for mass screening, especially in elderly males above the age of 50 years in India. The evaluation of free-to-total PSA (f/t PSA) ratio to distinguished in BPH and prostate cancer in the age group of 40-75 years in Chennai, Tamil Nadu. They found that f/t PSA ratio was decreased significantly in prostate cancer compared to BPH (Atish *et al.*, 2010). In 2006, Malati studied the serum total PSA levels in 583 healthy males, 1,090 patients with BPH, and 651 patients with adenocarcinoma prostate in Andhra Pradesh in South India. They found that the PSA level was significantly high in BPH patients and adenocarcinoma prostate patients when compared to healthy males. In Indian population studies there have been no authentic reports on PSA nomograms, Gleason’s scoring pattern, and the difference in biochemical recurrence among those who underwent definitive treatment in various ethnic and other population subsets in the country.
1.7.5. Digital Rectal Examination

The DRE, Latin *palpatio per anum* or PPA, is a relatively simple procedure. DRE is performed on all patients in whom PC is suspected (by elevated levels of serum PSA). The patient is placed in a position where the anus is accessible. The physician usually examines the external area (anus and perineum) for any abnormalities, such as hemorrhoids, lumps, or rashes. Then, as the patient strains down, the physician slips a gloved and lubricated finger into the rectum through the anus and palpates the inside for approximately sixty seconds. However, the probability that an abnormal DRE is highly suggestive of PC depends significantly on the PSA values (Catalona & Smith, 1994). Often, a palpable cancer is already advanced in both grade and stage and is potentially no longer organ confined. In experienced hands, DRE has a specificity of 83.6% and a sensitivity of 53.2% and an abnormality in either PSA or DRE alone confers a 20-25% chance of PC (Mistry & Cable, 2003). The overall positive predictive value (PPV) for cancer detection increases to 50% when DRE and PSA are used in combination (Chodak *et al*., 1989; Richie *et al*., 1993).

1.7.6. Prostate Biopsy

The need for Prostate Biopsy (PBs) should be determined based on the PSA levels and/or a suspicious DRE. Transrectal ultrasound is currently considered the normal standard of care. Although a Transrectal approach is used for most PBs, some urologists prefer to use a perianal approach (Presti *et al*., 2003). The Vienna nomograms offers an easy tool for selecting the optimal number of biopsy cores, based on age and total prostate volume in the PSA range of 2-10ng/mL. This system improved the cancer detection rate 66.4% compared to the old, systematic sextant biopsies (Remzi *et al*., 2005). TRUS-guided biopsies have an overall
sensitivity and specificity for PC detection of 32% (Conrad et al., 2002). The histopathology of prostate tissue can be definitively identified in most cases of PC. This method is the most commonly used prognostic indicator for PC and results in a Gleason score. The lower the Gleason grade, the better the prognostic outcome (Epstein et al., 2005; De Marzo et al., 2003). Finally, there is also disagreement regarding the thresholds of scoring afforded by different pathologists, since the Gleason grading scale that is used by them is semi-quantitative (Bostwick et al., 2000). For these reasons, the Gleason scores themselves have limited quantitative value.

1.7.7. Classification of Prostate Cancer

1.7.7.1. Gleason Grading

The Gleason grade was first described in 1966 by Donald F. Gleason (Gleason, 1966). More than 40 years after its introduction, the Gleason grading system remains one of the most powerful prognostic predictors in PC. However, the original descriptions of each pattern have undergone significant revisions over the years, first by Gleason et al., (Gleason & Mellinger, 1974) and, most recently, at the 2005 International Society of Urological Pathology Consensus Conference (Epstein et al., 2005). To account for this heterogeneity, Gleason proposed a grading system that is now predominantly used by pathologists, since it is an excellent prognostic indicator. The Gleason score can be assessed using biopsy material or prostatectomies. It is the sum of the two most common patterns (grades 1 to 5) of tumor growth found in a sample. The Gleason score ranges between 2 and 10, with 2 being the least aggressive and 10 the most. In 2005, Amin et al., recommended that the worst grade should be included, even if it is present in less than 5% of all cases (Amin et al., 2005) (Figure 1.5.).
Figure 1.5. Gleason’s pattern scale with the five Gleason grades of prostate cancer. Grade 1 appears on the top and grade 5 on the bottom of the drawing. Grading system-showing score of (2, 3+3, 4+3, 4+4 and 5+5).

1.7.7.2. Tumour Node Metastasis Classification

The tumour node metastasis (TNM) classification is based on the status of the primary tumour, ranging from organ-confined to fully invasive (T1 to T4), with or without lymph node involvement (N0 or 1) and the presence and degree of distant metastasis (M0 and 1a-c) (Ohori et al., 1994). The TNM staging system provides a basis for survival prediction, initial treatment selection, and patient stratification in clinical trials, accurate communication among healthcare providers, and a uniform method for reporting the result of cancer management (Ludwig et al., 2005). The 2009 TNM classification for PC (Sobin et al., 2009) is shown in (Figure 1.6.).
1.8. Prostate Cancer Treatment

The therapeutic management of PC has become increasingly complex, due to the various therapeutic options available, even in cases of clinically localized disease, which have equal oncological efficacy but with different, treatment-related side effects. Treatment recommendations vary by disease severity and life expectancy, since the side effects of treatment may outweigh the potential benefits for men whose cancers are unlikely to progress in their lifetime (Table 1.3.).
Table 1.3. Treatment recommendations for Prostate Cancer with respect to severity of the diseases.

Additionally, a multidisciplinary approach may be advisable from the beginning in patients with high risk PC, because it is very likely that adjuvant treatment will be necessary for locally advanced disease. The main treatments for PC from the Clinical Practice Guidelines in Oncology 2009 (Heidenreich et al., 2011) are summarized below:

1. **Watchful waiting (WW):** This term, which was coined in the pre-PSA screening era, refers to the conservative management of PC until the development of local or systemic progression, at which point the patient is afforded palliative treatment. The rationale
behind WW is the observation that PC often progresses slowly and is often diagnosed in older men for whom there is a high incidence of death from other disease.

2. **Active surveillance (AS):** AS is now an accepted management strategy for men with low-risk PC who previously faced radical whole gland treatment (surgery, external beam radiotherapy or brachytherapy (EBRT) (Choo *et al.*, 2002). AS involve monitoring the course of the disease with the expectation of intervening if and when the cancer progresses. It is often offered to men who have a limited life expectancy. Monitoring under AS involves PSA testing every 3 to 6 months, DREs every 6 to 12 months and possible, additional PBs.

3. **Radical Prostatectomy (RP):** This treatment involves the removal of the entire prostate gland between the urethra and the bladder and the resection of both seminal vesicles, along with sufficient surrounding tissue to obtain a negative margin. Regional lymph nodes may also be removed for examination to determine whether lymph node metastases are present.

4. **Radiation therapy:** Radiation therapy normally consists of EBRT or brachytherapy for localized PC. In EBRT, the patient receives radiation treatment from an external source over an 8 to 9 week period. Brachytherapy involves placing small radioactive pellets, sometimes referred to as seeds, into the prostate tissue.

5. **Hormonal therapy:** ADT alters the effects of male hormones on the prostate through medical or surgical castration (the elimination of the testicular function) and/or the administration of anti-androgen medications.
1.9. Molecular Genetics of Prostate Cancer

Evidence of a genetic influence to the susceptibility of PC has been established through familial relationship, segregation and twin studies are important genetic determinants of prostate cancer in the world. Besides age and race, family history is the only well-established risk factor for PC (Cunningham et al., 2003; Carter et al., 1992; Gronberg et al., 1997). Over the years, genetic epidemiological research has accrued much evidence in favor of a significant hereditary element for PC susceptibility. Familial aggregation of prostate cancer i.e., the occurrence of more than one PC case among first-degree relatives has been recognized as early as the 1950s. Since then, epidemiological studies have shown that first-degree relatives of PC patients have a 2- to 3-fold increased risk of developing PC (Gronberg et al., 1994). The highest risks of PC have been observed in men having multiple affected relatives, or relatives diagnosed at an early age. This familial risk of prostate cancer has been observed in multiple ethnic populations (Americans, Australians, and Europeans, Asian-Americans, Caucasians, Asian Pacific and African-Americans decedents) (Varghese & Easton, 2010). The increasing rate of PC has been compared between monozygotic and dizygotic twins, making it possible to assess genetic and environmental factors.

1.9.1. Role of Single Nucleotide Polymorphism in Predicting the risk of Prostate Cancer

There has been an increasing focus on the role of single nucleotide polymorphisms (SNP) in the development and progression of PC but also on their role in diagnostics and risk prediction. A SNP is a DNA sequence variation occurring when a single nucleotide (A, T, C, or G) in the genome differs from the normally expected nucleotide. These SNPs are known to underlie differences in our susceptibility to diseases. SNPs need to be determined only once and are
easy to determine, making them interesting biomarkers. The rising interest in the role of SNPs in PC development and progression is illustrated by the number of studies being published on SNPs in the PC field. Further, research has focused on identifying the genetic foundations of prostate cancer. It has been recognized that a number of forms of genetic changes coupled with epigenetic and gene expression changes can increase the prediction to develop prostate cancer. Identifying relevant genetic changes offers the ability to develop novel biomarkers to allow early and accurate detection of prostate cancer as well as provide risk stratification of patients following their diagnosis. In connection to the diagnosis, the concept of personalized or individualized medicine has gained significant attention.

Single nucleotide Polymorphisms are minute variations in the DNA sequence that are passed on from parents to children. They are the most common type of genetic variation in humans. Formally, an allele, that is, a variation in DNA sequence, is defined to be polymorphic if it occurs in at least one percent of a population (Feero *et al.*, 2010). Therefore, although overall humans are very similar at the DNA sequence level, because the genome is large there is substantial latitude for individual genetic variation. SNPs occur about once in every 800 base pairs (Qiu *et al.*, 1990). The Human Genome Project and advances in related technologies have supported the investigation of the relationship between genetic variation and many health outcomes, including prostate cancer. The SNPs identified in Genome Wide Association Studies (GWAS) are believed to be surrogates for the true causative locus within a linkage imbalance block that is biologically responsible for the association GWAS of PC have rapidly progressed after the identification of the 8q24 locus (Meyer *et al.*, 2011; Eeles *et al.*, 2013; Foulkes, 2008). In fact, there are now more than 80 altered SNPs associated with increased PC risk identified by GWAS on 20 different chromosomes (*Table 1.4.*).
<table>
<thead>
<tr>
<th>Locus</th>
<th>Nearby Genes</th>
<th>SNP</th>
<th>Reference Allele</th>
<th>Risk Allele</th>
<th>Per Allele OR (95% CI)</th>
</tr>
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</table>

Table 1.4. List of Single nucleotide polymorphisms associated significantly with increased risk of prostate cancer.
Therefore, a better understanding of the genetic and molecular characteristics distinguishing indolent from lethal prostate cancers is necessary to understand the genetic polymorphisms and metabolic pathways underlying prostate cancer development offers the opportunity to explore new therapeutic interventions with the possibility of offering patient-specific targeted therapy. Therefore, the study was designed to investigate the chromosome region 8q24 rs4242382 SNP associated with risk of prostate cancer in North Karnataka population.

1.10. The Role of Androgens in Prostate Growth

Growth of the prostate gland depends, like any cellular biology, on the balance between cell proliferation and cell death (apoptosis). If these two components are equivalent, as usually happens in normal prostate tissue, there is no increase in prostate growth. However, when the index of cell proliferation is greater than that of apoptosis, there is continuous growth of the prostate gland, and the number of cells increases. In the prostate, this balance between cell proliferation and apoptosis is regulated by androgens. Testosterone, which is the major circulating androgen in peripheral blood, is produced mainly at the testicular level. At the prostate level, testosterone is converted into 5-alpha-dihydrotestosterone (DHT) through the action of 5-alpha-reductasa iso-enzymes. Although both androgens are able to join the AR, DHT has a much higher affinity to the AR than testosterone (Liao et al., 1976; Bruchovsky et al., 1975; Krieg et al., 1979). The direct effect of testosterone on prostate epithelial cells is that it induces differentiation, while the indirect effect, proliferation, is mediated by the production of growth factors by the prostatic stroma. Androgens also directly stimulate the production of vascular endothelial growth factor (thus, inducing angiogenesis) in both normal prostate tissue and in neoplastic prostate tissue.
1.10.1. Docetaxel Based Treatment for Metastatic Castration Resistant Prostate Cancer (mCRPC)

1.10.1.1. Mechanisms of Androgen Resistance

It is well established that prostate cancer growth is largely dependent on the androgen-signalling pathway (Huggins et al., 2002). Androgen deprivation therapy (ADT), either through orchiectomy or with gonadotropin releasing hormone (GnRH)-agonists or antagonists, remains the mainstay of first-line treatment in locally advanced or metastatic prostate cancer. Unfortunately, resistance to hormonal treatment always occurs despite optimal castration. For prostate cancer cells to survive and proliferate in an environment deprived of androgen, they must either adapt the androgen receptor (AR) pathway to the androgen-deprived conditions or create alternative growth pathways (Jenster, 1999). Both of these mechanisms are commonly reported in the literature as AR dependent, sub classified as ligand dependent and/or ligand independent, or as mechanisms, which support tumour growth by passing the AR (Figure 1.7.).
Figure 1.7. Mechanisms of Androgen Independence Leading to Tumour Progression. T, testosterone; DHT, dihydrotestosterone; E, estrogens; P, progesterone; GC, glucocorticoids; Anti A, antiandrogens; AA, abiraterone acetate; MDV3100, enzalutamide.
Ligand-dependent castration resistance refers to tumour growth in the presence of a ligand. In approximately, 30% of castrate-resistant tumour cells, the AR gene remains amplified and supports tumour growth despite low concentrations of the naturally circulating ligands testosterone (T) and dihydrotestosterone (DHT) (Bubendorf et al., 1999; Visakorpi et al., 1995). It is thought that tumour cells in this environment become hypersensitive to the low level of androgens present in blood, enabling them to continue androgen-dependent growth (Koivisto et al., 1997; Feldman & Feldman 2001). Moreover, this phenomenon is associated with AR stability and nuclear localization (Gregory et al., 2001). The use of AR antagonists, such as bicalutamide and flutamide, does not seem to reverse tumour growth in this situation but may, in fact, enhance it (Kawata et al., 2010). Tumour cells may also acquire resistance via genetic mutation leading to the aberrant activation of androgen signalling (Buchanan et al., 2001). Although rare, genetic mutation of the AR can confer increased functional activity to the AR. The AR then becomes capable of binding with natural and non-classical ligands including estrogen, progesterone, adrenal androgens, corticosteroids (Zhao et al., 2000), DHT metabolites, (Koivisto, et al., 1998) and androgen antagonists (Culig et al., 1999). This binding further stimulates the AR and thus promotes tumour growth. Finally, prostate cancer cells may acquire independence from AR signalling by activating alternative survival mechanisms that bypass the AR pathway. These bypass mechanisms, many of which are under investigation, include the RAS/MAPK pathway, transforming growth factor-b, Wnt/b-catenin pathway, hepatocyte growth factor, fibroblast growth factor (FGF) pathway, and insulin-like growth factor (IGF) system. The phosphatidylinositol 3-kinase (PI3K)/AKT signal transduction pathway is also a key oncogenic pathway activated in many human cancers, including prostate cancer. The phosphatase and tensin homologue deleted on chromosome 10 (PTEN) genes is a
tumour suppressor gene and is the main inhibitor of the PI3K/AKT pathway. Deletion of PTEN and hence activation of PI3K/AKT has been shown to modulate AR activity, thus supporting tumour growth in mCRPC (Karantanos et al., 2013; Choucair et al., 2012). Because of these findings, patients who fail hormonal therapy are now referred to as castrate resistant as opposed to hormone resistant.

1.10.2. Castration-Resistant Prostate Cancer

Androgens regulate the prostate gland as the major stimulus for cell division in the prostate epithelium. Circulating androgens are essential to normal prostate development, as well as to the onset of PC through their interactions with the AR. In 1940, Huggins & Clark demonstrated that the removal of testicular androgens by surgical or chemical castration led to a reduction of the prostate tumour (Huggins & Hodges, 1972). While the initial growth of a prostate tumour is dependent on androgens, the transition to metastatic disease is generally followed by androgen-independence, which is often evoked by androgen deprivation therapy. Following ADT, the androgen-dependence of prostate tissue is manifested by rapid cellular apoptosis and an involution to the regressed state. However, ADT is usually associated with PC recurrence, thereby making continued ADT ineffective (Feldman & Feldman, 2001). This recurrent disease has been called, castration-resistant. Unfortunately, CRPC has remained essentially untreatable. Even when PC progresses to castration-resistant PC, AR activation and signaling remains sustained though a variety of mechanisms. Notably, castration-resistant tumours express AR, as well as AR target gens, such as PSA, indicating that the pathway activity has remained intact (Gregory et al., 1998). Nowadays, it is unclear when castration-resistant PC normally appears within prostate tumours. The conventional adaptation model proposes that castration-resistant cells arise through the genetic/epigenetic conversion of previously
androgen-dependent cells under conditions of androgen deprivation. The alternative, clonal selection model suggests that the emergence of castration resistance reflects the proliferation of a previously quiescent population of rare castration-resistant cells within an otherwise androgen-dependent tumour (Isaacs & Coffey, 1981).

1.10.3. Prostate Cancer Metastasis

Although common sites for secondary PC metastasis are the lung, liver, and pleura, when PC metastasizes, it goes first into the bone marrow stroma of the axial skeleton. This is the principal cause of PC morbidity and mortality. Furthermore, PC displays characteristic osteoblastic, rather than osteolytic, lesions (Logothetis & Lin, 2005). Local invasion is a fundamental, initial step in the metastatic process, as without it, tumour spread could not occur. To develop invasive potential, malignant cells must down-regulate their cell–cell and cell–matrix adhesive characteristics, become motile and acquire the ability to break down extracellular matrices (Liotta et al., 1986). Once the malignant cells have escaped the tumour capsule, they must enter vascular or lymphatic circulation. Then, these cells must migrate via the circulation system to find a new place, where they can attach and proliferate and/or coalesce with other metastasized cells to form micro-metastases (Clarke et al., 2009). This can only happen when the environment at the secondary site is favorable.

1.10.4. When to Start Cytotoxic Therapy

The widespread use of PSA monitoring has resulted in earlier detection of CRPC, often in asymptomatic patients. An important question for the management of asymptomatic disease is whether to initiate chemotherapy or to wait until symptoms occur. Although chemotherapy may halt or reverse progression, associated toxicity of the chemotherapy might lead to deterioration of Quality of Life (QoL). Comparative data evaluating the merits of delaying the
initiation of chemotherapy are lacking. As previously mentioned, survival benefit from treatment with docetaxel-based chemotherapy is equal for all subgroups of patients (Watson et al., 2010). Although the benefit is similar, there is a substantial difference in overall survival in patients with and without pain (14.4 months versus 21.3 months, respectively), but this does not necessarily imply benefit from early use of chemotherapy. Some patients had a decreased QoL after starting chemotherapy, and this was more often observed in patients with minimal symptoms (Krijnen et al., 1993). Delaying cytotoxic therapy may be a suitable approach in CRPC patients with rather indolent disease, for which the following criteria were proposed: PSA only progression as a single sign of metastatic disease with low baseline PSA and a slow prostate-specific antigen doubling time (PSA-DT), a normal (or slightly raised) alkaline phosphatase and normal or (slightly lowered) haemoglobin. In patients who are more likely to develop symptoms and progression at an early stage (based on high PSA-DT and/or high baseline PSA and/or bone scan progression and/or (visceral progression), the start of chemotherapy should not be postponed (Craft et al., 1999). Therefore, to optimise management of advanced CRPC, it becomes increasingly important to understand the predictive factors influencing the outcome. It is difficult to estimate the time expenditure in which the group of asymptomatic/low risk patients is likely to become symptomatic, or to develop other adverse prognostic features with associated poorer survival outcome. Postponing chemotherapy and gaining significant time without therapy, without hindering on survival expectancy when chemotherapy eventually starts, may be considered beneficial, whereas the risk of postponing treatment for only some months at which time the patient has obtained significant worse features and detrimental effects on survival probability must be avoided.
1.10.5. Proof-of-concept trials using docetaxel

Shortly after the reports of the mitoxantrone studies became available, proof-of-concept trials were being conducted to assess the feasibility and therapeutic potential of the taxanes. Several docetaxel-based regimens were investigated: a 3-weekly regimen, a weekly regimen (owing to the assumption that this regimen would be better tolerated in an elderly population), and a combination of docetaxel and estramustine (Taylor et al., 2014). The PSA response rates in these phase I-II trials evaluating docetaxel-based regimens were higher (41–68%) than those reported previously in the mitoxantrone trials. In addition, these trials were the first to report objective response rates of approximately 20–50% in patients with measurable disease (Okazawa et al., 1998; Dimopoulos et al., 2007; List et al., 2006). Furthermore, a median survival of up to 27 months was reported in patients who received 3-weekly docetaxel (Herman et al., 2011; Van der et al., 2009; Dredge et al., 2005). These results prompted the initiation of two large randomised phase III studies, TAX 327 and SWOG 99-16, to further evaluate the anti-tumour activity of docetaxel in the setting (Higano & Crawford, 2011). Hence, we explore docetaxel-based regimens offering a significant survival benefits when administered to patients in the setting.

1.11. Antioxidant Potential of Medicinal Plant

The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth’s inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components (Craig, 1999). Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase
(SOD), glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage (Aruoma, 1998; Bhatia et al., 2003; Peuchant et al., 2004) and health problems (Steer et al., 2002; Uchida, 2000). A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases (Shahidi et al., 1992), including cardiovascular diseases, cancers (Gerber et al., 2002), neurodegenerative diseases, Alzheimer’s disease (Di Matteo & Esposito, 2003) and inflammatory diseases (Sreejayan & Rao, 1996). One solution to this problem is to supplement the diet and herbal remedies with antioxidant compounds that are contained in natural plant sources (Knekt et al., 1996). These natural plant antioxidants can therefore serve as a type of preventive medicine.

1.11.1. Antioxidant vs. Cancer

Reactive Oxygen Species is a hallmark of human cancer. ROS and their functions with respect to the cancer initiation and signalling in cancer cells is a prime concern of cancer research. Tobacco smoke also plays a very important role in increasing the risk for inflammation and cancer due to its high carcinogenic potential and the synergistic effects with other particulate to generate ROS and catalyse redox reactions in the cells of humans, leading to oxidative stress and increased production of mediators of inflammation (Gardi & Valacchi, 2012). Many cancers arise from sites of infection, chronic irritation and inflammation. It is now becoming clear that the tumour microenvironment, which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration (Moller et al., 2008). In addition, tumour cells have co-opted some of the signalling
molecules of the innate immune system such as selectins, chemokines and their receptors for invasion, migration and metastasis (Lazennec & Richmond, 2010).

1.11.2. Antioxidants and Prostate Cancer: Current Status

Although little is known regarding etiology and factors that influence clinical outcome, ‘elevated oxidative stress’ in the cellular microenvironment is a common denominator in prostate cancer and aging. Oxidative stress causes damage to multiple cellular components such as DNA, proteins, and lipids, and is clearly implicated in prostate cancer. Cells have developed a robust antioxidant defence system to maintain cellular redox homeostasis and to protect from damage under conditions of oxidant attack. However, increased reactive species from inflammation or inhibition of defence mechanisms can easily overcome the capacity of the antioxidant system leading to perturbation of cellular redox balance (Thapa & Ghosh, 2012). Knowledge of prostate cancer pathobiology gave rise to the novel concept of antioxidants for its chemoprevention. Supplementation with antioxidants (direct antioxidants) or stimulation of cellular antioxidant systems (indirect antioxidants) can reduce oxidative injuries and thus prevent prostate cancer (Figure 1.8.).
Figure 1.8. Role of Reactive Oxidative Species (ROS) in prostate cancer initiation and progression.
1.11.3. Complementary and Alternative Medicine for Prostate Cancer

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind; a great deal of effort has therefore focused on using available experimental techniques to identify natural antioxidants from plants. (Speroni & Scartezzini, 2000; Matkowski, 2008). The first written records on the medicinal uses of plants appeared about 2600 BC from the Sumerians and Akkaidians (Samuelsson, 2009). Asia is the largest continent, with 60% of the world’s population living here. The region consists of the continent of Asia plus the islands in the Indian and the Pacific Oceans. It has abundant medicinal and aromatic plant species and traditional medicine in Asia has been practised since ancient times. The continent has well-documented traditional knowledge, long-standing practice of traditional medicine and the potential for social and economic development of medicinal and aromatic plants in primary health care and industrial scale production (Chapman & Chomchalow, 2003). Indian Ayurveda along with the Jamu, Siddha, Tibetan, traditional Chinese and Unani systems of medicine are an important source of health and livelihood for millions of people. Ayurvedic medicine is widely practised especially in India, Nepal, Bangladesh, Pakistan and Sri Lanka (WHO, 2001). In India, people of different ethnic groups inhabiting various terrains, possess their own distinct culture, religious rites, food habit and a rich knowledge of traditional medicine (Mahishi et al., 2005).

The observed discrepancies between preclinical and clinical studies regarding the use of antioxidants for prostate cancer chemoprevention and discuss challenges and opportunities that this system offers for its use as a prostate cancer prevention strategy. Practicing herbal medicine to cure a variety of diseases doing so will be highly rewarding. Thus the study was focused on plants belonging to two different families from around the Western Ghats of North
Karnataka to understand their therapeutic uses and their potential antioxidant activities. The selected plants *Leea indica* Merr. (Leeaceae), and *Allophylus cobbe* (L.) Raeusch. (Sapindaceae) were studied for preliminary phytochemical constituents, determination of total phenolic content, *in vitro* antioxidant properties and anticancer activities using *in vitro* model system on DU-145 (moderately metastasized) and PC-3 (highly metastasized) human prostate cancer cell lines and mouse embryo fibroblast MEF-L929 normal cell lines.