REVIEW OF LITERATURE
2.1 PROSTATE GLAND

Embryology

Prostate gland is a male accessory sex organ present below the urinary bladder and directly opens into the urethra. A fully developed gland is pyramidal in shape with the base directed towards the neck of the bladder and apex resting on the urogenital diaphragm (Marker et al., 2003). The organ is made-up of several glandular and non-glandular components that are tightly fused together within a common capsule (Mc Neal, 1988).

The prostate gland differentiates from the urogenital sinus (UGS), located just caudal to the neck of the developing urinary bladder, under the influence of fetal testosterone (Cunha et al., 1987). The fetal testis (Leydig cells) produces testosterone from day 12.5-post conception (dpc) onwards in the mouse, by 15 dpc in the rat and by 9 weeks of gestation in humans (Habert et al., 2001). UGS arises in humans at about 7 weeks of gestation and in male and female mice at approximately 13dpc. The male and female UGS are morphologically undistinguished until about 17.5 dpc in mouse, 18-19 dpc in rats and 10-12 weeks of gestation in humans. At this stage prostatic morphogenesis is initiated by circulating androgens produced by the fetal testes (Marker et al., 2003).

The early prostatic morphogenesis is the out growth of solid buds from the UGS epithelium (UGE) into the surrounding UGS mesenchyme
(UGM). The prostatic buds are solid cords of epithelial cells that grow into the UGM in a precise spatial pattern to form the lobular sub divisions of the prostate (Timms et al., 1994). In human fetus, the prostatic buds originate from different parts of prostatic urethra. The first epithelial out growth arises from the prostatic urethra around the tenth week of human gestation and by 13 weeks, there are approximately seventy primary ducts (Cunha et al., 1987).

**Anatomy of Prostate gland**

In human, prostate gland has 3 major zones (Mc Neal, 1983). The *transition zone* comprises 5% of the glandular tissues, this zone surrounds urethra, and about 20% of the prostatic cancer along with benign prostatic hyperplasia arises here. The *central zone* surrounds the ejaculatory ducts, it comprises 20-25% of prostate gland, and give rises to 5-10% of prostatic cancer. The *peripheral zone* lies posteriorly and laterally in the prostate and comprises 70-75% of the glands and surrounds the central zone, about 70% of adenocarcinomas arises from this area. The anterior fibromuscular stroma occupies anterior surface of the prostate and is composed of smooth muscles.

The *peripheral zone*, *central zone*, *transition zone* and the periurethral region are the glandular components. The non-glandular tissues of human prostate include preprostatic sphincter, striated sphincter, anterior fibromuscular stroma and prostatic capsule. The nerves and vascular supply are also included (Mc Neal, 1988). A prostatic ductal system is defined as a
single prostatic functional unit in which all glandular structures share a single drainage duct into the urethra. Each ductal system is made up of three segments, proximal, intermediate and distal regions. These regions consist of three types of cells, epithelial, stromal and neuroendocrine cells.

The epithelial and stromal compartments of the prostate ductal system enjoy a dynamic coexistence characterized by an active dialogue of cell-to-cell signaling, which influences proliferation, differentiation and apoptosis. The human prostate consists of more than 30 such ductal systems (McNeal, 1988). Very little is known about the presence of neuroendocrine cells in the prostate. They are present in all regions of the prostate at birth, but rapidly disappear from the peripheral regions after birth and then reappear at puberty (Cohen et al., 1993).

The neuroendocrine cells contain neurosecretory granules rich in various peptide hormones and biogenic amines. These include thyrotropin-releasing hormone (TRH), TRH like peptide, neuron-specific enolase (NSE), Chromogranin A (CgA), Serotonin (5-HT), Thyroid stimulating hormone (TSH) like peptide, Calcitonin (CT), Somatostatin (ST), and Parathyroid hormone (PTH) related peptide gene product (Abrahamsson and di Sant'Agnese, 1993).

The axis of the ductal system in the rat prostate is divided into three regions (Lee, 1997). Owing to the distance from the urethral orifice of
the duct, the entire length of the prostatic ductal system can be designated as proximal, intermediate and distal regions. In the distal region the epithelial cells are tall and columnar in shape with apically located nuclei; the cells undergo active cell proliferation, but have no secretory activity. The epithelial cells in the intermediate region are tall and columnar, but have basally located nuclei and are mitotically quiescent cell in this region which undergo active secretion. Proximal region cells have cuboidal epithelial cells with no secretory activity and mitosis; cells in this region actively undergo cell death or apoptosis under the influence of transforming growth factor (TGF) \( \beta_1 \) produced by adjacent stromal cells (Martikainen et al., 1990).

The prostatic stromal cells are a group of pleomorphic mesenchymal cell types. One of the most prominent features of prostate stroma is the multiple layers of smooth muscle cells surrounding the proximal region (Nemeth and Lee, 1996). The number of smooth muscle cells decline, from the proximal region towards the distal region. Apart from the presence of smooth muscles the stroma is also filled with fibroblasts. These fibroblasts have a direct contact with epithelial cells, which are often in contact with smooth muscle cells (Lee, 1997).

Functional cytodifferentiation of luminal epithelial cells occur with the expression of the prostate-specific secretory proteins. In human prostate,
secretory activity is detectable during fetal half-life (13\textsuperscript{th} week of gestation) (Xia \textit{et al.}, 1990), presumably due to the action of fetal testicular androgens.

\textbf{Secretions of Prostate gland}

The major function of prostate gland is to secrete prostatic fluid that facilitates the capacity of sperm fertilization. Prostatic fluid is the mixture of complex and heterogeneous, organic and inorganic compounds like zinc, magnesium, calcium, fructose and citrate, which are derived from the seminal fluid (see Mann and Lutwik Mann, 1981). Prostatic fluid is slightly acidic with pH about 6.5 (Huggins, 1947). It also contains number of proteolytic enzymes like PSA (Prostate specific antigen), PAcP (Prostatic acid phosphatase) and human kallikrein-2 and nitrogenous compounds like phosphoryl choline, polyamines like putresine, spermine, spermidine (see Beyler and Zaneveld, 1982).

\textbf{2.2 HORMONAL REGULATION OF PROSTATE GLAND}

\textbf{Gonadotropin-releasing hormone (GnRH)}

GnRH, a decapeptide is synthesized and released by hypothalamic neurosecretory cells and reaches the pituitary gland by way of a specialized portal system to induce the synthesis and secretion of the gonadotropic hormones, which regulate gonadal functions (Neill, 2002). GnRH-R
expression was found in breast, prostate, endometrial cells, ovarian, pancreatic and hepatoma cells (Imai and Tamaya, 2000; Finch et al., 2008).

**Luteinizing hormone (LH)**

LH stimulates testicular steroidogenesis and also acts directly on the prostate gland (Sriraman et al., 2001). Luteinizing hormone-releasing hormone (LHRH) stimulates the LH secretion, which is the key hormone in the regulation of reproductive functions. It has been evidenced that LHRH is expressed in prostate cancer (PCa) cells together with receptor and negatively regulates cell proliferation through the activation of inhibitory G protein (Gi)-cAMP (Cyclic adenosine monophosphate) intracellular signaling pathway (Moretti et al., 2003). High incidence of LHRH receptor gene expression was identified in human prostate cancers (Halmos et al., 2000).

**Androgen**

Androgens play a critical role in cell proliferation, differentiation, and maintenance of the prostate gland. The growth and development of normal prostate requires a functioning androgen signaling pathway, which is regulated by hypothalamic-pituitary gonadal axis. Testosterone are synthesized in the testes and released into the circulation in response to specific hormonal signals regulated by GnRH, FSH and LH. Testosterone is transported by steroid hormone binding globulin (SHBG) to the prostate,
where it is converted by 5α-reductase to its active metabolite 5α-dihydrotestosterone (DHT). In the prostate, androgens mediate their effects via high affinity to the androgen receptor (AR), a nuclear transcription factor that controls expression of genes involved in growth, differentiation, homeostasis and apoptosis.

The AR gene is located on the X-chromosome (Xq 11-12). This gene contains 8 exons, the 1st exon encodes the amino terminal transcriptional activation domain, 2nd and 3rd exon encodes zinc finger motif that form the DNA binding domain. Exon 4 encodes the carboxy terminal ligand-binding domain (Ueda et al., 2002). DHT is the potent androgen in the prostate and the binding affinity of DHT to AR is 5 times greater than that of testosterone. The androgens bind to the AR, then the ligand receptor complex translocates to the nucleus and binds specific androgen responsive elements on the chromosome, hence inducing DNA synthesis, cell proliferation and regulate variety of genes (Gobinet et al., 2002). The androgen responsive genes include PSA, human kallikrein-2, and prostatic binding protein (Habib and Grant, 1996). In the rat, prostatic acid phosphatase and aldolase increase markedly in response to androgen (Mainwaring et al., 1974; Forsgren et al., 1979).

Testosterone regulates citrate synthesis by stimulating pyruvate dehydrogenase (PDH) and mitochondrial aspartate aminotransferase (m-ATT)
and citrate metabolism by stimulating \( m \)-aconitase activity. Testosterone stimulates the uptake and phosphorylation of glucose in prostate cells by increasing hexokinase activity (Harkonen \textit{et al.}, 1982). Glucose utilization \textit{via} HMP shunt pathway is also stimulated by testosterone (Harkonen, 1981). Androgen deprivation induces rapid involution and recovery of human prostate vasculature (Godoy \textit{et al.}, 2011).

\textbf{Estrogen}

Although prostate is an androgen dependent tissue, estrogens influence both normal functions and pathological changes in this gland (Weihua \textit{et al.}, 2002). The developing prostate is particularly sensitive to the effects of estrogen during the early stages of organogenesis and ductal branching. This estrogenic influence can impact both on acute prostatic growth as well as on subsequent prostate development and function later in life (Prins and Korach, 2007). The exogenous exposure of estrogen in rats, results in the alteration of prostatic growth, secretory function and the activational response to androgen during childhood (Naslund and Coffey, 1986). However, after sexual maturation, both androgens and estrogens are important in maintaining the structure and integrity of prostate suggesting that estrogen synergistically with androgen regulates prostatic growth in adults (Cunha \textit{et al.}, 1987). Estrogenic activity is mediated by the physical interaction between estrogen receptors (ERs) and the hormone with
subsequent activation of the receptor. ERs belong to the superfamily of nuclear receptors to which AR and thyroid hormone receptor (TR) belongs. Estrogen receptors are distributed throughout the male reproductive tract (Hess et al., 2001), there are two isoforms of ER (ERα and ER-β) have been identified (Nie et al., 2002). In normal prostate, estrogen action is mediated by ERα in the stromal compartment and by ER-β in the epithelial compartment where it specifically regulates basal cell proliferation (Pelletier et al., 2000). ER isoforms β 2 and β 5 are associated with poor prognosis in prostate cancer, and promote cancer cell migration and invasion (Leung et al., 2010)

**Thyroid Hormone**

Optimum levels of thyroid hormones are essential for the maintenance of normal structure and metabolic integrity of prostate. Thyroid hormone especially tri-iodothyronine (T₃) is an important regulator of many cell types promote cell growth and differentiation. T₃ modulates proliferation, secretory function and AR concentration in androgen dependent (LNCaP) cell line (Esquenet et al., 1995). The study showed that exposure of LNCaP cells to T₃ for 6 days, stimulated PSA secretion by 2-3 folds. T₃ stimulates the PSA protein in the presence of androgens in LNCaP cells without affecting AR expression (Zhang et al., 1999). Thyroid hormone mediates its action through binding to the corresponding receptor, it is a member of steroid receptor
superfamily. Thyroid hormones differentially regulate prostatic glycoprotein metabolism. Prostatic β-glucosidase, β-galactosidase and β-N-acetyl glucosaminidase activities increased uniformly in hyperthyroid and decreased in thyroidectomised adult rats (Maran et al., 1998).

**Prolactin**

Prolactin is a major physiological regulator of prostatic epithelial cell function and metabolism (Costello and Franklin, 1994). Prolactin receptor has been identified in prostate (Aragon and Friesen, 1975). Prolactin stimulates mitochondrial aspartate aminotransferase (mAAT) and pyruvate dehydrogenase (PDH) activities, the two key regulatory enzymes involved in citrate synthesis by prostate epithelial cells (Costello and Franklin, 1994). Prolactin acts synergistically with androgens and has a stimulatory effect in pentose phosphate pathway enzymes studied in bonnet monkey prostate. This pathway is involved in the supplementation of the precursors for nucleic acids synthesis, which are utilized for the cell proliferation of the prostate gland (Arunakaran et al., 1992). Studies carried out in the human and animals *in vivo* and *in vitro* revealed presence of prolactin receptors in normal, benign and malignant prostate tissues (Wylot et al., 2006).
Oxytocin

Oxytocin, a nano-peptide secreted by the neurohypophysis is involved in parturition and lactation (Catheline et al., 2006). Oxytocin is also produced locally in a variety of tissues including the male and female reproductive tracts. Oxytocin produced within mammalian prostate, stimulates the 5-α reductase activity which converts testosterone to dihydrotestosterone (DHT) (Assinder, 2008) and this DHT in turn stimulates the growth of prostate. Oxytocin helps in muscular contractions during ejaculation (Bodanszky et al., 1992). The localization of the oxytocin receptor within the plasma membrane modulates oxytocin's proliferative response in the prostate (Whittington et al., 2007; Sendemir et al., 2008). Oxytocin induces the migration of prostate cancer cells through the involvement of the G protein (Gi) coupled signaling pathway (Zhong et al., 2010)

Growth hormone (GH)

GH or somatotropin secreted from pituitary somatotrophs is responsible for postnatal growth of the prostate gland (Ruan et al., 1999). GH and insulin-like growth factor (IGF-I) are important for normal human prostate growth. It is evidenced that chronic GH deficiency in adulthood is associated with reduced prostate volume (Colao et al., 2003). Also, acromegalic subjects do have an enlarged prostate that decreases significantly in size on treatment with somatostatin analogs (Colao et al., 2000). GH
receptor (GHR) is potentially involved in prostate cancer through stimulating IGF-I production in prostate epithelium (Wang et al., 2005; McKay et al., 2007). It has been evidenced that human GH and IGF-1 induces LNCaP cell proliferation (Bidosee et al., 2011).

**Insulin**

Insulin is a mitogen appears to be a growth factor for prostatic epithelial cells (Prisco et al., 1999). Elevated insulin levels are associated with increased risk of prostate cancer (Lehrer et al., 2002). Men with diabetes mellitus for 5 years or more, are prone to have higher incidence of prostate cancer than without diabetes (Will et al., 1999). Studies showed that increased serum levels of insulin and leptin in men with prostate cancer, underwent androgen deprivation therapy (Nowicki et al., 2001). Increased level of circulating insulin directly or indirectly affects different molecular signaling and can promote prostatic growth (Vikram and Jena, 2011).

**Insulin-like growth factors (IGF) family**

Insulin-like growth factors (IGFs) play a pivotal role in tissue homeostasis, regulating cell proliferation, differentiation and migration during development. IGF system components consist of peptide growth factors, IGF-I and IGF-II and six classes of IGF binding proteins (IGFBPs 1-6), which modulate the mitogenic effects of IGF. IGFBP-1 and IGFBP-3 have similar affinities for IGF-I and II while IGFBP-2 have a higher attraction for IGF-II
(Peehl et al., 1995). In general, IGFBPs inhibit IGF activity by binding to IGF and preventing IGF from binding to its receptor.

Normal prostate cell expresses IGFBP-2, -3 and -4, but not IGFBP-1. It requires IGF-I and IGF-II for proliferation (Cohen et al., 1991). IGFs are produced primarily by stromal cells and activate IGF-I receptor on prostatic epithelial cells (Cohen et al., 1991). The IGFBPs appear to lack cell surface receptors for regulating IGF-independent cellular functions. In this regard, IGFBP-3 and IGFBP-5 are most similar to one another and each have within their structures, a peptide stretch that is cell membrane permeable (Goda et al., 2008) providing a mechanism, where these proteins may enter cells to elicit IGF-independent effects. In that context, IGFBP-3 has had the most IGF-independent actions (Yamada and Lee, 2009).

**Transforming growth factor -β (TGF-β)**

Transforming growth factor beta (TGF-β) is a protein that controls proliferation, cellular differentiation, and other functions in most cells. TGF-β family is important for inducing differentiation and inhibiting prostate epithelial cell proliferation and for maintaining normal prostate homeostasis. TGF-β family includes TGF-β1 to β5, which are important in regulating the formation of extracellular matrix, and inhibiting cell proliferation and inducing apoptosis. TGF-β1 is important in regulating cellular growth, differentiation and apoptosis (Untergasser et al., 2003). TGF-β1, TGF-β2, TGF-β3, and TGF-β5 differentially enhance the expression of N-cadherin,
cell adhesion molecules, fibronectin, and tenascin in precartilage condensations, suggesting that TGF-β isoforms play an important role in the establishment of cell-cell and cell-extra cellular matrix interactions during precartilage condensations (Kondaiah et al., 2000; Goswami et al., 2003). The TGF-βs have an inhibitory role within the normal prostate, controlling proliferation and inducing apoptosis in epithelial cells (Martikainen et al., 1990).

Two types of TGF-β receptors namely type I and II (TβRI and TβRII) are involved in signal transduction (Wrana et al., 1994). TGF-β is overexpressed in advanced PCa and exerts diverse functions in stromal cells via both (Sma and Mad) SMAD-dependent and SMAD-independent signaling pathways (Zhu and Kyprianou, 2005). Loss of both TβRI and TβRII correlates with tumor stage, survival and recurrence rate (Zhu and Kyprianou, 2005). Recovery of TβRII function in human PCa cell line inhibits the growth of xenograft tumors through the induction of apoptosis and inhibition of cell proliferation (Song et al., 2006). TGF-beta1 suppresses IL-6-induced STAT3 activation through regulation of Jak2 expression in prostate epithelial cells (Starsichova et al., 2010).

2.3 PROSTATE CANCER

Prostate cancer (PCa) occurs frequently in men after the fifth decade of life and is a leading cause of cancer deaths in the Western population (Jemal et al., 2003). Incidence rates vary by more than 25-fold
worldwide, with the highest rates recorded primarily in the developed countries of Oceania, Europe, and North America. Males of African descent in the Caribbean region have the highest prostate cancer mortality rates in the world, which is thought to reflect partly due to the difference in genetic susceptibility (Miller et al., 2003; Bock et al., 2009). In contrast to the trends in Western countries, incidence and mortality rates are rising in several Asian and Central and Eastern European countries, such as Japan (Baade et al., 2009; Bray et al., 2010).

Prostate cancer mostly diagnosed in African, Americans (116/100000 persons/year), intermediate incidence rates are found in Caucasians (71/1000000) and lowest rate among Asians (Japanese, 39/100000; Chinese 28/100000; India, 8/100000) (Gronberg, 2003). Average annual cancer incidence rates in India ranged from 5.0 to 9.1 per 100000/year (Hebert et al., 2006). PCa incidence in Indian major cities Bangalore 2.4%, Chennai 4.7%, Delhi 3.1% and Mumbai 0.8% per 1 lac population (Mathur, 2010).

The male hormone testosterone, secreted by the Leydig cells of the testis, is the principle androgen that controls prostate function (Lipsett et al., 1966). Androgens have also been strongly implicated in the development of PCa. Prostate cancer transformation is a three-stage phenomenon of initiation, promotion and progression (Goldsworthy et al., 1996). Initiation occurs when
a carcinogen induces general or specific changes to DNA, these are transient and readily eliminated by DNA repair mechanism. If the genomic insult is extensive or of a chronic nature, or if DNA repair mechanism are compromised, unrepaired lesion are fixed as mutation in the target cell population via cell proliferation (Coleman and Tsongalis, 1995). Replication of the initiated cells, which often have gained selective growth advantages into focal aggregates, marks the beginning of promotion, while progression may take many years, it can also be accelerated by general or specific endogenous or exogenous factors. When it occurs, several sequential and parallel events are evident in the advancing neoplasm. These include of physiological properties that enhance invasiveness, morbidity and dissemination an escape from immune surveillance, and the emergence of new growth regulatory mechanism in distantly metastatic sites (Cheng et al., 1994).

Risk factors

The risk factors of prostate cancer can be classified as exogenous and endogenous factors.

Exogenous factors

Life style, dietary factor, specific environmental and geographical factors are the exogenous determining factors affecting the rate of tumor
promotion and progression (Henderson et al., 1982; Carter et al., 1990; Henderson et al., 1991; Giovannucci et al., 1993).

Life Style

Vasectomy and physical activity influences the risk of prostate cancer (Gronberg, 2003). Other than those factors alcohol consumption and cigarette smoking are also involved in the risk of prostate cancer (Matzkin and Soloway, 1993). Alcohol influences the metabolism and serum levels of sex hormones and specifically increases metabolic clearance of testosterone, therefore some role in the process of prostatic carcinogenesis (Denis and Hayes, 2001). There are several biologically plausible mechanisms suggesting that increased exposure to carcinogenic compounds in cigarettes, such as polycyclic aromatic hydrocarbons, heterocyclic aromatic amines, and nitrosamines promote prostate carcinogenesis (Hecht, 2006). Important hormonal factors may also be influenced by smoking, it is suggested that male smokers have elevated circulating levels of testosterone, androstenedione, and dihydrotestosterone (DHT) (Shiels et al., 2009), compared to non smokers.

Dietary Factors

Dietary calcium, specifically dairy calcium may increase the risk of prostate cancer. The increased calcium levels in the circulation inhibit the 1α, 25-dihydroxy vitamin D₃ synthesis. Vitamin D₃ involves normal cell,
differentiation and also inhibits prostate cancer cell growth and development (Krishnan et al., 2007). Vitamin D₃ can protect oxidative stress in nonmalignant human prostate epithelial cell lines (Bao et al., 2008). A high intake of both red meat and dairy product was associated with a statistically significant two-fold elevation in risk of metastatic PCa, compared to low intake of both products (Michaud et al., 2001; Muller et al., 2009) and intake of well cooked total meat was associated with a 1.26-fold increased risk of incident PCa and a 1.97-fold increased risk of advanced state of the disease (Koutros et al., 2008). The risk of prostate cancer is positively associated with calcium from dairy products (Allen et al., 2008). Calcium intake increases risk of prostate cancer among Singapore and Chinese men (Butler et al., 2010).

Obesity

Obesity is more consistently related to aggressive prostate tumors and that abdominal obesity may be associated with an increased risk of PCa even in relatively lean men (Hsing et al., 2001; Hubbard et al., 2004). Higher serum levels of leptin, the product of obesity gene, Ob have been linked to larger tumor volume (Gade-Andavolu et al., 2006). Leptin, is an adipocyte-derived cytokine that is closely associated with obesity, has associated with the motility and migration of human prostate cancer cells and expression of αvβ3 integrin on these cells (Huang et al., 2011).
Environmental Factors

An epidemiological study suggests that increased cadmium exposure is correlated with increased prostate cancer incidence (Waalkes and Rehm, 1994). *In vitro* studies demonstrate that cadmium causes malignant transformation of rat ventral prostatic epithelial cells (Ghatak *et al.*, 1996) and its exposure to cultured human prostatic epithelium, induces cell proliferation (Webber, 1985). Cadmium down-regulates expression of the X-linked inhibitor of apoptosis protein (XIAP) in prostate cancer cells (Golovine *et al.*, 2010). Arsenic metal ions induce significantly, increased the risk of prostate cancer (Barthel, 1981). Prostate stem cells have a survival selection advantage during arsenic exposure that favors their accumulation and facilitates their malignant transformation (Tokar *et al.*, 2010). Exposure to environmental estrogen agonists such as Dichloro diphenyl trichloro ethane (DDT), Poly chlorinated biphenyls (PCB), Methoxychlor and other herbicides and fungicides may significantly contribute prostate cancer risk (Barthel, 1981; see Thomas and Colborn, 1992; Prins, 2008).

Geographical pattern

Prostate cancer incidence is high in the Northern European countries, United States and Canada. Southern Europe and South Americans, African observed in moderate incidence while, Asian countries have the lowest incidence (Zaridze *et al.*, 1984). The incidence of prostate cancer is
rising rapidly in Sweden (Adolfsson et al., 2007). The rate of prostate cancer increases at least by 1% every year in Asian countries as the people adapting to western life style. The prostate cancer incidence in India, ranks 5\textsuperscript{th} in mortality rate (Quinn and Babb, 2002).

**Endogenous Factors**

The endogenous risk factors are genetic factors and endocrine factors.

**Family history**

Family history of prostate cancer is a risk factor for prostate cancer occurrence (Colloca and Venturino, 2011). Prostate cancer occurs almost exclusively in men over the age of 40 and most often after the age of 50. It is estimated that by age 70, about 65% of men have at least microscopic evidence of prostate cancers. Increased risk of prostate cancer is associated with the family history of the disease (Wittemore et al., 1995). The overall risk increases 2-3 folds in men with family history and it does not differ among Africans, American, Asian American and whites in US (Wittemore et al., 1995).

**Genetic Factors**

Prostate cancers can be divided into three groups as hereditary, familial and sporadic. More than 85% of all prostate cancers are sporadic and
only 10-15 per cent cancers are genetically determined. Afro-american men have worse staging and prostate cancer (PC) grading as well as a more aggressive disease than Caucasian. This is due to the number of CAG base triplet (polyglutamin) and GGC base triplet (polyglycin) repetition in the first exon of the AR gene. The AR gene is located on the short arm of chromosome X (Xq11–12) and the variability in the AR gene length is determined by polymorphism in the N-terminal region. The normal number of polyglutamin repetition is 8-35 and most men have 21 repetition (Afro-Americans, white and Asian men have 18, 21 and 22 repeats, respectively). Lower than 21 repetition of polyglutamin is connected with higher prostate cancer risk, earlier onset of disease and a more aggressive form, due to stronger binding of ligand and its long-lasting hyperstimulation of the androgen receptor (Marcelli et al., 2000).

AR gene mutations in the steroid binding domain was found in metastatic prostate cancer patients (Culig et al., 1993). The frequency of mutation generally appears higher in hormone refractory metastatic tumor compared with untreated lower grade primary tumors (Marcelli et al., 2000). Androgen receptor germline sequence variants cause prostate cancer risk (Lindstrom et al., 2010).
**Genes related to prostate cancer development** *(see Krala et al., 2011)*

<table>
<thead>
<tr>
<th>Localization</th>
<th>Genes/Locus</th>
<th>Remark</th>
</tr>
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<tbody>
<tr>
<td>1q25.3</td>
<td>RNaseL/HPC1</td>
<td>Age younger than 65 years, higher Gleason grade, advanced stage of diagnosis; strongest relationship with PC in families with higher than 5 affected men. Affects induction of apoptosis and susceptibility to infection</td>
</tr>
<tr>
<td>17p11</td>
<td>ELAC2</td>
<td>Unknown function</td>
</tr>
<tr>
<td>8p22–23</td>
<td>MSR1</td>
<td>Initiation of inflammation; affects induction and course of infection</td>
</tr>
<tr>
<td>Xq27–28</td>
<td>HPCX</td>
<td>Higher risk of PCa in men with affected brother than with affected father</td>
</tr>
<tr>
<td>20q13</td>
<td>HPC20</td>
<td>Higher age at PCa diagnosis</td>
</tr>
<tr>
<td>17q21</td>
<td>BRCA1</td>
<td>More frequent in younger men</td>
</tr>
<tr>
<td>13q12–13</td>
<td>BRCA2</td>
<td>Function in DNA reparation</td>
</tr>
</tbody>
</table>

The expression of estrogen receptor is commonly involved in the progression of prostate cancer (Dunsmuir *et al.*, 2000). The genetic polymorphism of genes in the estrogen metabolism significantly increases with familial prostate cancer risk. Moreover, one study suggests that a polymorphism in codon 10 of ER-α and variants of the GGGA polymorphism from the α-gene might be associated with increased risk of prostate cancer (Talcott *et al.*, 2003). Whereas, ER-β was decreased during malignancy progression owing to methylation of CpG dinucleotides in the promoter gene.
This suggests that ER-β is involved in the tumor suppression function (Zhu et al., 2004; Ji et al., 2005).

**Oncogenes and Tumor suppressor genes**

The oncogene and tumor suppressor genes are involved in neoplastic transformation of the prostate (Bostwick, 1996). Ras oncogene are activated by point mutations in codon 6 causing missense mutations leading to prostate cancer (Suzuki et al., 1994). The c-myc gene is a cellular proto-oncogene and is a nuclear phosphor-protein. The potential function is in promoting DNA replication, regulation of the G₀/G₁ cell-cycle transition, and control of cellular differentiation (Kato et al., 1992). Over expression of c-myc oncogene is seen in prostate cancer (Hawksworth et al., 2010).

In human cancer cells, the genomic alterations are characterized by abnormal methylation. The pattern of abnormal methylation includes hypermethylation, redistribution of methylation, and demethylation of normal methyl regions. Loss of 5’-methyl cytosine, or hypomethylation has been reported to occur in human prostate cancer, but its significance is not clear (Bedford and Van Helden, 1987).

The p53 gene located at 17p13.1 encodes a 53 kDa phosphorylated protein that negatively regulates cell growth (Levine et al., 1991). Inactivation of the gene, contributes to the genesis and progression of numerous cancers.
The frequency of p53 mutation in primary prostate cancer ranges from 1-42% (Downing et al., 2003).

Phosphatase and tensin homolog deleted on chromosome 10 (PTEN) a Protein tyrosine phosphates is located on chromosome 10q²³. PTEN appears to behave as tumor suppressor gene. This can suppress tumor cell growth by antagonism of protein tyrosine kinases, which may regulate tumor cell invasion and metastases through interaction at focal adhesions. Inactivation of PTEN gene by homozygous deletions occurs in approximately 13% of primary localized prostate cancer (Wang et al., 1998).

KAI1 gene located on human chromosome 11p¹¹.² has been shown to suppress the tumor metastasis when introduced into the highly metastatic Dunning R-3327 rat prostate cancer cell line AT6.1 (Gao et al., 1997). In addition, expression of the gene is reduced in human cell line derived from metastatic prostate cancer specimen (Gao et al., 1997). Another important metastasis suppressor gene (MSG) is located near KAI1 is CD44 (Dong et al., 1995). The CD44 gene 11p13 encodes an integral membrane glycoprotein that was initially discovered to be a lymphocyte homing molecule. Its expression is upregulated in prostate cancer (Iczkowski et al., 2003). Polymorphisms in p21 gene are overexpressed in prostate cancer (Facher et al., 1997).
Endocrine Factors

The polypeptide growth factors are important in the normal regulation of prostate development and growth. The inappropriate expression of some of these growth inhibitory factors appears to contribute to prostate cancer progression. Epidermal growth factor (EGF) promotes chemomigration of metastatic prostate cancer cells to lymph node and medullary bone sites (Rajah et al., 1996). The basic Fibroblast growth factor (FGF) has been detected in human prostate cancer specimen (Mydlo et al., 1998). Several studies suggested that alteration in the IGF signaling axis is associated with an increased risk of prostate cancer (Chokkalingam et al., 2001).

Androgens are involved in the development and progression of prostate cancer (Ross et al., 1986). Serum androgen levels tend to be elevated in high-risk populations, such as African, American; however, androgen levels are found to be normal in Japanese men than in Caucasians.

There are multiple evidences suggesting that estrogens are involved in prostate carcinogenesis. In worldwide the African-Americans have the higher risk of prostate cancer with elevated levels of serum estrone (E1) and estradiol (E2) levels even in healthy young men (Srinivasan et al., 1986). More epidemiological data support that early neoplastic epithelial cells develop in an environment of rising estrogenic stimulation and decreasing androgenic influence. CCDC62/ERAP75 is a new co-activator of ER and this
protein is mainly present in the nucleus and widely expressed in many PCa cell lines (PC-3, DU145, LNCaP, 22Rv1) than in the normal prostate epithelial cells (BPH-1). CCDC62/ERAP75 which is preferentially expressed in PCa cells enhances the ER-β transactivation of, target genes expression and E2-mediated LNCaP cell growth (Chen et al., 2009).

**Benign Prostatic Hyperplasia (BPH)**

In upto half of men in their fourth decade, the prostate begins to enlarge through a process of cell multiplication called benign prostatic hyperplasia (BPH). The symptoms of BPH can mirror late-stage prostate cancer because the enlarging inner portion of the prostate puts pressure on the urethra, which can potentially cause urinary problems. About 80% of men eventually develop enlarged prostates, but only some experience significant symptoms. BPH is a normal condition and is not life-threatening. Because the prostate enlargement in BPH is affected by testosterone, many men are concerned that it may be related to prostate cancer. Fortunately, evidences indicate that it has no effect one way or the other. The two conditions develop in different parts of the prostate. BPH occurs in the inner zone of the prostate, while cancer tends to develop in the outer area (Berry et al., 1984).

**Prostatitis**

Prostatitis is an inflammation of the prostate, often caused by bacterial infections. Symptoms include urgency, frequency, and pain in
urination, accompanied by fever and blood in the urine. Serum prostate specific antigen (PSA) levels are elevated, but information regarding the involvement of bacterial prostatitis in prostate cancer development is sparse (Blumenfeld et al., 1992).

**Screening and Diagnosis**

The PSA blood test is widely available for screening men for prostate cancer. Digital rectal examination (DRE), a physician palpates the prostate in order to feel lumps or masses. Biopsy, if preliminary tests raise the suspicion of cancer, physicians will perform a biopsy. Biopsy is used to diagnose prostate cancer, and is a very accurate method for predicting the severity of an existing cancer. Bone Scans and X-Rays reveal, whether the cancer has invaded the bones. Computed Tomography (CT) and Magnetic Resonance Imaging (MRI), scans can further pinpoint the location of cancer that has spread beyond the prostate.

**Symptoms**

Prostate cancer usually causes no symptom in the early stages. As the malignancy spreads, it may constrict the urethra and cause urinary problems. Later-stage urinary symptoms typically include, weak urinary stream, inability to urinate, blood in the urine, interruption of urinary stream (stopping and starting), and pain or burning sensation during urination.
Treatment

Hormonal therapy

Androgen deprivation therapy (ADT), with gonadotrophin releasing hormone analogues or surgical orchidectomy, is standard treatment for advanced PCa (Wilt et al., 2005) and antiandrogen, LHRH agonists, such as leuprolide also used for prostate cancer treatment.

Surgery

Radical prostatectomy is the removal of the prostate gland. The vessels that carry semen to the surrounding tissue may also be removed. With cancer that has spread beyond the prostate, the pelvic lymph nodes should be removed.

Radiation

Is used to destroy tumors. Complications include damage to adjacent organs such as the gastro-intestinal tract and bladder incontinence and impotence.

Common side effects of androgen suppression drugs

Osteoporosis is a disease, loss of bone density. This risk is higher with orchidectomy than with androgen suppressants. Some androgen suppressants, such as bicalutamide, may cause less bone loss. The use of
selective estrogens receptor modulators (SERMs) may actually be bone protective. A number of medications are available to help prevent or reduce bone loss. Bisphosphonates are used for preventing the bone loss. Other common side effects include, diarrhea, loss of muscle mass, psychological disturbances, fatigue, loss of sexual drive and sexual dysfunction, swelling of the breasts (gynecomastia), nausea and vomiting, hair loss and anemia (Debruyne, 2002).

2.4 ANDROGEN /ANDROGEN RECEPTOR IN PROSTATE CANCER

Locally defined disease is often successfully treated with surgery or radiotherapy; however, disease recurs in an estimated 15% to 30% of patients (Roberts et al., 2001). The androgen receptor (AR) is the mediator of the physiologic effects of androgen. It regulates the growth of normal and malignant prostate epithelial cells. Upon ligand binding, AR translocates to the nucleus, binds to DNA recognition sequences, and activates transcription of target genes, including genes involved in cell proliferation, apoptosis, and differentiation (Suzuki et al., 2003).

Androgen-dependent cancer

Androgens and AR are critical components for prostate gland development, prostatic function, and are involved in prostate tumorigenesis.
AR expression has been observed in primary prostate cancer and can be detected throughout progression in both hormone-sensitive and hormone-resistant cancers (Hernes et al., 2000), however, the importance of AR during late stages of prostate cancer is not entirely clear. Androgen ablation therapy is highly successful for the treatment of hormone-sensitive prostate cancer; however, hormone resistance significantly limits its benefits. Hormonal ablation therapy will control metastatic disease for 18 to 24 months (Denis and Murphy, 1993), but once metastatic prostate cancer ceases to respond to hormonal therapy, median survival decreases significantly (Eisenberger et al., 1998). The molecular mechanisms underlying the progression of prostate cancer to hormone-independent disease remain unclear.

**Androgen-independent cancer**

There are reports suggesting that there is an heterogenous expression of AR throughout prostate cancer. Initial studies showed that AR mRNA is present in androgen-sensitive prostate cancer cell lines, but is absent or expressed at low levels in androgen-independent cell lines (Trapman et al., 1990). de Vere White et al. (1997) showed a loss of AR expression in 33% of prostate cancer patients following combined androgen blockade therapy. Hobisch et al. (1996) showed complete loss of AR in prostate cancer lymph node metastases. Kinoshita et al. (2000) showed a significant loss of AR expression in advanced prostate cancer and found that methylation of the AR
promoter which lead to metastatic hormone resistant tumors. These results suggest that loss of AR expression during prostate cancer progression may be associated with the development of androgen independence in a subset of patients.

A variety of AR mutations have been identified in prostate cancer cell lines and human tumors with the most common mutations occurring in the ligand binding domain. Mutations in the ligand binding domain alter the specificity of the AR enhancing the binding of estrogens, progesterone, and antiandrogens; and thereby decreasing its dependency on androgens while stimulating cell growth (Culig et al., 1993). In addition to AR mutations, a variety of growth factors, including insulin-like growth factor I, epidermal growth factor, and keratinocyte growth factor, can activate androgen-responsive genes via the AR, suggesting that androgen independence occurs due to the over expression of growth factors in the local environment (Culig et al., 1994). Clearly, mutations of the AR and activation of AR via growth factors play a role in androgen independent growth. Mechanisms for AR down regulation in prostate cancer have been proposed, including promoter silencing by DNA methylation (Jarrard et al., 1998) and transcriptional repression by transcription factors nuclear factor-κB and nuclear factor-1 (Song et al., 1999).
2.5  EGF/EGFR

The activation of epidermal growth factor receptor (EGFR) is not only critical for cell proliferation but also contributes to other processes that are crucial to cancer progression, including angiogenesis, metastatic spread, and the inhibition of apoptosis (Hirata et al., 2002). EGFR is a 170-kDa transmembrane protein that displays intrinsic Tyrosine Kinase activity (Marmor et al., 2004). It is a member of a group of four closely related receptors called the human EGF (or HER) family. EGFR has been shown to bind a variety of ligands including EGF, transforming growth factor-α and amphiregulin (Yarden and Sliwkowski, 2001). Ligand binding triggers receptor dimerization, promoting the autophosphorylation of specific tyrosine residues within the intracellular domain, creating binding sites for transduction proteins containing Src homology 2 or phosphotyrosine-binding domains (Hynes and Lane, 2005). Several downstream signaling pathways are subsequently activated including the Ras/mitogen-activated protein kinase cascade, phosphatidylinositol-3 kinase/ Akt, protein kinase C, and the signal transducers and activators of transcription factors (Marmor et al., 2004). These pathways promote cell proliferation, prevent apoptosis, and increase cell migration. The receptors and ligands of the EGFR family mediate complex interactions between tumor cells and the neoplastic environment that ultimately results in enhanced tumor growth and progression. On the other hand, the ectodomain shedding of EGFR ligands including epidermal growth
factor (EGF), transforming growth factor-a (TGF-a), heparin binding EGF-like growth factor (HB-EGF), and amphiregulin (AR), act in an autocrine or paracrine fashion and activate EGFR via a ligand-dependent mechanism (Borrell-Pages et al., 2003). These growth factors and their receptors have been reported to be overexpressed in advanced prostate cancer including EGF, transforming growth factors (TGF)-α and β, fibroblast growth factors (FGF), and insulin-like growth factors (IGF) (Culig et al., 1996).

2.6 PI3K/AKT pathway

Phosphatidylinositol-3 kinase (PI3K) is a heterodimeric enzyme composed of 110-kDa catalytic subunit and 85-kDa regulatory subunit which serves as a major signaling component downstream of growth factor receptor tyrosine kinases (Luo et al., 2003). PI3K catalyzes the production of the lipid secondary messenger phosphatidylinositol-3,4,5-triphosphate, which in turn activates a wide range of downstream targets, including the serine/threonine kinase Akt (Luo et al., 2003). Full activation of Akt/PKB is PI3K dependent and requires both recruitment to the plasma membrane and phosphorylation on two key residues, Thr 308 and Ser 473 (Lawlor and Alessi, 2001; Cantley, 2002). The PI3K/Akt pathway regulates multiple cellular processes, including cell proliferation, differentiation, survival, growth, motility and angiogenesis.

Intrinsic apoptotic program in mammalian cells is activated, with the consequent release of apoptogenic factors such as cytochrome C, which is
critical for the progression of apoptosis (Parone et al., 2002). Akt, a major
downstream effector of growth factor-mediated cell survival was shown to
inhibit apoptosis induced by a variety of apoptotic stimuli. Akt has shown to
inhibit molecular events that precede cytochrome C release (Gottlob et al.,
2001), suggesting that Akt inhibits apoptosis by maintaining the integrity of
mitochondria. It has been demonstrated that members of the Bcl-2 protein
family are critical regulators of mitochondrial integrity. An apoptotic stimulus
may activate one or more of the “BH3- only containing” proapoptotic
members of the Bcl-2 family, which proceed to directly or indirectly activate
one or both of the terminal Bcl-2 family death effectors BAX and BAK at the
mitochondria (Scorrano and Korsmeyer, 2003).

The antiapoptotic Bcl-2 family members, Bcl-2 and Bcl-xL,
directly antagonize the activity of the BH3-only proteins. Akt has shown to
negatively regulate, the activity of several proapoptotic members of the Bcl-2
family such as BAX and BAK. Akt elevates mitochondrial hexokinase
(mtHK). Hexokinase (HK) catalyzes the phosphorylation of glucose to yield
glucose-6-phosphate (G-6-P), which constitutes the first committed step of
glucose metabolism (Gottlob et al., 2001). Mammalian cells express upto
three high-affinity HK isoforms, two of which, HKI and HKII, associate with
the cytoplasmic face of the outer mitochondrial membrane (OMM). An
amino-terminal hydrophobic domain found only in HKI and HKII mediates
this interaction. HKI and HKII binds to the voltage-dependent anion channel
(VDAC) in the outer mitochondrial membrane thus inhibiting the mitochondrial binding of BAX (Pastorino et al., 2002). Therefore Akt regulates mitochondrion-associated proteins, such as HKs, which may antagonize the activation of BAX and BAK at the mitochondria following an apoptotic insult, thereby maintaining mitochondrial integrity and preventing the release of apoptogenic factors.

Akt prevents apoptosis by phosphorylating and inactivating caspase-9, and the proapoptotic Bcl-2 family member, Bad (Datta et al., 1997; Cardone et al., 1998). Akt even phosphorylates the transcription factor, forkhead, causing it to be localized in the cytoplasm. The Forkhead transcription factor family has been shown to be directly phosphorylated by Akt and the inactivation of the Forkhead family member FKHRL1 promotes cell survival (Zhong et al., 1999).

Apart from regulating apoptosis Akt also promotes cell proliferation also. Progression through the cell cycle is controlled by the activity of protein kinase complexes consisting of cyclins and cyclin dependent kinases (cdks) and associated regulatory proteins (Malumbres and Barbacid, 2001). Cyclin dependent kinases (cdks) are activated by binding to specific cyclin proteins that are synthesized periodically during the cell cycle. Progression from G1 to S phase of the cell cycle requires formation of complexes between cdk4 or cdk6 and D-type cyclins during early to mid-G1,
followed by formation of complexes of cdk2 and cyclin E during late G1. Accumulation of the D-type cyclins in G1 is required for cell cycle entry and is regulated by extracellular growth factors (Sherr and Roberts, 1999). During G1/S transition, Akt regulates the level of cyclin D, c-myc, p27kip1 and p21waf1 by preventing their proteosomal degradation. GSK-3β phosphorylates cyclin D1 at Thr 286 which promotes its degradation via ubiquitin-mediated pathway (Diehl et al., 1998). Thus, Akt phosphorylating and inactivating its substrate GSK-3β prevents the degradation of cyclin D1 which facilitates the G1/S progression. Akt can also inactivate the cdk inhibitor proteins p21 and p27, thereby promoting cdk activity and cell cycle progression (Liang and Slingerland, 2003). It is reported earlier that PI3K/Akt pathway provides major survival signals to prostate and many other cancer cells (Datta et al., 1999; Kandasamy and Srivastava, 2002; Downward, 2004). Constitutive activation of Akt is frequently described in many types of human cancers (Khwaja, 1999). Increase of p-Akt expression, particularly at serine 473, has been shown to correlate with higher Gleason score and is an excellent predictor of poor clinical outcome in prostate cancer patients (Kreisberg et al., 2004).
2.7 RAS/RAF/MAPK PATHWAY

The Ras/Raf/MEK/ERK cascade couples signals from cell surface receptors to transcription factors, which regulate gene expression. This pathway is often activated in certain tumors by chromosomal translocations such as BCR-ABL, mutations in cytokine receptors such as Flt-3, Kit, Fms or over expression of wild type or mutated receptors, e.g., EGFR (Mc Cubrey et al., 2007). This pathway has diverse effects which can regulate cell cycle progression, apoptosis or differentiation (Steelman et al., 2004).

This pathway consists of a small G-protein of the Ras family and three-tiered kinase cascade Raf/ MEK/ERK. The Ras protein is anchored to the cell membrane. The activated receptors assemble the multimeric proteins...
at the cell membrane that contain guanosine exchange nucleotide factor (GEF) and Son of sevenless (SOS) proteins. GEFs promote Ras to release GDP and bind to GTP. The resulting conformational change Ras/GTP to interact with the effector molecules such as the RAF. This leads to the translocation of Raf to the cell membrane where it is activated.

The activation of Raf occurs via a multimeric step. The mammalian Raf gene family consists of A-Raf, B-Raf and Raf-1 (C-Raf). Raf is a serine/threonine kinase and is normally activated by a complex series of events including: (i) recruitment to the plasma membrane mediated by an interaction with Ras (Yan et al., 1998); (ii) Dimerization of Raf proteins (Luo et al., 1996); (iii) phosphorylation/dephosphorylation on different domains (Fabian et al., 1993); (iv) disassociation from the Raf kinase inhibitory protein (RKIP) (Dhillon et al., 2002) and (v) association with scaffolding complexes (e.g., kinase suppressor of Ras, (KSR) (Chang et al., 2003). There are at least thirteen regulatory phosphorylation sites on Raf-1 (Steelman et al., 2004). Some of these sites e.g., S43, S259 and S621 are phosphorylated when Raf-1 is inactive. This allows 14-3-3 to bind Raf-1 and confer a configuration which is inactive. Upon cell stimulation, S621 becomes transiently dephosphorylated by an unidentified phosphatase. Phosphatases such as protein phosphatase 2A (PP2A) dephosphorylate S259 (Dhillon et al., 2002). 14-3-3 then disassociates from Raf-1. This allows Raf-1 to be phosphorylated at S338, Y340, and Y341, rendering Raf-1 active. The S338 residue present
in Raf-1 is conserved among the three Raf isoforms. S338 phosphorylation on Raf-1 is stimulated by Ras. The scaffolding protein RKIP has been shown to inhibit Raf-1 activation and downstream signaling (Corbit et al., 2003). RKIP is a member of the phosphatidylethanolamine-binding protein (PEBP) family. Interestingly RKIP can bind either Raf or MEK/ERK but not to Raf, MEK and ERK all together.

Activated Raf phosphorylates the MEK which in turn activates the ERK protein. MEK1 (multi-gene family is evolutionarily mitogen-activated protein kinase/ERK kinase protein kinase) is a tyrosine (Y-) and S/T-dual specificity (Alessi et al., 1994). Its activity is positively regulated by Raf phosphorylation on S residues in the catalytic domain. All three Raf family members are able to phosphorylate and activate MEK. MEK1 and MEK2 will later phosphorylate and activate extracellular-signal-regulated kinases 1,2 (ERK or MAPK), which are S/T kinases. ERKs can directly phosphorylate many transcription factors including Ets-1, c-Jun and c-Myc. ERK can also phosphorylate and activate the 90 kDa ribosomal S6 kinase (p90Rsk).

2.8 INTERLEUKIN-8

Chemokines are chemotactic cytokines that cause the directed migration of leukocytes, and are induced by inflammatory cytokines, growth factors and pathogenic stimuli. Chemokine signalling results in the transcription of target genes that are involved in cell invasion, motility,
interactions with the extracellular matrix (ECM) and survival. The chemokine superfamily consists of over 40 ligands and approximately 20 receptors (Rossi and Zlotnik, 2000). They can be classified into four groups: -CXC-, -CC-, -C- and -CX3C – according to amino acid position at the N terminal. Several chemokines have been shown to be present in cancer tissue including CCL2, CCL3, CCL4, CCL5, CCL8, CCL17, CCL18, CCL20, CCL21, CCL22, CCL27, CCL28, CXCL1, CXCL2, CXCL8, CXCL9, CXCL10, CXCL12, and CXCL13 (Mantovani *et al.*, 2004; Allavena *et al.*, 2008).

Interleukin-8 (IL-8), alternatively known as CXCL8, is a proinflammatory CXC chemokine. Transcription of the IL-8 gene encodes for a protein of 99 amino acids that is subsequently processed to yield a signaling competent protein of either 77 amino acids in non immune cells or 72 amino acids in monocytes and macrophages. The biological effects of IL-8 are mediated through the binding of IL-8 to two cell-surface G protein–coupled receptors, termed CXCR1 and CXCR2 (Murphy and Tiffany, 1991). IL-8 signaling has been shown to promote the transactivation of the epidermal growth factor receptor in ovarian cancer (*Knall et al.*, 1996) and vascular endothelial cells (*Schraufstatter et al.*, 2003). IL-8 signaling regulates the activity of the mitogen activated protein kinase (MAPK) signaling cascade that constitutes a number of serine/threonine kinases that are colocalized via their interaction with scaffolding proteins in close proximity to cell-surface receptors.
IL-8 signaling has been shown to induce the activation of this classic MAPK signaling cascade, with downstream phosphorylation of Erk 1/2 detected in both neutrophils (Knall et al., 1996) and cancer cells (MacManus et al., 2007). In neutrophils, phosphatidylinositol-3 kinase activity has been identified as a key intermediate in coupling IL-8 receptors to MAPK signaling (Knall et al., 1996), whereas studies conducted in ovarian and lung cancer cell lines show that IL-8 signaling transactivates the epidermal growth factor receptor, promoting the downstream activation of MAPK signaling, mediated through growth factor receptor binding protein 2/SOS-promoted activation of the monomeric small G protein, Ras-GTPase (Luppi et al., 2007). Activation of the ERK-MAPK signaling ultimately describes a putative pathway linking IL-8 signaling to the activation of E2F and activator protein transcription factors, whose function is to primarily regulate the transcription of many genes implicated in cell proliferation and metastasis.

2.9 UROKINASE-TYPE PLASMINOGEN ACTIVATOR (uPA)

The epidermal growth factor receptor (EGFR, erbB1, or HER1) is a 170-kDa member of the erbB family of PTKs, which are transmembrane receptors with important roles in development, differentiation, proliferation and migration (Rusch et al., 1997). The activation of EGFR by ligand binding causes dimerization and autophosphorylation of the receptor and subsequent
recruitment of downstream molecules, leading to mitogenic signaling (Yarden, 2001). Upregulation of epidermal growth factor receptor (EGFR) and subsequent increases in extracellular-regulated kinase (ERK) and Akt signaling are implicated in prostate cancer progression (Gan et al., 2010).

Urokinase-type plasminogen activator (uPA) is a serine protease that is causally involved in cancer progression, especially invasion and metastasis. The human uPA gene is located on chromosome 10q (10q22) (Helenius et al., 2001) and encodes a 53 kDa protein. The protein is initially synthesized as a catalytically inactive single chain peptide. Conversion into the active form can be brought about, at least in vitro, by a number of proteases such as plasmin, cathepsin B and cathepsin L. The active form of uPA consists of a two-chain molecule in which the N-terminal A-chain is linked to the B-chain by a single disulphide bond. The A-chain (amino acids 1-158) contains a growth-factor-like domain (amino acids 1±49) while the B-chain contains the catalytic site (Andreasen et al., 1997).

uPA can be regarded as a multifunctional protein that is involved in both proteolysis and signal transduction. As a protease, its best known reaction is catalysis of conversion of the zymogen plasminogen into active plasmin. Plasmin, in contrast with uPA, has multiple substrates. It can promote degradation of diverse extra cellular matrix (ECM) substrates such as fibrin, fibronectin and laminin (Andreasen et al., 1997). It can activate the
precursor forms of certain matrix metalloproteases (MMPs) such as MMP-3, MMP-9, MMP-12 and MMP-13 (Carmeliet et al., 1997). uPAR expression was an adverse prognostic factor for prostate cancer (Thomas et al., 2009).

The formation of the active MMPs allows further degradation of the ECM, especially interstitial and type IV collagen. Plasmin can also activate or release specific growth factors such as fibroblast growth factor 2 and transforming growth factor β (Rifkin et al., 1990). These pleiotropic growth factors have the potential to enhance tumour progression by stimulating angiogenesis and enhancing both cell proliferation and migration. uPA-catalysed proteolysis occurs in vivo while the protease is attached to a membrane anchored receptor known as the uPA receptor (uPAR). uPAR, which is a member of the Ly-6 family of molecules, is a 55-60 kDa glycoprotein. It consists of three homologous domains and is bound to the cell membrane by a glycosylphosphatidylinositol moiety. The primary binding between uPA and uPAR involves the growth factor domain of uPA (amino acids 12-32) and the N-terminal end, or domain 1, of the receptor. Other regions of ligand and receptor may also participate in binding, for example amino acids 136-142 of uPA (Guo et al., 2000) and domains 2 and 3 of uPAR (Ossowski and Aguirre-Ghiso, 2000). Binding of uPA to its receptor has two main consequences. First, it leads to both enhanced and focused proteolysis. Secondly, ligand receptor interaction results in signal transduction, including activation of mitogen-activated protein kinase, extracellular signal-regulated
kinases 1 and 2 and other signalling pathways. uPA signalling activates Fos and Jun transcription factors (Ossowski and Aguirre-Ghiso, 2000).

Since metastasis is the principal cause of mortality in patients with cancer and since uPA is a critical mediator of the process, uPA is a good candidate for investigation as a marker for predicting the likely formation of metastasis. uPA expression is increased in the plasma of patients with prostate cancer compared with those with benign prostatic hyperplasia (Hienert et al., 1988). uPA levels are particularly high in patients with advanced hormone refractory stages of this disease (Kirchheimer et al., 1984). However, the mechanism that regulates the expression of uPA gene expression at different stages of tumor progression has not been fully elucidated.

### 2.10 MATRIX METALLOPROTEINASES (MMPs)

The matrix metalloproteinases (MMPs) are a family of proteolytic enzymes present in both normal and pathological tissues in which matrix remodelling is involved, including embryonic development, wound healing, arthritis, angiogenesis, and tumour invasion and metastasis (Liotta et al., 1991). The enzymes contain a zinc atom at their active site and depend on calcium for their activity. The MMPs degrade the components of the extracellular matrix, with MMP1 degrading fibrillar collagen and the gelatinases (MMP2 and MMP9) being important in degrading the basement membrane. MMPs play a role in the invasion of normal tissues by tumors and...
their subsequent metastatic spread. The local production of MMP-9 and other proteases, such as plasminogen activator, by prostate cancer cells or stroma facilitates the degradation of the extracellular matrix and results in tumor invasion and subsequent metastasis (Festuccia et al., 1998). The proteolytic effect of MMPs facilitates the migration of endothelial cells through the altered extracellular matrix toward the source of the angiogenic stimulus. Hence MMPs are an integral component for the angiogenic process. It has been reported that there is a link between EGFR function and MMPs expression that may contribute to the invasive phenotype. There was an increased level of EGF in the urine of patients with bladder cancer. In human bladder tumour cell lines RT112, EGF at 10 or 50 ng/ml induced the MMP-9 expression (Nutt et al., 2003). In nonmalignant (S1) and malignant (T4-2) human breast epithelial cells it was reported that MMP-9 was regulated by Raf/MEK/ERK signaling in 3D cultures. Suppression of either MEK or MMP-9 using shRNAs allowed T4-2 cells to form quiescent structures (Briand et al., 1996; Weaver et al., 2002).
Apoptosis or programmed cell death is involved in development, elimination of damaged cells, and maintenance of cell homeostasis. Deregulation of apoptosis may cause diseases, such as cancers, immune diseases, and neurodegenerative disorders. The concept of apoptosis emerged with its unique and dynamic morphological features that are distinguishable from senescence or necrosis, such as cell shrinkage, plasma membrane blebbing, chromatin condensation, nuclear membrane breakdown, and formation of small vesicles from the cell surface also known as apoptotic bodies (Kerr et al., 1972). After apoptosis, the apoptotic bodies are rapidly...

Adapted from Waugh and Wilson, (2008)
Tanaka and Galliot, (2009)
engulfed by phagocytes, and thus a potential inflammatory response is avoided (Kerr et al., 1972). This deliberate physiological cell suicide concept was proved molecularly in the 1990s by Horvitz and colleagues in C elegans (Horvitz et al., 1994).

All apoptosis signaling pathways converge on a common machinery of cell destruction that is activated by a family of cysteine proteases (caspases) that cleave proteins at aspartate residues. Dismantling and removal of doomed cells is accomplished by proteolysis of vital cellular constituents, DNA degradation, and phagocytosis by neighboring cells (Strasser et al., 2000).

Caspases are synthesized as precursor proenzymes which are proteolytically processed to their active forms. Active caspases are composed of a heterodimer comprising a large subunit (P20 for caspase-1, P17 for caspase-3) that contains the catalytic cysteine residue, and a smaller subunit (P10 for caspase-1, P12 for caspase-3) that contains determinants which govern substrate specificity. Pre-caspases are activated by cleavage at critical aspartate residues that themselves conform to the substrate consensus for caspases. All pre-caspases have an N-terminal prodomain that is also removed during activation. For some caspases (caspase-3, -6, -7 and -14) the prodomain is short (10-40 residues) while for the other caspases it is extensive and contains recognizable domains. The extensive prodomains play important
roles in caspase regulation and function as signal integrators for apoptotic or pro-inflammatory signals (Hofmann et al., 1997). Two pathways of caspase activation have been described. The first one is mediated by death receptors or extrinsic pathway, controlled by caspases 8/10 which in turn activate downstream effector caspases such as caspase-3 and caspase-7 (Henson et al., 2001). This pathway is initiated by extracellular hormones or agonists that belong to the tumor necrosis factor (TNF) superfamily, including TNF-α, Fas/CD95 ligand, and Apo2 ligand/TRAIL. These agonists recognize and activate their corresponding receptors, members of TNF/NGF receptor family, such as TNFR1, Fas/CD95, and Apo2. Then, via a series of protein-protein interactions involving domains, which include the death domain and the death effector domain, the receptors will recruit specific adaptor proteins to form a complex called the death-inducing signaling complex (DISC) (Jiang and Wang, 2004). DISC recruits and activates the initiator caspases, caspase-8 or caspase-10, probably by bringing the procaspases close enough in proximity so that they can cleave each other. These activated initiator caspases trigger a caspase cascade and subsequent cell death by activating downstream executioner caspases, such as caspase-3 and caspase-7 (Jiang and Wang, 2004).

In second pathway diverse apoptotic signals converge at the mitochondrial level, including the release of cytochrome C from mitochondria into the cytosol. One of the primary regulators of the mitochondria-mediated
pathway of apoptosis is the family of B cell lymphoma-2 (Bcl-2) proteins (Green and Reed, 1998). Bcl-2 is the founding member of a large family of proteins that either promote or inhibit apoptosis (Cory and Adams, 2002; Danial and Korsmeyer, 2004). The family is typically divided into three groups according to structural homology and function. The first group includes the antiapoptotic proteins Bcl-2, and Bcl-XL. The second group includes the proapoptotic proteins Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist killer (Bak). The third group is also proapoptotic and encompasses the Bcl-2 homology 3 (BH3)-only proteins, including Bcl-2-interacting mediator of cell death (Bim), Bcl-2 antagonist of cell death (Bad), and Bcl-2-interacting killer (Bik).

Upon exposure of cells to apoptotic stimuli, cytochrome C is released from mitochondria into the cytosol, where it is one of several factors implicated in the proteolytic activation of caspase-3 by caspase-9 (Slee et al., 1999). Biochemical analysis has identified two cytosolic proteins, apoptotic protease-activating factors (Apaf)-1 and 3, which form the complex with cytochrome C that activates caspase-3. The caspase recruitment domain (CARD) domains in Apaf-1 and the prodomain of caspase-9 interact and, in the presence of cytochrome C and either ATP or ADP, this induces autocatalytic activation of the caspase which then activates the downstream caspase effector cascade involving caspases-3, -6, and -7 (Slee et al., 1999). The pro-apoptotic Bcl-2 family member Bax, cause permeabilization of the
inner membrane and mitochondrial depolarization by binding to the adenine nucleotide translocator (ANT). This process allows entry of water and solutes into the matrix and leads to mitochondrial swelling thus leading to hyperpolarization of the inner mitochondrial membrane. This drop in membrane potential follows the release of cytochrome C.

**Apoptotic pathway**

Adapted from Kennedy et al., (1999)

2.12 NEEM

The medical properties of Neem have been known to Indians since time immemorial. The earliest Sanskrit medical writings refer to the benefits of neem’s fruits, seeds, oil, leaves, roots and bark. Each has been used in the Indian Ayurvedic and Unani systems of medicines, and is now being used in the manufacture of modern day medicinals, cosmetics, toiletries and
pharmaceuticals. The neem tree has been known as the wonder tree for centuries in the Indian subcontinent. Traditionally neem has been used for skin and blood purifying conditions. Neem has shown to have antiviral (Singh, 1990), anti-fungal and anti-bacterial properties. Neem is used in treatment of dermatitis eczema, acne, bacterial, fungal infections and other skin disorders (Negi, 1992). It helps support a strong immune system and is used in cases of inflammatory skin conditions. Alcoholic extract of the leaves was found to possess a significant blood sugar lowering effect, which are very useful against diabetes. Ancient ayurvedic practitioners believed high sugar levels in the body caused skin disease; Neem's bitter quality was said to counteract the sweetness. Neem may provide antiviral treatment for smallpox (Chetty and Rao, 1989), chicken pox and warts especially when applied directly to the skin. Neem produces pain-relieving, anti-inflammatory and fever-reducing compounds that can aid in the healing of cuts, burns (Nistherswar, 1992), sprains, ear aches, and headaches, as well as fevers. There are reports suggesting that neem extracts aid in suppressing malaria support its use in treatment (Kumar and Jain, 1998). Neem has broad applications to human and animal health, as well as organic farming. Preparations from the leaves or oils of the tree are used as general antiseptics. Neem oil is commonly added to a variety of creams and salves. It is effective against a broad spectrum of skin diseases including eczema, psoriasis, dry skin, wrinkles, rashes and dandruff. It is effectively used as a mosquito
repellent. Because of its unpleasant smell, it is best when it is added to a formula with other essential oils, such as citronella. Neem oil is an effective and environmentally safe pesticide when it is diluted and sprayed on crops through irrigation systems. It is a healthier alternative to artificial chemical pesticides. Neem oil does not harm the soil and it increases the yield.

![Image of Neem](image)

**Taxonomical description of Neem**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Order</td>
<td>Sapindales</td>
</tr>
<tr>
<td>Family</td>
<td>Meliaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Azadirachta</td>
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**Ecology**

Neem is a large tree growing about 25 m in height with semi-straight to straight trunk, 3 m in breadth and spreading branches forming a broad crown. Neem is tolerable to most soil types including dry, stony, shallow
soils, lateritic crusts, highly leached sands and clays. With an extensive and deep root system, the neem grows in the regions with an annual rainfall below 400 mm, largely depending on the ground water levels (Anonymous, 2006). It grows on almost all types of soil including clayey, saline and alkaline soil, but does well on black cotton soils and deep well drained soil with good sub-soil water. Neem trees have the ability to neutralize acidic soils by a unique property of calcium mining (Hegde, 1995). Neem can grow in many different types of soil, but it thrives best on well drained deep and sandy soils (pH 4-10). It is a typical tropical/subtropical tree and exists at annual mean temperatures between 21-32°C. It can tolerate high to very high temperatures but cannot with stand a temperature below 4°C (leaf will start to shed and will die ultimately). There are an estimated 25 million trees growing all over India (see Rembold, 1996), of which (17.8%) are found in Tamilnadu and it is in the second place next to Uttar Pradesh (55.7%), and Karnataka (5.5%) occupying third places, respectively (Bahuguna, 1997).

The pinnate leaves of the neem are alternating, and they can be about 20 to 40 cm long, with about 20 to 30 dark green to medium green leaflets about 3 to 8 cm in length. The leaves when they are very young can be purplish or reddish in color. More often than not, the terminal leaf is found to be missing, and the petioles are found to be very short. The mature leaflets are often asymmetric, and their margins are dentate, except for the base of their basis copal half, which is very strongly cuneate and reduced.
Commercial Uses

Almost every part of the neem tree roots, leaves, flowers, seeds, trunks and branches has multiple uses.

Timber

The sapwood of neem tree is grayish white, while the heartwood is reddish brown. The wood is aromatic in nature though it is not that very lustrous still it can be easily sawn. Neem timber is very durable and is resistant to termites and woodworms (Bhowmik et al., 2010).

Bark

Neem bark contains tannins which are used in tanning and dyeing. Compounds extracted from neem bark are used in production of some dental-care products like toothpaste (Singh and Singh, 1992).

Leaves

Neem leaves possess excellent medicinal properties. Neem leaves are used in some parts of India as fertilizer especially, in the south Indian states (Mohanty et al., 2008). In some countries, neem leaves are used as in tobacco and tomato fields (El Shafiea and Basedow, 2003). They can be very effectively used to kill weeds by spreading them over plant roots to retain
moisture. Neem leaves can also be used to protect stored woolen and silk clothes from insects.

**Neem Cake**

Neem cake is widely used in India as fertilizer for sugarcane, vegetable and other cash crops. It can be used as livestock feed, fertilizer and natural pesticide. It provides organic nitrogen at the same time it also inhibits the nitrification process, when mixed with urea, before applying to the fields. The uses of neem coated urea in proportion of 90:10 saves up to 30% of the total chemical nitrogen requirement of the crops. This results in cost reductions of agricultural production.

**Fruits**

Neem fruits are bitter, purgative, antihemorrhoidal and anthelmintic (vermifuge) in nature.

**Flowers**

The flowers are used in vitiated conditions of pitta (balancing of the body heat) and kapha (cough formation). They are astringent, anthelmintic and non toxic (Bhatnagar et al., 1973).
Seeds

Neem seeds are also described as anthelmintic, antileprotic (Yoganarasimhan et al., 1979) and antipoisonous (Mukherjee et al., 2008). Seeds, along with leaves and dry neem cake, are an active ingredient in mosquito-coils.

Oil

Neem oil, derived from crushing the seeds, is antidermatonic, a powerful vermifuge and bitter in taste. It has a wide spectrum of action and is highly medicinal in nature. Oil is used in aroma therapy, it has been effective in killing head lice in children (Bhowmik et al., 2010).

Neem as an antifertility agent

Several potential approaches for induction of infertility have been investigated over a long period, including chemical, hormonal and immunological approaches. However, no suitable method has emerged that is effective and free from side effects. The juice of fresh green leaves of A indic a was believed to suppress “Kam Vasana” (desire for sex). In ancient India it was consumed by sanyasees in shrines and the pupils studying in Gurukul for the same purpose. The antifertility activity of A indica leaves were studied in male mice (Deshpande et al., 1980). Freshly prepared the aqueous extract of old and tender neem leaves was reported to have potent
spermicidal action. The aqueous extract of crushed green leaves of *A indica* was orally fed to mice every day for 1 month to study its effect on male reproduction function. It was observed that control mice showed 100% fertility rate. Whereas in *A indica* treated animals, the antifertility effect was 80%. Then after 45 days of withdrawal of treatment, the percentage of pregnancies was found to be 100%. Thus, *A indica* leaves have shown reversible male antifertility activity. The powder of *A indica* leaves is reported to cause histological and biochemical changes in the testes of rats (Joshi *et al*., 1996; Aladakatti *et al*., 2001). The ethanolic neem leaf extract (NLE) has been reported to induce abnormal head morphology and reduce mean sperm count in murine (Khan and Awasthi, 2003). Neem leaf extract is also reported to inhibit motility and viability of human spermatozoa treated *in vitro* (Khillare and Shrivastav, 2003).

Apart from neem and its products to have anti fertility effect in male animals it even regulates female fertility. The aqueous neem leaf extract (NLE) (10.0 mg/mL) induced degeneration of rat oocytes. After 1.0 h of NLE treatment shrinkage of oocyte volume was observed as a first visual change. Shrinkage of oocytes resulted in leakage of transparent cytoplasmic fluid out of corona that progressed as the treatment time of NLE was increased. Almost half of the oocyte volume was filled with leaked cytoplasmic fluid after 3.0 h of NLE treatment. NLE induced apoptosis to the oocytes through the activation of caspases. The NLE induced morphologic apoptotic changes were
inhibited when the oocytes were cotreated with NLE (10.0 mg/mL) along with caspase-3 inhibitor (1.0 µmol/L) for 3 h. This suggests that NLE also regulates the female fertility (Chaube et al., 2006). Neem oil is used as a vaginal contraceptive (Sharma et al., 1996) and its reversible antifertility effects have been reported in rats and bonnet monkeys (Upadhyay et al., 1994).

2.14 NEW INSIGHTS ON THE ANTICANCER PROPERTIES OF NEEM LEAF EXTRACT

*A indica* A. Juss, is one of the most versatile medicinal plants that have gained worldwide prominence owing to its medicinal properties. Almost all parts of the neem tree such as leaves, flowers, seeds, fruits, roots and bark are known to possess a wide range of pharmacological properties, but the medicinal utilities have been described especially for neem leaf. Extracts of neem leaf have been reported to be non-toxic, non-mutagenic and found to possess immunomodulatory as well as anti-inflammatory and anticarcinogenic properties (Subapriya et al., 2005). It has been previously reported that alcoholic extracts of neem leaf are more effective than aqueous extracts and exhibit a wide range of pharmacological properties (Chattopadhyay, 1998; Subapriya et al., 2005). There are many studies showing the ethanolic extract of neem leaves to possess anticancer activity. Ethanolic neem leaf extract (ENLE) exhibited anticancer activity against N-
methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced oxidative stress and gastric carcinogenesis (Subapriya et al., 2003).

In another study, effect of two different doses (250 and 500 mg per kilogram body weight) of 80% ethanolic extract of the leaves of *A indica* were examined on drug metabolizing Phase-I and Phase-II enzymes, antioxidant enzymes, glutathione content, lactate dehydrogenase, and lipid peroxidation in the liver of 7-week-old Swiss albino mice. Also anticarcinogenic potential of *A indica* leaf extract was studied on benzo (a) pyrene-induced fore-stomach and 7,12-dimethyl benz (a) anthracene (DMBA)-induced skin papillomagenesis. Findings revealed that 250 mg per kilogram body weight of *A indica* significantly inhibited the chemical carcinogenesis at peri-initiational stages of carcinogenesis through modulation of phase II detoxification enzymes, elevation of antioxidant enzymes level and by inhibiting lipid peroxidation and lactate dehydrogenase-induced damages. Chemopreventive response was measured by the average number of papillomas per mouse, as well as percentage of tumor-bearing animals. There was a significant inhibition of tumor burden (Dasgupta et al., 2004).

Administration of ENLE significantly reduced the incidence of DMBA-induced HBP carcinomas and tumour burden. ENLE downregulated anti-apoptotic Bcl-2 expression and upregulated pro-apoptotic Bim, caspase 8 and caspase 3 expression in the buccal pouch indicating that it has apoptosis
inducing effects in the target organ (Subapriya et al., 2005). ENLE exerted its chemopreventive potential and anticarcinogenic properties to DMBA induced HBP carcinomas by inhibiting cell proliferation and inducing apoptosis by the downregulation of PCNA, mutant p53 and Bcl-2 protein, associated with the upregulation of cytokeratin (Subapriya et al., 2006). *A indica* effectively inhibits tumors of colon cancer (Roy et al., 2006) and skin carcinogenesis in Balb/c mice (Koul et al., 2006).

Previously in our laboratory, a pilot study was done to examine the anticancer activity of ENLE and it has been shown to induce cell death in prostate cancer cells (PC-3) by inducing apoptosis as evidenced by a dose-dependent increase in DNA fragmentation and a decrease in cell viability. As well by up-regulating the expression of pro-apoptotic Bax protein and down-regulating the expression of Bcl-2 (Suresh et al., 2006).

Manikandan et al., (2008) have shown that neem leaf fractions exert greater inhibitory effect on hamster buccal pouch carcinogenesis. Both ethyl acetate and methanolic fraction of neem leaf extract showed *in vitro* antioxidant activity as well as *in vivo* antiproliferative and antiangiogenic effects and possessed significant chemopreventive potential. Similarly neem leaf fraction inhibited DMBA induced mammary carcinogenesis by modulating phase I and phase II xenobiotic metabolizing enzymes, oxidative stress as well as by inhibiting cell proliferation and by inducing apoptosis (Vinothini et al., 2009). During the last five decades, apart from the
chemistry of the neem compounds, considerable progress has been achieved regarding the biological activity and medicinal applications of neem.

In addition to ENLE, even aqueous neem leaf preparations have been shown to have anticancer activities. The aqueous extract of neem (*A. indica*) possessed anticarcinogenic effect against 7,12-dimethylbenz (a) anthracene (DMBA) induced buccal pouch carcinogenesis in Syrian male hamsters. Administration of neem leaf extract effectively suppressed oral carcinogenesis initiated with DMBA as observed by the reduced incidence of neoplasms. Lipid peroxidation, glutathione (GSH) content and the activities of glutathione peroxidase (GPx), glutathione S-transferase (GST) and γ-transpeptidase (GGT) were used to biomonitor the chemopreventive potential of neem. The aqueous extract of neem *A. indica* significantly decreased lipid peroxidation whereas GSH, GPx, GST and GGT were elevated in the oral mucosa of tumour bearing animals. Hence the neem leaves extract has exerted its chemopreventive effects in the oral mucosa by modulating lipid peroxidation, antioxidants and detoxification systems (Balasenthil *et al.*, 1999).

Neem Leaf Preparation (NLP) activates various immunocompetent cells that may be responsible for tumor growth restriction (Haque *et al.*, 2006). Neem dry powder (0.5 mg) is considered as one unit of NLP, generally used for the immunization of mice. Growth inhibition of murine Ehrlich carcinoma (EC) and B16 melanoma (B16Mel) were observed following
treatment of mice (Swiss and C57BL/6) with aqueous extract of neem (1 unit/mice/week for 4 weeks) either before or after inoculation of $1 \times 10^6$ tumor cells. Two groups of mice were injected with either NLP (1 unit/mice/week) or PBS for 4 weeks and erythrocyte free spleen cells were prepared from these mice after 7 days of last treatment to study CD4+ and CD8+ phenotypes of T lymphocytes. It was observed NLP treatment in spleen as well as in blood increased significantly both helper and cytotoxic T lymphocytes (Baral and Chattopadhyay, 2004).

NLP also acts as an adjuvant by inducing an active antitumor immunity in murine model against B16 melanoma (Baral et al., 2005) and breast tumor associated antigen (Mandal-Ghosh et al., 2007). Moreover, NLP mediated immune activation protects mice from leucopenia, caused by cancer chemotherapy (Ghosh et al., 2006). NLGP up-regulates CXCR3A to promote the lymphocyte proliferation and migration to induce better infiltrated cell mediated killing. NLGP triggers the signaling pathway involved in the CXCL10 release in IFNγ independent manner to obtain greater migration of cytotoxic T/NK/NKT cells at tumor site so that the tumor cells be killed and the progression of cancer may be prevented (Chakraborty et al., 2008).

Neem leaf glycoprotein (NLGP) acts as an adjuvant. An adjuvant facilitates presentation of antigens for effective T and B cell responses. Vaccination with neem leaf glycoprotein, matured carcinoembryonic antigen (CEA) pulsed dendritic cells (DCs) (DCNLGPCEA) enhanced antigen-
specific humoral and cellular immunity against CEA and restricts the growth of CEA\(^+\) murine tumors. NLGP helps in better CEA uptake, processing and presentation to T/B cells. NLGP induced IFN\(\gamma\) secretion and involved in specific cytotoxic reactions to CEA\(^+\) colon tumor cells. DCNLGPCEA vaccine generates anti-CEA antibody response, which is principally IgG2a in nature. This antibody participates in cytotoxicity of CEA\(^+\) cells in antibody-dependent manner. The anti-CEA cellular and humoral immunity protects mice from tumor development and mice remained tumor free with second tumor inoculation, indicating generation of effectors memory response (Sarkar et al., 2010).

The air-dried powdered neem leaves were defatted and repeatedly extracted with methanol (MeOH) at optimum temperature. Chromatographic resolution of the MeOH extract yielded one sulfonoglycolipid (SQDG) and three flavone glycosides characterized as quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside and quercetin-3-O-b-glucopyranoside. THE SQDG tested on acute lymphoblastic leukemia (ALL) cell line was found to induce anti-proliferative and apoptotic effect. The SQDG had DNA binding capacity to calf thymus DNA as evaluated by circular dichroic studies. The anticancer effect of methanolic extract of neem leaves is probably due to the presence of SQDG and DNA binding ability of SQDG (Chatterjee et al., 2010).