INTRODUCTION

1. THE PROSTATE

The human male reproductive system harbors the prostate gland in the sub peritoneal compartment. One can find symphysis pubis to its posterior, rectum to anterior and at the inferior end, urinary bladder is localized. It is conical in shape and displays a classical “walnut shape”. It surrounds the proximal urethra while exiting the bladder (Figure 1) (Lee et al., 2011).

![Prostate Anatomy](image)

Figure 1: Anatomy of human prostate and associated structures. Adapted from (Timms, 2008).

Prostate gland is very small in size with no discernible organization of the lobules. Late 19th till early 20th century, there was a common belief that the prostate gland had various lobes by pure analogy to laboratory animals and no visual validation in the humans (McNeal, 1981). McNeal for the first time divided the prostate gland into clear morphological zones, rather than lobes. Till date this model holds true wherein the prostate gland comprises 4 distinct zones. The following are the key features of the current anatomical model (Figure 2):

- The fibromuscular stroma is situated anteriorly to the gland and is formed during prostate embryogenesis due to interaction of gland budding around urethra.
The glandular region occupying the posterior part of the tissue consists of three zones. They are central zone (surrounds ejaculatory duct), transition zone (surrounds urethra) and peripheral zone (main body of the gland).

Understanding the above-mentioned zones, one can directly point out that 75% of the normal prostate gland is represented by peripheral zone (PZ). These three zones—central, peripheral and transition—differ biologically and histologically (Greer, 2007). The 25% part is covered by central zone that surrounds the ejaculatory ducts behind the urethral sphincter. It is relatively resistant to carcinoma and other prostatic diseases. The location of transition zone (TZ) is inner part of the prostate. The transition zone, which makes up approximately 5-10% of the prostate tissue surrounds the urethra. Both the PZ and TZ are derived from the urogenital sinus while the histologically distinct CZ (central zone) originates from the mesonephric duct (Tewari, 2013). Secretory epithelium lines the ducts and acini of all these zones. In addition to basal cells and luminal cells, interspersed endocrine-paracrine cells are also found in these zones. Few scientists accredit the presence of periurethral glandular region (PGR), as the last anatomically discrete area within the glandular prostate (Gillenwater, 2002).

**Figure 2:** Anatomical division of the prostate part. (According to McNeal)
Now keeping in our mind the classification background of these zones, we can now easily understand the underlying relationship of these zones to prostatic disease (Myers et al., 2010). Benign prostatic hyperplasia (BPH) develops at TZ whereas the PZ is the origin site for both prostatitis and prostate cancer (PCa) (McNeal, 1981). In whole-mount coronal section of the prostate one can clearly see the duct-acinar architecture using special stains to enhance the beautiful anatomical areas, although CZ is not readily separable from the rest of the gland (Myers et al., 2010). On performing histological studies one can see the branched ducts of the prostate gland. Going further into the diversity of the cellular types present in the prostatic epithelium, we can demarcate these cells based on their relevance for carcinogenesis. The cells that form the pseudostratified columnar epithelium are of varying height. It is divided into two layers: a luminal secretory layer and basal layer (Figure 3).

**Figure 3:** (a) Cell types in human prostatic duct. Adapted from (Abate-Shen & Shen, 2000). (b) Hematoxylin and eosin stained section of a normal prostate gland (image obtained from http://www.pathologyoutlines.com).

The luminal cell is the most predominant cell type, secretory in nature and it is a differentiated androgen-dependent cell. Prostatic specific acid phosphatase is an enzyme (glycoprotein) that is synthesized by prostate gland is secreted by these luminal cells. Prostatic specific antigen, an enzyme is also secreted by luminal cells. Androgen receptor
(AR) or the keratins 8 and 18 are the characteristic cell markers for these proteins (Hudson et al., 2001). Basal cells are found embedded between the basement membrane and luminal cells and form a continuous layer in the gland. The basal cells are responsible for regeneration of epithelium. The basement membrane comprises variety of structural proteins, growth factors and adhesion molecules. With the stroma below, this membrane a literal barrier with respect to epithelial lining (Maitland et al., 2011). Coming to the final cell type: the neuroendocrine cell. They are representative of a very minor cellular population and their embryonic origin is an uncertain fact, but these are believed to produce paracrine signals that regulate growth and differentiation of luminal cells (Abate-Shen & Shen, 2000, Yuan et al., 2007).

Prostate gland is indispensable for the male reproductive system. It secretes complex proteolytic fluid that makes up 1/3rd of the seminal fluid. The fluid composition has various lipids, amines, enzymes and metal ions that are required for normal functioning of the spermatozoa (Kumar & Majumder, 1995).

2. BENIGN PROSTATIC DISORDERS

A number of benign diseases can influence the prostate. These aren’t life threatening, often treated with surgery or drugs but can make one uncomfortable with the pain associated with them. A correct identification of these pathologies is the key for the administration of the therapeutics.

3. PROSTATIC HYPERPLASIA

Prostatic hyperplasia or also called benign prostatic hyperplasia is a common Idiopathic condition seen in men after the age of 50. BPH is a benign (non-cancerous) condition in which enlargement of prostate gland is seen. As stated earlier, prostate gland is divided into 3 zones namely, PZ (Peripheral Zone); CZ (Central Zone) and the TZ (Transition zone). Benign prostatic hyperplasia (BPH) arises mostly in the periurethral zones and transition zones. Due to proliferation in stroma and epithelium, its development results in the formation
of small glandular nodules that ultimately merge to form macroscopic BPH. Responsible factors for developing macroscopic BPH are advancing age and androgens (Untergasser et al., 2005, Siiteri & Wilson, 1970).

A classical mixed picture of BPH is mostly seen in individuals. A hyper proliferation of stromal as well as epithelial element is seen to form a nodule. However, presentation of the disease varies from individual to individual depending upon the area (Stromal/Epithelial) of overgrowth.

By the age of 80, nearly 80% of the male population suffer from BPH. Though BPH severely affect the quality of life, the condition does not increase the risk of Prostate Cancer. Symptoms include frequent urge to urinate especially during night, difficult in urination, dribbling urine, loss of bladder control (Strittmatter et al., 2014).

Diagnosis of BPH includes an early diagnosis which is necessary to rule out Prostate cancer. Diagnosis is generally made on the history of lower Urinary tract infection symptoms. Other examinations include digital rectal exam, urine test, blood test, prostate specific antigen (PSA) blood test. Though PSA levels increase in BPH, however, elevated PSA level is not a confirmatory test for BPH because elevated PSA is seen in other conditions as well. For example, UTI and Surgical Procedure (Kumar et al., 2013).

Though BPH is an idiopathic condition, however, several mechanisms are involved in the development and progression of the disease, which includes Ageing –After the age of 50 due to the increase in the activity of Aromatase and 5 alpha reductases, testosterone levels in the body decrease. Also, due to the hyperactivity of Aromatase and 5 alpha reductase, Estrogen and DTH levels are raised which are responsible for growth of cells in prostate (Gandaglia et al., 2013). Thus we can say that advancement in age is very well acknowledged high risk factor which results in BPH. As discussed earlier, overgrowth of cells is seen in the epithelial and stromal cells of prostate. Hence enlargement is seen in the median and lateral lobes of prostate as both these lobes are composed mainly of epithelial and stromal cells. On the
contrary, the anterior lobe is devoid of glandular tissue and hence enlargement is rarely seen in the anterior lobe.

3.1. MANAGEMENT OF BPH

There are various treatment regimes followed in the treatment of BPH. Some of them are discussed below:

3.1.1. LIFE STYLE MODIFICATION

This is an important parameter in the treatment of BPH. Life style modifications include Exercise, Diet containing less amount of protein and lipid, less liquid consumption before bedtime, reduced alcohol consumption (Silva et al., 2016, de Jong et al., 2014b, de Jong et al., 2014a).

3.1.2. MEDICINE

Medicinal part includes two main group of medicines namely Alpha blocker and 5 alpha reductase. The alpha-blocker group contains Alfuzosin, Doxazosin, silodisin, Tamsulocin, and Terazocin. All the drugs are equally effective but slightly vary in terms of side effects (Bechis et al., 2014).

3.1.3. SELF-CATHETERIZATION

Self-catheterization can help to relieve the symptoms like bladder obstruction. To empty the bladder completely, catheterization is used. Due to complicated side effect of UTI catheterization is not the treatment of choice in most of the cases of BPH (Wyndaele, 2002).

3.1.4. SURGICAL PROCEDURES

In advanced cases where medical treatment is not effective Surgical intervention is done. Various surgical procedures are done which include TURP (Transurethral resection of the prostate), TUIP (Transurethral incision of the prostate), PVP (Photo selective (laser) vaporization of the prostate) and open prostatectomy. Among all the surgical procedures TURP is the best choice of treatment. Recently, other minimal invasive treatments are emerging with better results. These techniques have various advantages over traditional
methods, like less chances of infection, less pain and low hospital admission duration. An alternative treatment option for benign prostatic hyperplasia with respect to TURP is prostatectomy that is executed by laser. A high-energy molecule is used in the laser treatment to remove the overgrown tissue. There are various advantages of laser therapy like less pain, less time duration (Pinheiro & Martins Pisco, 2012, Rieken & Bachmann, 2014).

4. PROSTATITIS

Prostatitis is the inflammation of the Prostate gland. This inflammation is of unknown origin. Symptoms include dysuria, Painful micturition, pain- Pain in the groin region, lower back and the pelvic region. Nocturia- Frequent urination during the night time. Painful ejaculation. Other symptoms include body ache, fever etc. Types of Prostatitis- 1. Acute Prostatitis 2. Chronic Prostatitis (mostly observed in peripheral zone) 3. Asymptomatic inflammatory prostatitis. Bacterial Prostatitis is seen in 10% of the cases while in other 90% of the cases are etiological in origin (Kirby, 2003, Kirby et al., 1982, Krieger et al., 1999).

Diagnosis- 1. Urine microscopic examination, Blood test- this is done to rule out infection. Blood test includes CBC, KFT etc. 2. Imaging test- CT-scan imaging provides more detailed information about the disease. Management- treatment depends upon the severity and the type of symptoms. As the best management for chronic prostatitis is unknown, the treatment is usually symptomatic with the use of anti-inflammatory drugs, anti-pyretic drugs and use of antibiotics. The use of Antibiotic drugs is widely done though the role of Antibiotic treatment in prostatitis is not completely known. Antibiotics include: alpha-blocker drugs. Hormonal manipulation, physiotherapy and chronic pain therapy are some of the other choice of therapy (De Marzo et al., 2007, Delcos et al., 2007).

5. PIA: LESIONS OF PCa

A background lesion of prostate cancer, also termed proliferative inflammatory atrophy (PIA), means long standing chronic inflammation of the prostate. In response to unknown stimuli, hyper proliferation develops and it leads to the development of carcinoma of the
prostate. There is a connection between prostate cancer and PIA, which is shown by these two facts:

PIA more frequently occurs in the peripheral zone than in the transition zone, and is directly transferred to malignant or pre-malignant epithelium which suggests a connection between PIA and PCa (De Marzo et al., 1999).

5.1. FEATURES OF PIA

Atrophied epithelium in contrary to the expected quiescence is shown to exhibit increased proliferation and reduced apoptosis as compared to normal epithelium in the case of PIA (Woenckhaus & Fenic, 2008). Its location and proliferative index may have hypothesized that PIA could be a prostate cancer precursor. The progression (in terms of morphology) from normal epithelium to PIA and further its change into low grade PIN and high grade PIN has been depicted in the figure 4 given below:

![Figure 4: Schematic model showing early neoplastic progression. Figure shows morphological changes. The last stage(d) shows atrophied basement membrane and epithelial cells engrossing tissue. Adapted from (De Marzo et al., 2007).](image)

Furthermore, this transition results in the loss of detoxification of cells which occurs due to the silencing of a detoxifying enzyme Glutathione-S transferase. These results in the genomic damage of prostatic epithelial cells which is a manifestation of inflammatory oxidants or nutritional carcinogens. This ultimately results in consecutive somatic genome damage which enhances pathogenesis of PCa (Nelson et al., 2001, Wagenlehner et al., 2007).
6. PIN (PROSTATIC INTRAEPITHELIAL NEOPLASIA)

PIN is a dysplastic lesion which consists of irregular epithelial cells showing cytological atypia. Due to proliferation and crowding of cells epithelium shows false multilayered appearance, however the texture of glands and duct are seen normal (Haggman et al., 1997a, Joshua et al., 2008). On the basis of morphological examination PIN is considered as premalignant lesion or carcinoma in situ and is further classified into two categories: LGPIN or PIN1 (Low grade intraepithelial neoplasia) and HGPIN or PIN2 and 3 (High grade intraepithelial neoplasia). The differences between them are (Drago, 1992):

- LGPIN has lower risk of prostate cancer while HGPIN has high risk to cause PCa.
- 20% of the LGPIN will result in invasive cancer without any requirement of further treatment, whereas HGPIN is associated with 80 percent of invasive disease and active surveillance required.
- In LGPIN basal cell layer in intact and gives negative AMACR staining, while in HGPIN it is highly disrupted giving positive staining.
- In LGPIN nucleoli invisible, variable with enlarged size, while in HG-prostatic intraepithelial neoplasia it is of uniform size and visible.

6.1. HGPIN (HIGH-GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA)

HGPIN is the pre malignant condition of the prostate gland, which leads to the prostate carcinoma. HGPIN is usually asymptomatic unless accompanied by some other pathology. Histological pattern seen in HGPIN are Tufted, micro papillary, cribriform and flat. Cellular atypia is seen, such as increased nucleo-cytoplasmic ratio, prominent nucleoli, and increase in the size of nucleus (Montironi et al., 2000, Herawi et al., 2006, Curado et al., 2007).

6.1.1. DIAGNOSIS OF HGPIN

Biopsy-HGPIN may be diagnosed by a pathologist with the help of a needle biopsy. Another method of diagnosis includes blood test and USG of prostate.
6.1.2. RELATION BETWEEN HGPIN AND PROSTATE CARCINOMA

Both high grade PIN and prostate cancer are phenotypically and morphologically similar. Primarily found in the peripheral zone and observed in areas which are in continuation of prostate cancer, HGPIN shows high rates of aneuploidy and angiogenesis as cancer progresses. Both are multifocal and heterogeneous. HGPIN, therefore, appears to be an intermediate step between benign and malignant disease in the molecular spectrum of prostate cancer (Qian et al., 1997, Sinha et al., 2004) (Figure 5).

![Figure 5: Framework showing different types of prostatic disorders. A- Prostatitis (Epstein & Netto, 2008). B- Benign prostatic hyperplasia (Gallardo-Arrieta et al., 2010). C- Prostate inflammatory atrophy (outlined area) adjacent to benign normal gland (De Marzo et al., 1999). Arrows indicate inflammatory infiltrate; D- High grade prostatic intraepithelial neoplasia (Epstein & Netto, 2008).](image)

6.1.3. CLINICAL SIGNIFICANCE OF HGPIN

If HGPIN is diagnosed without showing any malignant state in needle biopsy, then this can be manifested as being clinically useful. However, biopsy needs to be repeated after 6 months as HGPIN is a precursor of prostate Malignancy (Tewari, 2013, Jones, 2012). Patient age, family history of PCa, PSA levels in serum, and DRE findings should also be considered while clinically managing prostate cancer.
HGPIN is further divided into 2 sub categories: Unifocal and Multifocal. Patients who are diagnosed with multifocal HGPIN initially on extended PNB are at a greater risk for development of prostatic adenocarcinoma than those with unifocal HGPIN. Nevertheless, the magnitude of the risk of PCa in men with HGPIN and the optimal follow-up strategies remain controversial. In early studies, using limited biopsy schemes, HGPIN was associated with high rates of PCa and it was suggested that its presence indicated an immediate need for repetition of the biopsy (Zlotta et al., 1996). However, when a more extensive biopsy scheme was initially used, the cancer detection rate was considerably lower. This was due to the fact that the number of cores sampled during the initial biopsy affected the likelihood of detecting PCa in subsequent biopsies (Herawi et al., 2006). For this reason, some researchers believe that repeat PBs might be unnecessary in the current era and that follow-up for these men can be accomplished using serial digital rectal examinations (DREs) and PSA measurements (Moore et al., 2005). HGPIN does not contribute to the serum concentration of PSA or modify the percentage of free PSA (fPSA) (Morote et al., 2000); however, PSA velocity assist in identification of men possessing high likelihood of suffering from prostate cancer and who have a real need for repeating the biopsy (Loeb et al., 2007).

Several attempts have been made in the past to improve the current management of HGPIN patients. For instance, the number of positive HGPIN cores at the moment of diagnosis has been associated with the risk of cancer, suggesting that patients with unifocal HGPIN should be managed expectantly, whereas those with multifocal HGPIN could benefit from a more aggressive surveillance including re-biopsy (Akhavan et al., 2007).

Overexpression of certain molecules in HGPIN tissue has been found to correlate with the likelihood of finding a PCa in subsequent biopsies. One of these predictors is the \textit{TMPRSS2:ERG} gene fusion. Park et al. assessed the presence of this molecular rearrangement through the measurement of ERG expression levels by immunohistochemistry on biopsies from 461 patients, showing that patients with ERG expression were more likely to develop PCa (Park
et al., 2014). The expression of Alpha-methylacyl-CoA racemase (AMACR) was found to be weakly positive in radical prostatectomy specimens and, negative to weakly positive in biopsy specimens containing HGPIN without carcinoma and, while its expression was highly positive in HGPIN lesions that were adjacent to adenocarcinoma (Helpap, 2006). The immunohistochemical study of GSTP1 expression also exhibited similar results (Montironi et al., 2000). Finally, Prostate Tumor Overexpressed 1 (PTOV1) may be linked to PCa in detecting the risk of carcinoma in repeat biopsies following diagnosis of HGPIN (Morote et al., 2008).

Markers in biological fluids have also been described. For example, an increased serum level of early prostate cancer antigen (EPCA) has been associated with a higher cancer risk in men with isolated HGPIN (Zhao & Zeng, 2010). In urine, PCA3 has been suggested as a candidate diagnostic marker with a good performance before the first repeat biopsy. In one study, PCA3 predicted PCa well in HGPIN cases (AUC=0.80) and would have avoided 72.2% of repeat biopsies, compared to serum PSA (Auprich et al., 2012). However, this study presented the shortcomings of a small sample size and Chin et al. have shown, in a larger cohort, that efficacy of the PCA3 score to rule out PCa in men with HGPIN is lower than in men with milder pathological conditions, for example prostatitis, BPH (Morote et al., 2000).

In summary, the recognition of this HGPIN is clinically important because of its association with PCa. Although the relationship between both has not yet been conclusively demonstrated, HGPIN has been widely accepted as a precursor lesion to PCa and, consequently, men with a first PB positive for HGPIN usually undergo a close clinical follow-up over several years, comprising the measurement of serum PSA, DRE, ultrasound and repeat PBs. Evidently, many of these patients will have consistently negative results year after year, thus many of these biopsies could be avoided if clinicians were provided with better tools to predict the presence of PCa in this specific set of cases. Clearly, there is still a
need for new biomarkers that could differentiate between indolent HGPIN cases and those who actually present PCa.

7. PROSTATE CANCER

7.1. EPIDEMIOLOGY

Prostate cancer is the second most common cancer of urological subsets in men. In 2012 more than 1.1 million men were diagnosed with prostate cancer (PCa) worldwide, which accounts for 15 percent of the cancers diagnosed, with almost 70 percent of them (759,000) occurring in higher developed regions (Ferlay et al., 2015). According to an estimation by ACC (American Cancer Society), it shows that 164,690 new cases of prostate cancer will be diagnosed and 29,430 men will die of PCa in 2018 (Society, 2018). According to Indian Council of Medical Research (ICMR) and different state cancer registries, in India also it is the second most common cancer in males. The incidence rate is lower than USA and Europe, but higher than other parts of Asia and Africa. As per Urological Oncology (March 24, 2017), the incidence rates are constantly and rapidly increasing and the cancer estimated data (projected cases estimated 26,120 and 28,079 for the year 2010 and 2015 respectively) shows that by 2020 the number of cases will become double (Figure 6 and 7).

Figure 6: Prostate cancer incidence and mortality. According to the report of GLOBOCAN 2012 (IARC) Adapted from http://globocan.iarc.fr, www.wcrf.org
Figures 7: Prostate cancer incidence and mortality according to World Cancer Report (IARC 2014).

7.2. PROSTATE CANCER RISK FACTORS

7.2.1. AGEING

Advancing age is most prominent risk factor, which leads to prostatic malignancy. The average age of prostate cancer diagnosis ranges between 55 to 70 years while prostate-related death is 66 years and 80 years, respectively.

The environmental and internal stress exposures might be the causative factors of these noteworthy incidences. Methylation of DNA, Genome imprints, and modifications of histones are some of the epigenetic factors which are responsible for the changes related to prostate cancer and ageing. (Damaschke et al., 2013).

7.2.2. FAMILIAL PREDISPOSITION

Prostate cancer have been well studied in populations which are genetically susceptible and have family history. The risk of developing prostate cancer is almost doubled in men affected with first-degree relative (parents, brothers and sisters, children), and even more high risk for early onset of prostate cancer in those men who are devoid of such relative (Chen et al., 2014). In prospect of identifying several candidate familial loci, studies on linkage has been
explored on massive scale to study reasons behind the pedigree of prostate malignancy. Towards our understanding of HOX biology, the most convincing locus is the HOXB13 (G84E) which is known to have role in prostate development and prostate cancer. It executes this by binding to androgen receptors. The recent and exciting identification of germline HOXB13 (G84E) mutation description is found to be associated with early onset of disease (Ewing et al., 2012). HPC1 Hereditary Prostate Cancer 1, Predisposing for Cancer of the Prostate, HPC X-Linked, Cancer of the Prostate and Brain and Prostate Cancer, Hereditary,20 are some of the examples of susceptible genetic loci responsible for pedigree of prostate cancer (Dean & Lou, 2013). A strong association between family history of breast cancer and prostate cancer risk, especially among first-degree relatives is visible prior to the age of fifty. Moreover this was also pronounced in the case of aggressive prostate cancer. Epidemiological studies indicate that dominantly inherited susceptibility genes which plays major roles are Breast Cancer 1 (BRCA1) and Breast Cancer 2 (BRCA2), and mutation in these have been associated with clustering of cancer in men. Recent studies reportsthat by the age of 65, they may be susceptible for an 3.5-fold and 8.6-fold increased risk of prostate cancer (Castro & Eeles, 2012).

7.2.3. ENVIRONMENTAL FACTORS

According to the survey done by the epidemiological study of Prostate cancer (EPICAP), the most well recognized risk factors for prostate cancer development are environmental and genetic factors. Due to adoption of westernized lifestyle a distinct geographical distribution of prostate cancer incidence is observed which is implicative of environmental causes in addition to hereditary factors (Sfanos & De Marzo, 2012).

7.2.4. DIET AND STEROID HORMONES

Diet and steroid hormones are also probable risk factors for the development of prostate malignancy. High dietary intake of fat (meats, oils, nuts and dairy products such as cheese
and milk) has been associated with development of prostate cancer (Lophatananon et al., 2010). The mechanism behind this may be peroxidation of lipids, which leads to the production of free radicals causing DNA damage and moreover changes in the serum androgens level. Conversely, fruit or vegetable consumption was found to exhibit significant protection from prostate cancer risk. Foods especially tomato and tomato products have found to have potential anti-cancer effects (Giovannucci, 2002). Lycopene, found in tomatoes, is a carotenoid and has potent antioxidant properties. Studies have evaluated its potential anticancer effects showing diminished risk of prostate cancer. The histological tissue grading system also shows its potential role in aggressive PCa which was related with its inverse level in serum (Giovannucci, 2002).

Vitamin D and androgens (especially testosterone and its derivatives) are the group of steroid hormones that are most likely found to be associated with prostate cancer risk. Men with vitamin D deficiency increase the risk of clinical prostate cancer (Schwartz, 2005).

7.3. PROSTATE CANCER SIGNS AND SYMPTOMS

Usually there are no symptoms during the early stages of prostate cancer. However if the symptoms develops it includes: (https://www.cancer.net/cancer-types/prostate-cancer/symptoms-and-signs):

- Difficulty starting or holding back urination, urinating more frequent, urges to urinate especially at night
- Loss of bladder control, weak, dribbling, or interrupted flow of urine
- Burning or pain during urination
- Blood in the urine (Hematuria)

The advanced stage symptoms include:

- Blood in semen
- Difficulty in having an erection (erectile dysfunction)
Painful ejaculation and a decrease in the amount of fluid ejaculated

Rectal pressure combined with pain

Pain or numbness in the hips, legs or feet

If the cancer spreads to the spine and compresses the spinal cord following symptoms are seen.

Swelling, pain or stiffness in the lower back, legs or pelvic area is seen.

### 7.4. PROSTATE CANCER DIAGNOSIS AND PROGNOSIS

Early stage prostate cancer usually has no symptoms and it is most frequently detected through screening tests such as prostate specific antigen (PSA) blood test and digital rectal examination. However, an augmented PSA level in the blood does not automatically point to prostate cancer, because PSA levels can be altered by other prostate circumstances including benign prostatic hyperplasia (BPH) or during infection. In fact, 75% of men with an elevated PSA (4-10 ng/ml) in the blood do not have prostate cancer, and prostate cancer has been diagnosed in 25% of men with normal or below normal PSA levels (≤ 4.0 ng/ml) (Crawford et al., 1996).

The serum PSA (prostate specific antigen) test and abnormal DRE (digital rectum examination) despite being widely used as a first-line for screening PCa in the last decade (Banez et al., 2003), lacks sensitivity and specificity (Mingkun Han, 2016) as it is unable to determine whether PCa is in indolent or aggressive form (Mingkun Han, 2016). These diagnostics tests are suboptimal resulting in overdiagnosis and overtreatment of many clinically insignificant prostate cancers (Norgaard et al., 2017). In preview of limitations of PSA, several prostate cancer biomarkers have been identified till date, (He et al., 2005, Zhang et al., 2003, Riprijanovska, 2014, Iglesias-Gato et al., 2015, Sun et al., 2011, Ummanni et al., 2012, Zhou et al., 2002, Herrmann et al., 2003, Pin et al., 2013, Khan et al., 2010) which needs to be clinically validated prior to the routine use (Davalieva et al., 2015a, Davalieva et al., 2015b, Pin et al., 2013, Schiffer, 2007, Evans et al., 2009, Kim & Kislinger,
To overcome these limitations and improve the early detection and prognosis, still there is critical need to identify additional biomarkers (Ornstein & Tyson, 2006).

Proteomics-based early detection strategies for cancer diagnosis are becoming increasingly popular (Hanash, 2003, Simpson et al., 2008, Wulfkuhle et al., 2003, Bitarte et al., 2007, Cowan & Vera, 2008). In order to identify the differentially expressed proteins during transition from normal to neoplastic stage, proteomic analysis is a highly recommended approach as it has provided new insights into the changes that occur in early phase of cancer progression, giving rise to candidate biomarkers for prognosis and improved response to treatment (Wulfkuhle et al., 2003, Pin et al., 2013).

Recently, serum has become an increasingly desirable source for clinically useful biomarkers discovery with advances in proteomic technologies (Ornstein & Tyson, 2006). Serum proteome is considered dynamic, oppositely to the stable nature of the genome, since the expressed proteins, native, fragmented, or post translationally modified, quickly change in response to pathogenic stimuli (Tessitore et al., 2013). Though tissue biopsy is the gold standard of diagnosis of PCa, it has the most invasive sampling, low tolerability and carries significant morbid risk, (Loeb, 2010). Serum screening is highly desirable because of the minimally invasive and low cost procedures for collecting samples. Hence, the current investigation in prostate cancer biomarkers is mostly oriented to the identification of minimally invasive blood samples.

The mechanisms leading to the initiation and progression of PCa are largely unknown (Senthilkumar K. & Kanagaraj P., 2006), and this led the scientific community to develop preclinical models in order to study components of cancer initiation and progression and to further develop effective preventions and therapeutic interventions (Pienta et al., 2008). The events of prostate carcinogenesis can be studied well enough using experimental animals as models. N-Methyl,N-Nitrosourea (MNU), a highly reliable carcinogen in combination with testosterone can induce PCa in Wistar rats (Goncalves et al., 2010, Goncalves et al., 2013,
Sharmila et al., 2014, Peixoto et al., 2015), with lesions occurring mainly in the dorsolateral lobe of rat prostate, which shows homology with the peripheral zone of human prostate, area where prostate cancer is most frequent (Shappell et al., 2004, McNeal, 1988). The early stage prostate tumorigenesis in Wistar rat model closely mimics the human condition recapitulating many features of human PCa including enlarged prostate lobes, marked stromal hyperplasia with moderate inflammation, ductal dilations, epithelial dysplasia, intra-epithelial neoplasia (PIN), and finally development of adenocarcinoma (McCormick & Rao, 1999, Bostwick et al., 1996, Haggman et al., 1997b, Bosland et al., 1990).

We employed 2-DE and peptide mass fingerprinting based on MALDI-TOF-MS techniques adopting proteomics approach, tissue diagnosis for pathophysiological characterization and disease differentiation were assessed by histopathology and to corroborate the sustainable evidences of proteomics observations and to ascertain the functional association and regulation of corresponding genes; transcriptional genomics were performed using qRT-PCR with the aim of identifying novel, PCa associated proteins (biomarkers) during early stages of disease.

OBJECTIVES

Hence, in the background of above facts the main objectives undertaken for my doctoral work were:

- Proteomic analysis to identify different proteins by 1D and 2D in control and diseased animals.
- To characterize the proteins of interest by MALDI-TOF-MS (and/or other techniques) and determine their sequences.
- In silico analysis of peptide sequences and identification of corresponding genes.
- In silico analysis and designing of novel potential drug molecules against Human Prostate-specific Gene-1 protein.