

## THE ROLE OF THE CLINICAL AND MOLECULAR ASSAYS IN PROSTATE CANCER DETECTION

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## ABSTRACT

To assess the correlation between the clinical and molecular assays in identify early and robust prostate cancer detection. Early detection, management of cancer and decision about the disease are important for beneficial treatment of prostate cancer. We used a computerized search of the Medline/ PubMed databases with the key words prostate cancer, biomarker, and early detection. Clinical management of cancer is facilitated by a conventional test such as prostate-specific antigen and digital rectal exam for application in clinical practice. Although these tests have significantly reduced the mortality with prostate cancer, but have some drawbacks and false positive rate. Fortunately, there are strong correlations between the clinical and molecular assays in identifying early and robust cancer detection, because molecular assays are less invasive and reliable. The use of genetic markers has the potential to providing useful prognostic or predictive information into clinically useful diagnostic tests to improve clinical decision-making and enhance therapeutic success. Different clinical and molecular assays are for detecting prostate cancer and use the biomarkers as potential tumor markers could be a useful predictor in the screening and monitoring to avoid over treatment prostate cancer.

**Keywords:** Prostate cancer, Biomarker, Clinical assay, Molecular assay.© 2017 The Authors. Published by Innovare Academic Sciences Pvt Ltd. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>) DOI: <http://dx.doi.org/10.22159/ajpcr.2017.v10i6.17490>

## INTRODUCTION

Common prostate disease includes benign prostatic hyperplasia (BPH), prostatitis and prostate cancer. Prostate cancer is the second type of cancer in men (aged 65-79 years) [1]. In fact, about 60% of all prostate cancers are diagnosed in men over the age of 66 years, and 97% are diagnosed in men at 50 years. The roles of race, age, family history, and diet are critical factors for developing prostate cancer [2,3]. Men of black African-Caribbean origin are 60% more to get prostate cancer compared with white Caucasian men [3]. Asian men have a lower risk than white men [4]. There is also the higher incidence of men with a relative history (father, brother, or relative with breast cancer) now or in the past to develop the disease. This studies have shown that Asian men have a 50% lower incidence of prostate cancer than Caucasian men. Furthermore, the influence of social and environmental factors, particularly a diet high in animal fat and protein and lifestyle condition increase the risk of prostate cancer in men [4,5]. The result of meta-analysis by Raslau *et al.* described the male flight attendants, civil and military pilots are at least 2-fold increase the risk of prostate cancer, compared with other people [6]. Physical and chemical occupational exposures, cosmic radiation and disruption sleep patterns are the critical factors for types of cancers among flight personnel [6-8]. The recent discovery of inherited and acquired genetic a marker has the potential to aid with cancer initiation and progressions provide an opportunity to understanding this heterogeneous disease and apply these findings to guide clinical decision-making.

## LITERATURE REVIEW

We used the Medline/PubMed databases of the National Library of Medicine for the comprehensive information on the clinical and molecular procedures for prostate cancer detection. The terms used for the search included prostate cancer, biomarker, early detection, and the combinations of these terminologies.

## Diagnosis and detection

Prostate as a singular organ grows with the glandular region in human and primates. It has heterogeneous nature and many genes involved in prostate cancer development. Prostate cancer cells often grow and divide abnormally within the prostate. Most of the cancers have been

classified into four stages [9]. In some case, the cancer cells grow more quickly and may spread to other parts of the body. There are several clinical tests for the diagnosis of prostate cancer. The prostate-specific antigen (PSA) blood test, digital rectal exam (DRE), magnetic resonance imaging, and sonography are the first-line options to detect the presence of prostate cancer when no problems or symptoms are present [10]. Sonography appears to be a very important that can be performed on patients without any side effect or radiation. These tests allow experimental approaches for reducing the number men presenting with metastatic cancer [11]. During a DRE, doctors can examine the prostate for any irregularities in shape, size, and outline of the prostate. During a PSA test, the level of PSA, a protein produced by the prostate is measured by a monoclonal immunoradiometric assay or a polyclonal radioimmunoassay. For the first time in 1991, Catalona and colleagues applied the PSA screening for detection prostate cancer [12]. PSA is found in blood plasma at low levels but can increase in prostatic disease. PSA levels are correlated with prostate cancer and can be measured from a small of peripheral (venous) blood. PSA blood levels under 4 ng/ml are usually normal; results between 4 and 10 ng/ml are intermediate and results over 10 ng/ml are considered high [13]. Clinicians recommend that both PSA and DRE should be confirmed annually at age 40 and older without any symptoms. Although these tests cannot diagnose prostate cancer specifically, the way to determine for prostate cancer cells is a prostate biopsy. The PSA and DRE can determine the need for doing a biopsy [14]. Transrectal or transperineal ultrasound guide biopsies with a probe placed in the rectum to collect the small tissue samples [15]. If prostate cancer is found under the microscope, the pathologist assigns it by Gleason scores. Gleason grade is used in clinical predictors of cancer progression for measuring the degree of differentiation of tumor cells. On scores from (2-4) tend to low grade; Gleason scores (5-6) middle grade and Gleason scores (7-10) tend to be more aggressive. Men with low-grade Gleason  $\leq 6$  tumors have a low risk of metastasis, and Gleason  $\geq 7$  have a high risk of lethal prostate cancer significantly. Staging can describe the extent of the prostate cancer and provide an idea of management and treatment. Types of prostate cancer are; localized prostate, locally advanced, and metastatic. Localized prostate cancer is located only within the prostate. In locally advanced the most cancer cells are within the

prostate, but some cells are growing outside the prostate and escape to the surrounding tissue and cancer can spread from the prostate to nearby tissue such as bladder or rectum. Metastatic cancer, the cells are growing outside the prostate and spread to other areas in the body into the lymph nodes or bone. A rapid diagnosis of cancer is critical for the clinician to the immediate initiation of treatment [16]. There are several major treatment options for a patient diagnosed with localized or locally advanced prostate cancer; active surveillance, surgery, radiotherapy, and hormone therapy. During active surveillance, cancer can be monitored exactly to developing of signs.

Radiation therapy is used two different ways; to cure cancer, destroy tumors that have not spread to other body parts; to reduce symptoms, alleviate pain by reducing the size of a tumor. Radiation can be delivered two ways; externally and internally. External radiation therapy delivers radiation using a linear accelerator beam and helps to kill cancer cells and surrounding tissue. Internal radiation called brachytherapy or seed implants involves placing tiny radioactive metal seeds or pellets into the prostate patient. Radiotherapy is combined with other treatments, such as surgery and chemotherapy [17]. Surgery is the effective treatment, while the tumor is within the prostatic capsule. A radical prostatectomy is the surgical removal of the entire prostate gland plus some surrounding tissue. At present, robotic surgery and laparoscopy are available [18]. In some cases, patients have experienced urinary continence, erectile dysfunction, and biochemical recurrence within 5 years after surgery. The type of treatment used will depend on the men's age, economic position, general health, stage, location, size, and type of cancer [18]. Furthermore, treatment with hormone therapy to lower testosterone level is typically done; this treatment stops the production of testosterone and can make prostate cancer grow more slowly [19]. However, PSA and DRE are considered the great clinical routinely tests by urologists for establishing and correct diagnosis; which have multiple limitations. PSA 2-9 ng/ml has poor correlation with grade and disease progression [20]. In some cases, PSA can be raised in BPH (overgrowth), aggressive cancers or infections or those are not cancerous, thus leading to a delay in diagnosis and unnecessary biopsies. They are lacks sensitivity and specificity [20].

Prostate cancer antigen 3 (PCA3 or DD3) is a prostate-specific gene [13]. In the PCA3 test, the urine collected after post-DRE and measures the concentration the ratio of PCA3 RNA molecules and PSA RNA molecules [21]. The PCA3 analysis is easily and good biomarker prostate cancer after DRE [13]. Prostate cancer possibly decreased when a PCA3 score is >25. It is suitable for men negative biopsy results and PSA levels and DRE are positive. Detection and treatment pathways in prostate cancer are shown in Fig. 1.

#### MOLECULAR GENETIC ALTERATIONS IN PROSTATE CANCER

The most common cancers occur by allelic variability at some locus. Molecular genetic alterations such as protein expression, chromosomal rearrangement, oncogene activation, tumor suppressor gene alterations, DNA mitochondrial mutations, and gene polymorphism in the repeat of androgen receptor are associated with risk of prostate cancer. Much attention lately is focused on the genome in populations of the major gene loci that contribute to the disease. Such studies use a tool neutral variation between individuals in DNA sequence or polymorphisms. These polymorphic markers are present at high density in the human genome. The previous studies have suggested that both environmental and genetic factors and individual difference in the susceptibility to carcinogens may be imputed to the genetic polymorphisms of genes required in the metabolic detoxification of exogenous and endogenous carcinogens and chemotherapeutic agents. There are several important genes such as glutathione-S-transferases (GST) which are involved in DNA repair and protect DNA from damages by detoxifying the carcinogens, metabolism of hormones and chemical agents. Different GST isoform is expressed in the prostate and some tissues [22-23]. Several genetic polymorphisms and total or partial deletions of GST family (GSTM1, GSTT1, GSTM3, GSTP1, and GSTZ1) have been identified that

associated with a reduction of enzymatic activity [24]. The previous studies have suggested that individuals with null genotypes of GSTs may be unable to inactivate electrophilic carcinogens efficiently and higher risk for prostate cancer [25]. An additional finding was that GSTM1 null genotypes were significantly 45.5% higher in cancer and BPH group compare with normal men. In cohort studies Cotignola *et al.* have demonstrated that more than two polymorphism alleles of GSTs (GSTM1 null, GSTT1 null, and GSTP1 A>G) were at high risk for relapse after radical prostatectomy in Argentina patients [16]. These results are consistent with the finding of reported in Caucasian men by Nock *et al* [26]. The GSTP1 genes are down-regulated in prostate carcinogenesis. This gene is involved in the detoxification of electrophilic carcinogens and has a polymorphic site at codon 105 (exon 5), where (A>G) transition causes an Ile-to-Val substitution. The presence of valine residue to the hydrophobic binding site for electrophilic substrates has been associated with decreased enzyme activity and a tendency to increase neoplasms [22]. Knowledge of GST variation has led to understanding the role of GST family genes in somatic mutation and tumor formation [16]. Other the most frequently mutated genes are p53 and ki-67 [20]. p53 mutations are present in half of the tumors [27]. The p53 plays a critical role in human cancer as a transcription factor in cell cycle progression and DNA damage or cellular stress. The lack of p53 leads to cell cycle arrest and apoptosis. Furthermore, the novel role of ki-67 in a regulation of the cell cycle and a nuclear antigen was identified. A high-level Ki-67 expression is a prognostic marker of prostate cancer recurrence after radical prostatectomy [28]. In a study by Verma and colleagues were observed both p53 and ki-67 expression were significantly up-regulated in malignant prostate cancer as compared to benign tissue and relation with Gleason's grading [28,29]. These results give support to the use of these marker genes together for personalized medicine as an ideal marker for cancer management.

#### MITOCHONDRIAL DNA IN PROSTATE CANCER

Any type of tests can be identified by mutations in the mitochondrial genome in different cancer [30]. For the first time, Jeronimo *et al.* have also demonstrated the presence of tumor specific mitochondrial genome in the plasma and tumoral tissue from prostate cancer patients [30]. Tumor cells contain thousands of copies of double-stranded DNA in the inner mitochondrial matrix. Therefore, the presence of highly copy number is quite specific way to increase the sensitivity of early detection. In some cases, mutation within protein coding regions in NADH dehydrogenase subunits 3-5 from complex 1 is the hotspot in cancer. D-loop is a regulatory site for both replication and expression of mitochondrial DNA. D-loop mutations are identified in many body fluids due to high copy number as a diagnostic tool in neoplastic tissue. D-loop variations and microsatellite instability can influence cell physiology and result in a benign or malignant phenotype in cancer patients [31]. Various studies have shown that the number of CAG repeat  $\leq 21$  inpatients with prostate cancer was smaller than BPH and normal group. Balic and coworkers showed the number of CAG  $\leq 18$  have 3-fold higher risk for prostate cancer in Hispanic men [31]. Furthermore, some investigations have confirmed in China and Indian populations; polymorphic CAG repeats gene associated with high-risk cancer.

#### MIRNA IN PROSTATE CANCER

Mendel and Matais reported that extracellular nucleic acid could be detected from healthy and sick individuals. Nucleic acids as a tumor marker have been developed for the purpose of screening for early malignant diseases and monitoring cancer progression. Some tumor markers (mRNA, microRNA, and proteins) are detectable to differentiate between normal and cancer tissue [32,33].

Although proteins have significantly identified of biomarker science, there are several drawbacks such as methods of detection, availability, and stability. General techniques for measurements of protein biomarkers antigen-antibody complex formation are detected by ELISA

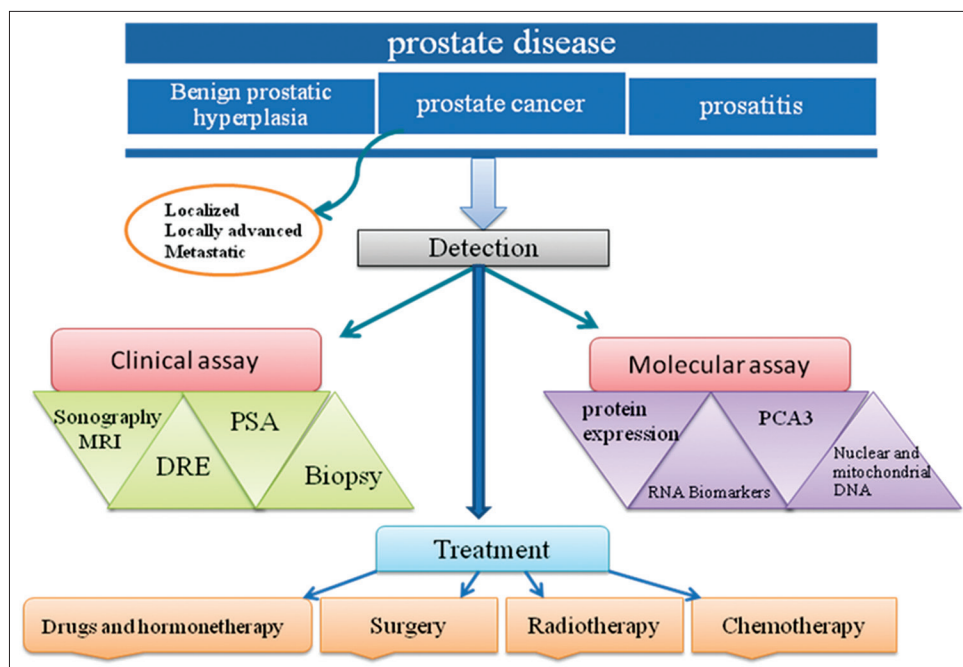


Fig. 1: Detection and treatment options for prostate disease

test. mRNA expression has been suggested as a potential marker for detection of prostate cancer.

A miRNAs have been identified as new generations of biomarkers not only to be developed into sensitive and robust markers but also to monitor disease progression and efficacy of treatment [34]. Using current the public database miRBase, 2500 mature miRNA have been discovered. They are ideal candidates for innovating new early detection and diagnosis for cancer [1,34,35]. A miRNAs are a group of small noncoding RNA molecules that are transcribed by polymerase II to producing the primary miRNA (pre-miRNA). Subsequently, RNase III generates precursor (pre-miRNA) with 70-100 nucleotides. Then, pre-miRNA is exported to the cytoplasm, and Dicer enzyme cleaves the miRNA into 22 nucleotides in length [36-38]. Different miRNAs with the capacity regulate gene expression through binding to complementary sequences in the 3'-untranslated region of the target gene [39]. Interestingly, some microRNAs have multiple targets, and some others can be targeted by several miRNA. Approximately 52% of miRNA genes are located the intergenic area, 40% are within introns, and 8% are within exons.

Today's, several reports have indicated that miRNAs are present in other fluids such as serum, blood, saliva, sputum, amniotic fluid, urine, and plasma [40,41]. Serum or plasma studies showed up-regulated of miR-375, miR-141, miR-21, miR-93, miR-106a, miR-874, miR-1207, and miR-26 in patients with prostate cancer [42]. With this idea, miRNA can be released into the blood in stable form and are highly effective manner, noninvasive diagnosis and predictable the response to therapy [32,43]. Urine is even more accessible than blood, and larger sample volumes can be obtained [41]. Urinary biomarkers can be detected from the bloodstream [41]. These small molecules persist for a long time because of the stable miRNA-protein or miRNA-lipid complexes. A miRNA molecules could play important roles in differential expression, inflammation, proliferation, cell cycle, apoptosis, cancers initiation, progression, metastases, and drug resistance [1]. The first report was in 2007 by Valadi *et al.* also showed the encapsulation of miRNAs into exosome bodies and can be secreted into blood circulation [44,45]. The exosome is a secreted vesicle that attached to the surface of acceptance cells and enters the cells via endocytosis, which regulates cell to cell communication and release miRNAs. Extracellular microRNAs in circulating exosomes are robust biomarkers in cancer. Higher exosomal miR-141 is significantly associated with metastatic prostate cancer and

also a high-level exosomal expression of miR-1290 and miR-375 are associated with cancer progression [42].

In patients with prostate cancer, tissue samples down-regulated in 5 miRNAs (miR-23b, miR-100, miR-145, miR-221, and miR-222), whereas miR-135-b, miR-194 were increased [46,47]. Recently, some miRNAs expressions were shown to be significantly correlated with the Gleason scores and tumor stage. In fact, down-regulated circulating levels of miR-221 were associated with Gleason score, progression and recurrent. Penney and coworkers found 157 mRNA expressions in prostate cancer could be used to predict both Gleason score and the relative risk of lethality [48]. Some evidence has shown the role of miRNAs as an oncogene or tumor suppressor gene in cancer initiation, lymph node metastasis, progression, and drug resistance in the type of cancer [49]. MiR-21 (17q23), as an oncogene, is the most frequently in the development of many types of human cancers and in prostate cancer and patient follow-up [50-52]. Some studies reported that invasive tumors have over expression of miR-21 by targeting PTEN, TPM1, and PDCD4 genes in prostate cancer specimens [53,54]. The expression levels of miR-21, miR-125b, and miR-141 were highly expressed in prostate cancer as oncogenesis [55,56]. Some microRNAs such as miR-15, miR-16, miR-31, miR-330, miR-145, miR-146a, miR-203, and miR-205 expression were significantly down-regulated as tumor suppressor gene in prostate cancer.

In 2011, Cuzick *et al.* showed that cycle cell progression scores and PSA levels are involved in the relative risk of mortality and avoid to over-treatment, especially unnecessary surgery and biopsies [57]. Volinia *et al.* reported upregulation of miRNAs (miR-17-5p, miR-21, miR-25, miR-30c, miR-146, miR-181b-1, miR-199a-1, miR-29b-2, miR-20a, miR-32, miR-92, and miR-214) in prostate cancer and 6 miRNAs downregulation in their study [58]. Importantly, Ambis *et al.* found that miR-26a, miR-32, miR-196a, miR-181a, miR-25, miR-92, miR-93, Let-7i expression are increased in prostate cancer cells compared to normal cells and also determined some miRNAs correlated with androgen regulation, which is similar to Volinia and coworkers findings [58,59]. In another study, Porkka *et al.* and Ozen *et al.* found downregulation of miR-23, miR-29, miR-99, miR-125, miR-30, Let-7 family in prostate cancer tissue [36,46]. According to Brase *et al.*, circulating miR-375 and miR-141 levels correlated with high Gleason score and spread to lymph node [60]. Interestingly, miR-375 plays the dual role in prostate carcinogenesis and progression. In patients with the higher Gleason



score and lymph nodes metastases significantly higher expression levels of miR-375 were observed, whereas in PC-3 cells the level of miR-375 was down-regulated [1]. Moreover, CCND2 was identified as the miR-375 target in prostate cancer. MiR-375 and CCND2 are involved in cell cycle regulation. Furthermore, in patients with metastatic prostate cancer, miR-375 is significantly up-regulated in serum exosome relative to those of patients without non-recurrent prostate cancer. Many studies reported that miR-143 and miR-145 are very important in the development of prostate cancer and advanced disease stage [61]. Upregulation miR-96 and downregulation miR-221 is associated with the Gleason score and recurrent after surgery. Therefore, noninvasive assays are appropriated for use in clinical that are reliable and inexpensive.

## CONCLUSION

Cancer is a complex disease and difficult to diagnoses and treat. Prostate diseases in humans may arise through a combination of basic processes of tissue changes and the sum of genetic and environmental risk factors to which the individual is exposed. Together, these determine the individual disease risk.

Many studies are currently being focused on the dissection of genetic influences on common complex diseases such as cancer disease. The genetic make-up of individuals contributes considerably to the risk of disease within the context of age, various systemic risk factors, lifestyle and an environment. The approach of genome searching allows for identification of yet unknown genes with a major effect the pathophysiology of cancer disease.

Early detection is the best method of combating cancer and can be treated successfully. Whereas, advanced cancer is difficult to treat and has a poor prognosis. Early prostate cancer usually has no symptoms but can be characterized by molecular genetic markers and clinical screening. Identification of patients who have increased familiar incidence of cancer and gremlin mutation is the most importance. In this regards, teamwork collaboration with clinicians, geneticists, pathologists and epidemiologists to identify clinical and pathology features without morbidity provide unique opportunities for patient's decision making.

Development of new molecular approaches is the most promising way to reducing mortality. Molecular biomarkers in clinical oncology have described as a group of detectable and characteristic alterations in DNA and proteins. The connection between molecular detections and clinical tests is the direct one to understanding the prostate cancer onset and progression. Biomarkers are an interesting and promising marker to improve outcomes while reducing the overtreatment of disease. Therefore, identification of more reliable and sensitive marker than PSA and DRE is emerging. Other techniques are time-consuming or less attractive for routine monitoring. The studies performed to date mainly focused on detection and applicability of biomarker. A few studies focused on quantification methods, searching for fast, easy methods that are widely applicable in clinical practice. Although several limitations impede the clinical implementation of the biomarker until now such as information related to sample collection, the time between collection and processing and storage time.

The knowledge about biomarkers that are released outside the cell, abundant expression, board dynamic range, amplifiable signals and some qualities that make them attractive candidates. Circulating DNA, miRNAs, and mRNA can be correlated with Gleason score, metastases to other organs, susceptibility and predict recurrence disease. Urinary and tissue biomarkers and gene assays allow patient specific molecular and genetic characterization of cancer phenotype; will likely become incorporated into major oncologic guidelines and standard urologic practice.

Furthermore, they help to increase the quality of life and choosing the best treatment for optimization of personalized medicine and response

to therapy. We hope that advances in science and further extensive studies are necessary to solve these problems in health-care system.

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