Use of TP4303 to identify prostate cancer cells in voided urine samples

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Introduction: *Prostate cancer is the second most common malignancy in men worldwide. Genomic VPAC receptors are expressed on malignant prostate cancer cells and can be targeted and imaged optically by a peptide labeled fluorophore. The objective of our study was to assess the feasibility of detecting cancer of the prostate using a voided urine sample.*

Materials and methods: *Patients ≥ 40 years old, with lower urinary tract symptoms and serum PSA > 4 ng/mL formed the study group. The first 50 mL of voided urine sample was collected and processed. The cells that were shed in the voided urine were fixed and stained with a peptide TP4303 and incubated. The slide was then stained with DAPI which binds with the DNA in the nucleus. All patients underwent a standard 12-core TRUS-guided prostate biopsy.*

Results: *A total of 318 patients were included in the study, of these 158 were histologically confirmed cancers. Voided urine samples were positive for VPAC receptors in 154 (97.46%) of these. The remaining 160 patients had no cancer on the HPR examination and none of these patients were positive for VPAC receptors.*

Conclusions: *This study validates our belief that patients with prostate cancer shed malignant cells in the urine that can be identified by targeting the VPAC receptors. If these results are further validated by multicentric studies, then this could form the basis for indications for a preliminary prostate biopsy in patients with elevated serum PSA but normal digital examination or in patients needing a repeat biopsy.*

Key Words: prostate cancer, genomic VPAC receptors, diagnosis, voided urine

Introduction

Prostate cancer is a common non-cutaneous malignancy affecting men all over the world. In the United States alone, it accounts for 29% of all diagnosed cancers in males and ranks second most common cause of death due to cancer. It accounts for 13% of all cancer deaths.[1,2] In 2023, 1,958,310 new cancer cases and 609,820 cancer deaths are projected to occur in the United States.[3] In India, the incidence of prostate cancer is gradually on the rise.[4] Estimation of serum prostate-specific antigen (PSA) test remains the most important biomarker for the detection and follow up of prostate cancer. The test is well tolerated, quick, cheap and standardized. Urologists all over the world are familiar with test results and can easily translate the results into a certain risk level for having the disease or into the risk of tumor progression. Disease-specific mortality has been reduced by using serum PSA test for screening.[5-7] However this gain has come at a considerable cost leading to 70%-80% of potentially unnecessary prostate biopsies, depending on the PSA cut off level used.[5] PSA-based diagnosis/screening leads to overdiagnosis of cancer, especially the detection of "non-life threatening" disease, which often results in overtreatment in such patients.[8]

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As of today, prostate cancer is suspected whenever digital rectal examination (DRE) findings are abnormal or serum PSA levels are elevated. Currently, prostate biopsy is the only way to confirm the diagnosis of clinically localized prostate cancer following an abnormal PSA and/or DRE. Prostate biopsy be it transrectal or transperineal is associated with risks, including local infection, systemic sepsis, and hemorrhage.[9] Prostate biopsy as of today remains the gold standard for the detection of prostate cancer and is the basis for preoperative pathologic grading and cancer volume estimation. At times cancers can also be missed due to inadequacies associated with prostate tissue sampling, resulting in a sensitivity deficit (false negatives).

Clinical interest in the use of multiparametric magnetic resonance imaging (mpMRI) has occurred with the increased magnetic quality from 0.3T-0.5T to 1.5T and even up to 3T, with further incremental gains from endorectal coil (ERC) technique and multiphasic sequencing. mpMRI has managed to change the paradigms of prostate cancer detection and risk classification.[10]

A non-invasive and definitive test for prostate cancer would be most welcome to patients and clinicians, whether by imaging or molecular biofluid analysis. New biomarkers for the detection and staging of prostate cancer have become an absolute must. Urinary biomarkers for prostate cancer are the subject of ongoing research and represent a promising alternative or addition to serum-based biomarkers.[11] Urine-based tests being non-invasive might be suitable for both clinical and (mass) screening purposes, and also for prediction and to gain prognostic information.

Prostatic cancer cells shed into the prostatic urethra and collected through voided urine samples in prostate cancer patients could be imaged optically, by targeting the VPAC1 receptors (combined vasoactive intestinal peptide {VIP} and pituitary adenylate cyclase-activating peptide {PACAP} family of cell surface receptors) with the same peptide labeled fluorophore. Trabulsi et al[12] reported their preliminary study on the detection of prostate cancer non-invasively, by a simple and reliable assay by targeting genomic VPAC receptors expressed on malignant prostate cancer cells shed in voided urine. The assay detected VPAC positive cells in 98.6% of the patients having a prostate cancer diagnosis, (n = 141), and none (0%) of the males with benign prostatic hyperplasia (BPH) (n = 10). In this prospective study, we have assessed the feasibility of detecting cancer of the prostate using a voided urine sample and targeting the genomic VPAC receptors expressed on malignant prostate cancer cells.

Materials and methods

This study was conducted with consent obtained from the university/institutional ethical committee. Patients attending urological services ≥ 40 years old, with lower urinary tract symptoms and serum PSA > 4.0 ng/mL formed the study group. Patients with urinary tract infection, hematuria, history of urothelial carcinoma, and radiotherapy were excluded. All the patients in the study group were advised to give the first 50 mL of voided urine sample for testing. The urine samples were kept at 22°C and processed at 22°C for up to 4 hours. However, if the processing was likely to be delayed due to some reason for more than four hours of urine collection then, the samples could be stored at −10°C for up to 72 hours.

Processing of urine samples[11]

The samples were identified only by the patient identification No., date of collection and presenting diagnosis. Samples were centrifuged at 2000xg for 10 minutes and all but approximately 250 µL of supernatant was discarded. The cells were then suspended and cytocentrifuged, and fixed in 97% ethyl alcohol. TP4303 solution (0.5 µg) was added to the cells to cover the entire cell area, approximately 1 cm in diameter. The slide was then kept in the dark, at 22°C for approximately 20 minutes and then thoroughly rinsed with deionized water and air dried. On the cells was then added, 20 µL of 4′,6-diamidino-2-phenylindole (DAPI, Fisher Scientific, PA, USA) which strongly binds to A-T rich region of DNA in the cell nucleus.

A coverslip was then placed and the slide was observed using a fluorescent microscope. Cells with TP4303 interaction presented themselves with dark orange fluorescence around the nucleus and thereby indicated the presence of VPAC receptor molecules around the cell surface. In the absence of VPAC receptors, only the DAPI-bound cell nucleus was seen in dark blue. Normal epithelial cells that may only have minimal or no expression of VPAC therefore do not interact with TP4303 and show only cell nuclei, Figure 1.

All patients in the study group underwent a standard 12-core prostate biopsy using transrectal ultrasonography (TRUS) guidance. The specimens were labeled properly denoting the zone of the prostate and sent for histopathological estimation. The results of the histopathological studies were then compared to the results of the urine biomarker.

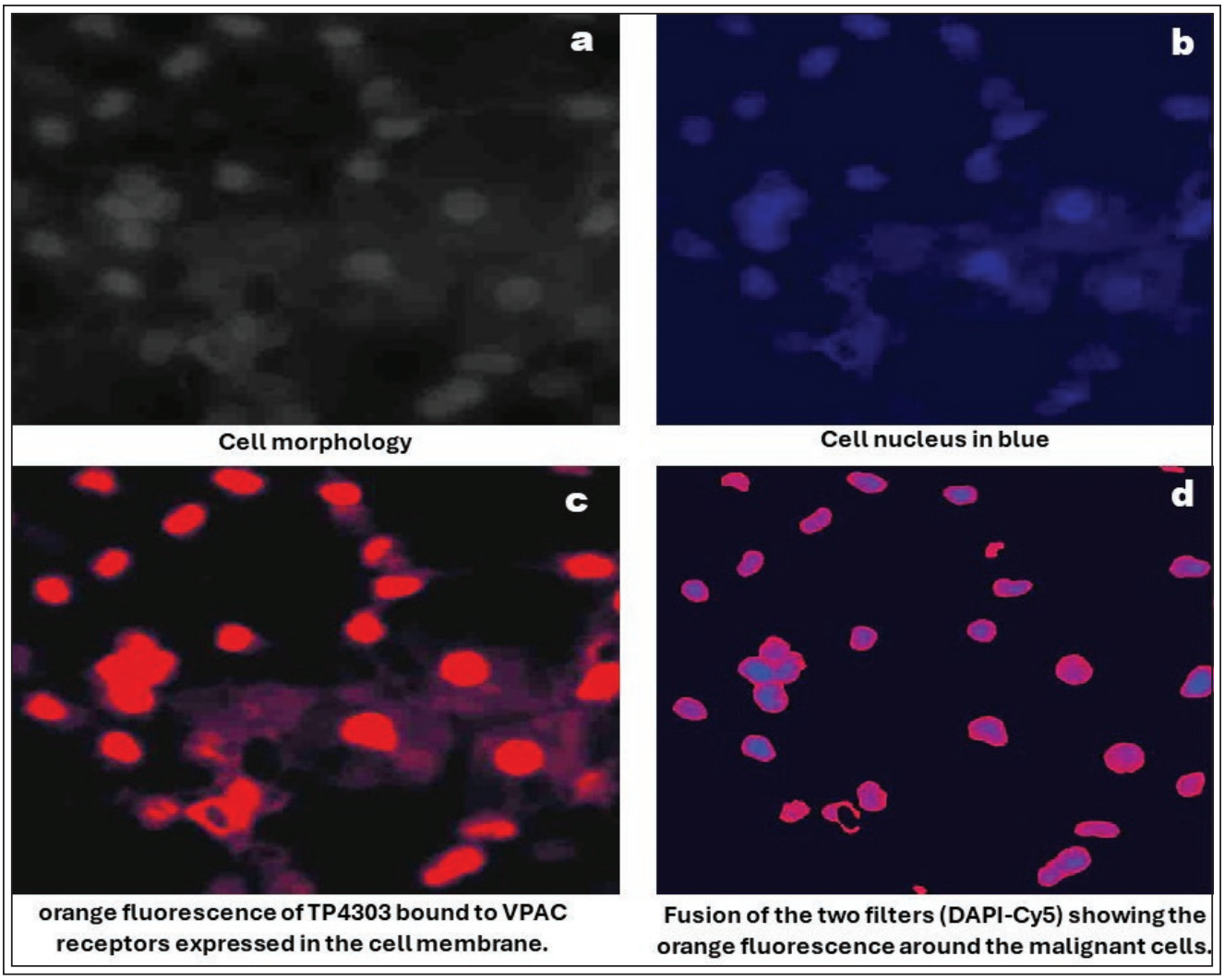

Figure 1. Optical imaging of cells prepared from voided urine of patients.

Results

During the study period, 318 patients were included in the study group. The mean age of these patients was 70.1 ± 6.36 (range 43-85) years and the mean serum PSA was 9.41 ± 6.55 (range 2.68-1200) ng/mL, Table 1. Twelve core TRUS-guided prostate biopsies revealed adenocarcinoma (Gleason score ≥ 7 or group grade 2 or more) in 158 patients and BPH in the remaining patients.

TABLE 1. **Clinical demographics of patients in the control and study group**

No	Category	Mean age years	Mean PSA ng/mL	Benign histology	Adeno carcinoma	Total
1	Control group (160)	70.1 ± 6.36 (range 43-85) years	9.41 ± 6.55 (range 4-500) ng/mL	-	-	160
2	Study group (158)	73.2 ± 4.35 (range 43-85) years	34.53 ± 7.93 (range 4-500) ng/mL	4	154 (97.46%)	158

TABLE 2. **Biomarkers results of patients in the control and study group**

No	Category	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Total
1	Control group (160)	-	-	-	-	160
2	Study group (158)	99.9%	97.56%	97.47%	97.47%	158

The biomarker study revealed the absence of malignant markers in all 160 patients in the control group. Of the 158 patients with adenocarcinoma, 154 (97.46%) were positive for malignant markers in the biomarker study. The sensitivity of the test was 100%, specificity was 97.56%, positive predictive value was 97.47%, and negative predictive value was 100%, Table 2. Patients with negative biopsies were followed up with an estimation of serum PSA, however, none of our patients who had negative biopsies required to undergo a repeat biopsy as the serum PSA returned back to normal.

Discussion

Urine is a naturally available fluid medium, that contains a variety of substances, some of which are filtered from the circulation, such as metabolic waste products and small proteins (< 20 kDa) secreted by numerous cell types, as well as larger proteins and cells that originate from urogenital organs downstream of glomerular filtration.[13] Using low-speed centrifugation, solid components of urine could be easily separated from the liquid fraction. The resulting pellet usually contains formed elements such as cells, casts, mucin, and debris; whereas, the supernatant liquid retains the soluble components including proteins, exosomes, biochemicals, and cell-free nucleic acids. The composition of urine is highly dynamic and shows great variability across individuals due to numerous factors such as age, diet, gender, and physical activity.[14,15]

Using urine for clinical assays has its advantages. Collection of urine is non-invasive and carries no risk or harm to the patient. Urinary samples can be obtained at frequent intervals and in large quantities, making urine amenable to repeat sampling procedures. Urine may contain prostatic secretions and exfoliated prostate epithelial cells due to the anatomic proximity of the prostate to the bladder and urethra.[14,15] Several prostatic biomarkers are usually released into the urine, including cell-associated markers and secreted cell-free markers. The presence of prostatic biomarkers in urine can be enriched by physical manipulation of the gland during DRE. As a source of biomarkers for localized and early-stage prostate cancer, urine may be better suited than blood that contains markers from virtually all body tissues, leading to high background interference that can hinder detection ability. In contrast, urine is enriched in material coming directly from the prostate gland; it does not require crossing blood-tissue barriers; and it contains fewer confounding elements.[16]

Prostate cells that appear in the urine are luminal epithelial cells that have been shed from the gland, however, it is not fully understood the exact nature of urinary prostate cells and how these cells are released into the urine. Shedding of prostate cells presumably occurs during normal cell turnover. The prevalence of intact prostate cells in urine sediment has been little investigated. The morphological features of prostate cells have been well-defined in several cytological studies.[17,18] Prostate cells found in the urine sediment have a distinct appearance that can be identified microscopically using conventional cytology staining methods; they generally are round with a high nuclear to cytoplasm ratio, prominent nucleoli, and often present in small clusters. Despite these characteristic features, identifying prostate cells solely based on morphology is difficult even for trained cyto-pathologists because of their overlapping appearance with other cell types found in the urine sediment as well as their scarcity in regularly voided urine specimens.

Trabulsi et al[11] chose to target VPAC receptors known to express in high density on prostate cancer cells at the onset of oncogenesis to develop a simple, completely non-invasive, inexpensive and reliable test to screen or detect prostate cancer. It has been observed that one gram of a growing tumor, sheds nearly 0.4% ($3.4x10^6$ cells) every 24 hours.[19] Complete or parts of these cells pass through the prostatic ducts and appear

in the urine even without performing DRE. It has been demonstrated that the genomic VPAC receptors are expressed on the cell surface of the prostate cancer cells.[11] TP4303 is known to specifically target the cell surface VPAC receptors and help in identifying prostate cancer cells.[11] It has also been seen that the cells that are targeted by TP4303 were truly prostate cancer cells.[19]

Our study has shown that patients with adenocarcinoma of the prostate shed cancer cells in the urine that could be easily identified by targeting the VPAC receptors. Trabulsi et al[11] reported the detection of VPAC-positive cells in 98.6% of the patients having a prostate cancer diagnosis, (n = 141), and none (0%) of the males with BPH (n = 10). This study of ours validates our belief that patients with prostate cancer shed malignant cells in the urine that can be identified by targeting the VPAC receptors.

Conclusion

Our study is simple and can be feasible in most centers dealing with patients with prostate cancer. Our study is a small study and the results need to be validated by a multicentric study with a large number of patients. If validated this study could form the basis in future i) to indicate a repeat prostate biopsy in patients with an elevated serum PSA and negative TRUS-guided biopsy, and ii) to indicate a reduced number of cores on TRUS-guided biopsy.

Disclosure

Drs. Madhukar L. Thakur and Leonard G. Gomella have patents with Thomas Jefferson University for the use of TP4303 for the detection of shed cancer cells. □

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