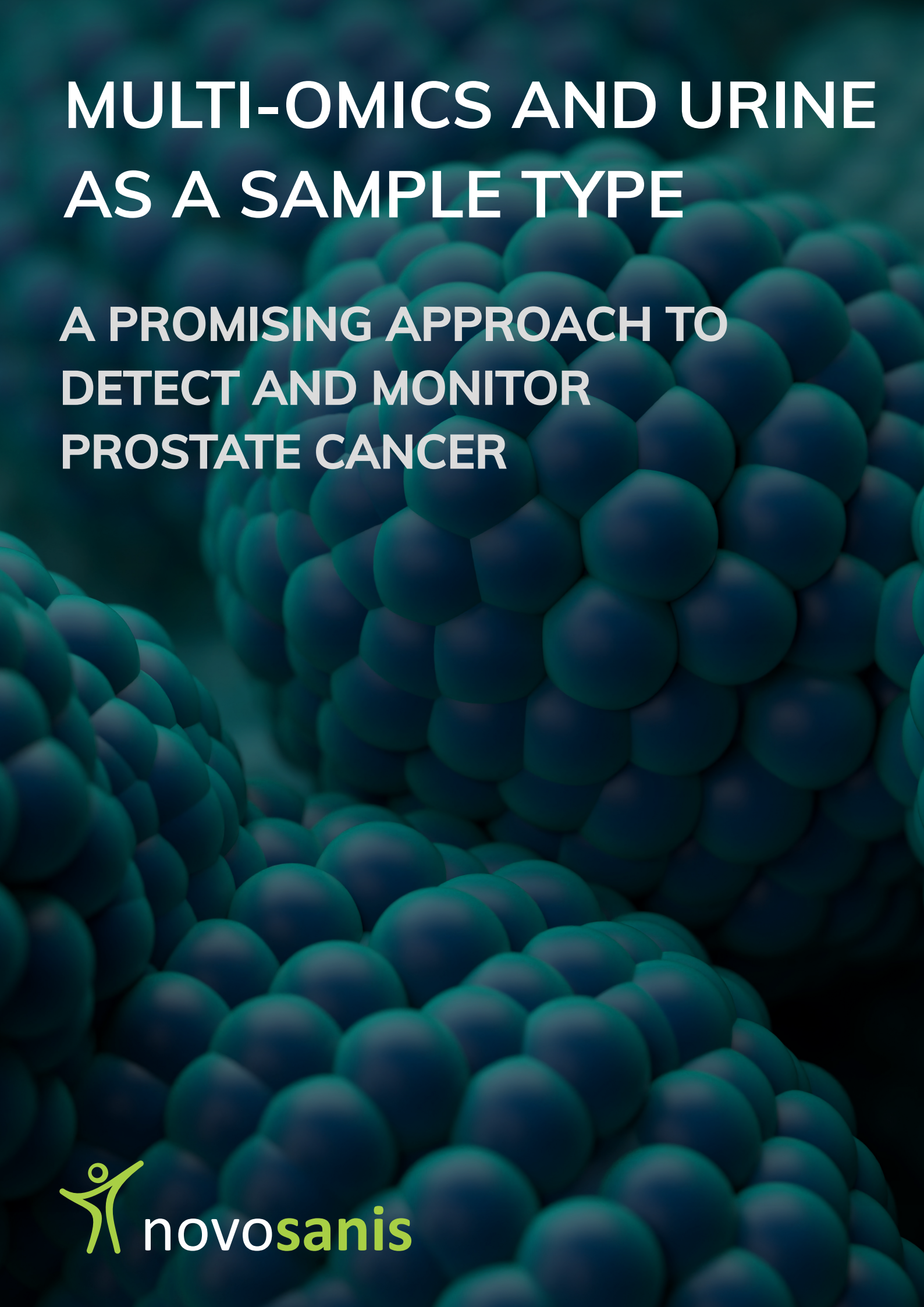
MULTI-OMICS AND URINE AS A SAMPLE TYPE

A PROMISING APPROACH TO DETECT AND MONITOR PROSTATE CANCER



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INTRODUCTION

Prostate cancer is the second most frequently diagnosed cancer and fifth leading cause of death among men worldwide. The cancer type is highly heterogenous, manifesting in pathological, genomic, functional and intratumorally differences[1,2].

The study of multi-omics has gained interest over the years as it can provide a full picture of a disease from the original cause (genetic, environmental or developmental) to the functional consequences[3]. Several biomarker candidates have been identified for prostate cancer, including DNA, RNA, proteins, exosomes and metabolites.

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URINE: A GAME CHANGING LIQUID BIOPSY IN CANCER BIOMARKER DETECTION

A tissue biopsy is the traditional approach used to diagnose many cancers. However, obtaining a tissue sample has limitations. The procedure is invasive, painful, expensive, time intensive and requires the intervention of a clinician. Further, due to intratumor heterogeneity, a tissue biopsy may not always reflect the entire tumor landscape. As a result, researchers are continuously exploring alternative methods to detect cancer types.

The use of minimally invasive procedures such as liquid biopsies and detection of circulating tumor markers in body fluids, like blood and urine are gaining interest.

Several studies have shown that the use of urine as a liquid biopsy for cancer detection and monitoring is promising due to the ease of sampling and high acceptability compared to blood and tissue. Urine cell free tumor DNA has proven to be of value in biomarker studies of bladder, kidney and prostate cancer, but surprisingly also in breast, colon and lung cancer[4].

URINE AS AN EMERGING LIQUID BIOPSY FOR PROSTATE CANCER BIOMARKERS

Limitations with current prostate cancer detection methods

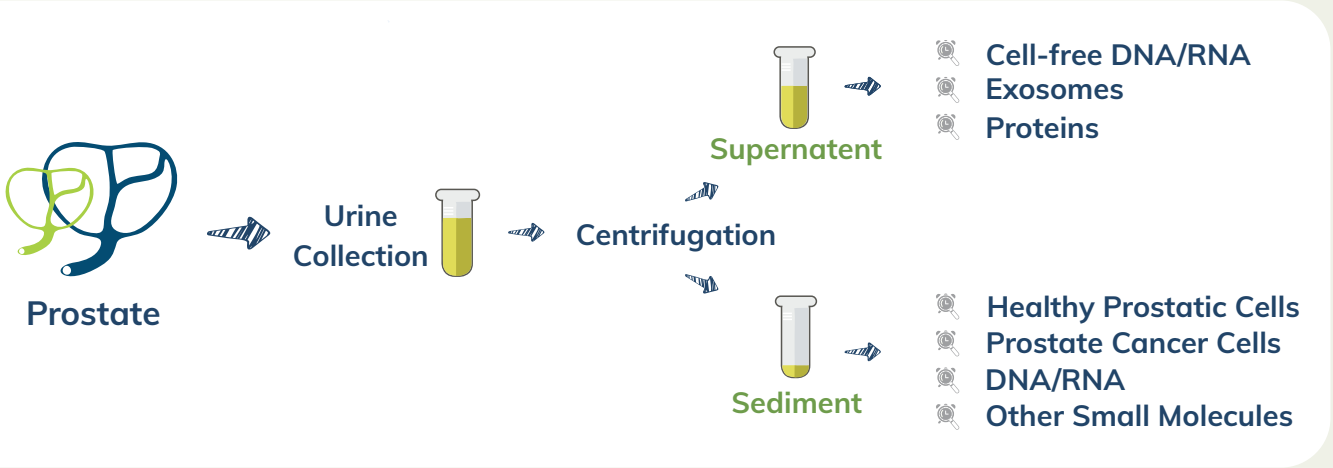
Early detection of prostate cancer involves measurement of serum prostate specific antigen (PSA) levels and/or inspection of the prostate via digital rectal examination (DRE). PSA, as a biomarker has shown to be fairly sensitive to detect early-stage cancer as well as predict potential response to treatment. However, the test has low specificity[5,6,7].

Other conditions, such as benign prostatic hyperplasia, prostatitis and prostate infection can also result in elevated serum PSA levels[5,7,8,9]. Consequently, PSA testing can lead to overdiagnosis, and a high number of unnecessary biopsies, adding pressure to healthcare systems. It may also result in patient discomfort, and uncertainty[5,6,7]. Alternatively, in some prostate cancer patients, PSA levels are not elevated, leading to false negatives[10].

Urine as an attractive sample type for prostate cancer detection

Given these limitations, finding new and specific biomarkers are necessary to detect prostate cancer in a more effective way. Urine as a sample type is promising. This body fluid is particularly attractive as it allows non-invasive collection, as well as offers the possibility of repeated sampling.

As urine is produced by the kidneys, which remove waste products from the entire body, this sample type carries information from several body areas such as the renal and urinary tract, as well as the prostate. In addition, urine may contain contents of distant organs via plasma obtained by glomerular filtration in the kidneys[5,7,11,12,13,14].



URINE AS AN EMERGING LIQUID BIOPSY FOR PROSTATE CANCER BIOMARKERS

Biomarkers in urine for prostate cancer

There are several biomarker candidates for prostate cancer in urine, including prostate cancer cells, DNA, RNA, proteins, exosomes and other small molecules.

Genomics

Interdisciplinary field of biology focusing on the structure, function, evolution, mapping, and editing of genomes.

Epigenomics

Study of the complete set of epigenetic modifications on the genetic material of a cell, the epigenome.

Transcriptomics

Study of an organism's transcriptome, the sum of all its RNA transcripts.

DNA - RNA
mRNA
microRNA
Non-coding RNA

Proteomics

Study of the entire set of proteins that is produced or modified by an organism or system.

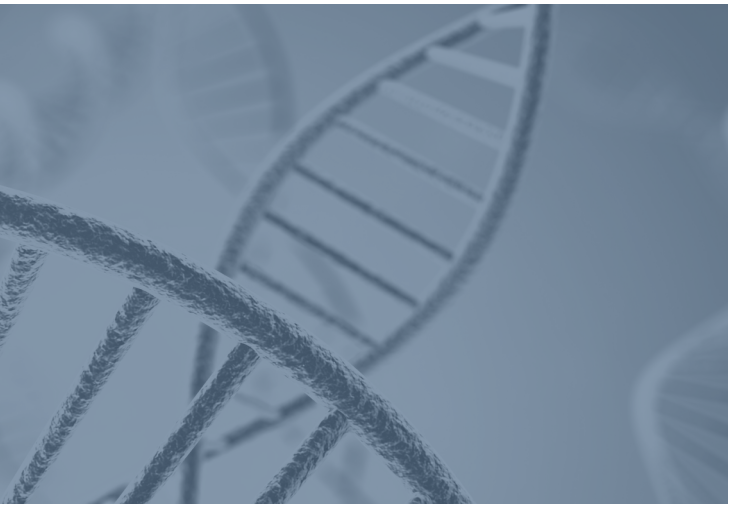
Proteins
Antibodies
Receptors
Enzymes
Peptides

Metabolomics

Study of chemical processes involving metabolites, the small molecule substrates, intermediates and products of cell metabolism.

Lipids
Carbohydrates
Enzymes
Metabolites

EXOSOMES / NUCLEOSOMES



GENOMICS/EPIGENOMICS

Urinary DNA-based markers include single nucleotide polymorphisms (SNPs), chromosomal aberration, copy number variations, loss of heterozygosity, gene amplification, microsatellite instability, and alteration in promoter region methylation[12,13].

Epigenetic alterations, including abnormal DNA methylation, are among the most common molecular alterations in human cancer. DNA methylation, unlike RNA and protein alterations, is relatively stable in body fluids and occurs in well-defined regions, unlike DNA mutations. This also makes DNA methylation easier to detect by sensitive PCR-based assays[6].

For example, the most common (>90%) genetic alteration reported to date in PCa is the epigenetic silencing of the glutathione-S transferase P1 (*GSTP1*) gene, as a result of promotor hypermethylation[6,12,13,15].

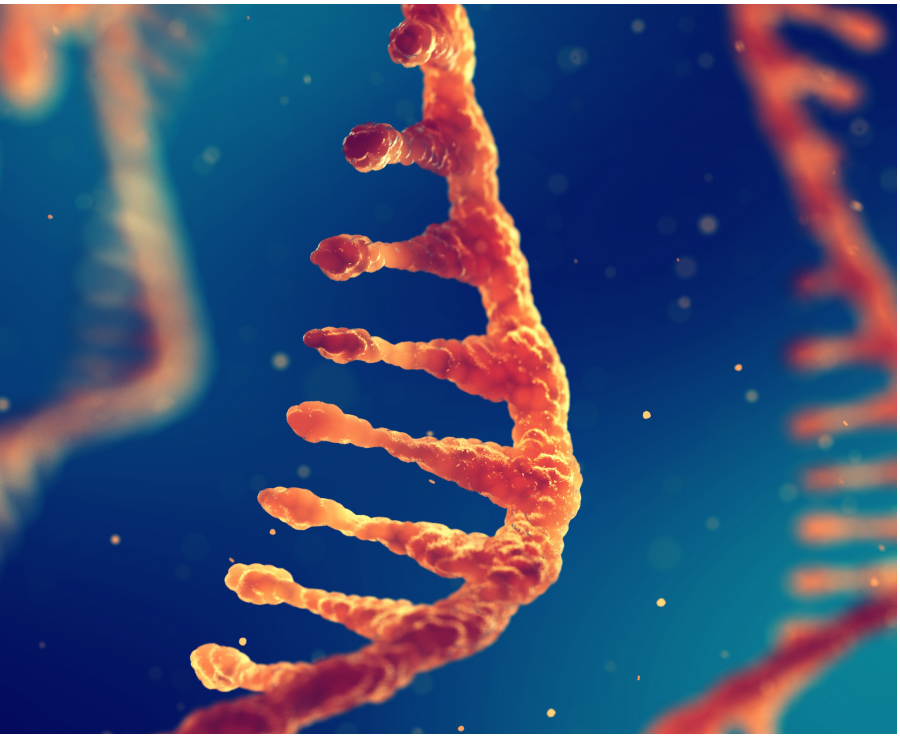
Some other less researched, but highly sensitive DNA-based biomarkers are adenomatous polyposis coli (*APC*), Ras association domain family member 1 (*RASSF1*), Ras association domain family member 2 (*RASSF2*), retinoic acid receptor beta (*RARB*) and transcription factor AP-2 epsilon (*TFAP2E*) (see Table).

DNA from prostate cancer can also be present in urine without prior DRE as cell-free DNA. The most investigated biomarkers in cell-free DNA are AR amplification, TMPRSS2-ERG fusion, PTEN gene deletion, MYCL amplification and NOTCH1 locus amplification[14].

DNA methylation markers

DNA marker	Full name	Methylation
APC	Adenomatous polyposis	Promotor methylation
GSTP1	Glutathione-S-transferase P1	Promotor hypermethylation
RARB2	Retinoic acid receptor beta 2	Methylation
RASSF1	Ras association domain family member 1	Promotor methylation
RASSF2	Ras association domain family member 2	Promotor methylation
TFAP2E	Transcription factor AP-2 epsilon	Methylation

URINE AS AN EMERGING LIQUID BIOPSY FOR PROSTATE CANCER BIOMARKERS



TRANSCRIPTOMICS

Urinary RNA-based biomarkers include coding and non-coding transcripts and regulatory RNAs, such as miRNAs.

The most common urinary marker is Prostate cancer antigen 3 (*PCA3*), a prostate-specific long non-coding mRNA, formerly known as differential display code 3 (*DD3*).

Although PCA3 does not encode a protein, PCA3 mRNA transcripts originating from prostate cells are detectable and quantifiable in urine[14,16]. The PCA3 gene is overexpressed in 95% of all primary prostate cancer specimens and absent in benign prostate tissue and other tumor types[7,12].

Another prominent RNA-based urinary biomarker, which is highly specific for prostate cancer is the TMPRSS2-ERG (transmembrane protease, serine 2 – E26 transformation specific (ETS) related oncogene ERG) fusion gene[12,13,15,16]. TMPRSS2-ERG levels have shown to be related to the pathological stage of prostate cancer[12]. Various commercially available tests have been developed based on PCA3 and/or TMPRSS2-ERG.

Other noteworthy RNA markers that are known to be overexpressed in prostate cancer are mRNAs alpha-methylacyl-coenzyme-A racemase (*AMACR*), Golgi membrane protein 1 (*GOLM1*), human telomerase reverse transcriptase (*hTERT*), homeobox C6 (*HOXC6*), prostate-specific G-coupled receptor (*PSGR*), prostate-specific membrane antigen (*PSMA*), and TTTY-15-USP9Y fusion gene, as well as numerous miRNAs and long non-coding RNAs[7,13].

Another study suggested eight mRNAs (*HOXC4*, *HOXC6*, *DLX1*, *TDRD1*, *ONECUT2*, *NKAIN1*, *MS4A8B* and *PPFIA2*) as potential biomarkers candidates in urine obtained after DRE[14]. The discovery of miRNAs has opened up a new field in cancer research with potential novel applications in diagnostics and therapy. However, to date only a few studies have investigated the connection between miRNAs and PCa[12].

RNA-based markers

RNA marker	Full name	Type RNA
AMACR	Alpha-methylacyl coenzyme-A racemase	mRNA
GOLM1	Golgi membrane protein 1	mRNA
hTERT	Human telomerase reverse transcriptase	mRNA
HOXC6	Homeobox C6	mRNA
PCA3	Prostate cancer antigen 3	Lnc mRNA
PSGR	prostate specific G-coupled receptor	mRNA
PSMA	Prostate specific membrane antigen	mRNA
TMPRSS2-ERG	Transmembrane protease serine 2-ETS-related gene	Fusion gene/protein
TTTY15-USP9Y	Testis-specific transcript Y linked 15 - ubiquitin specific peptidase 9 Y-linked	Fusion gene

Did you know

More men die with prostate cancer than because of it.

Jacklin C. et al. Cancer Treatment and Research Communications, 2021.

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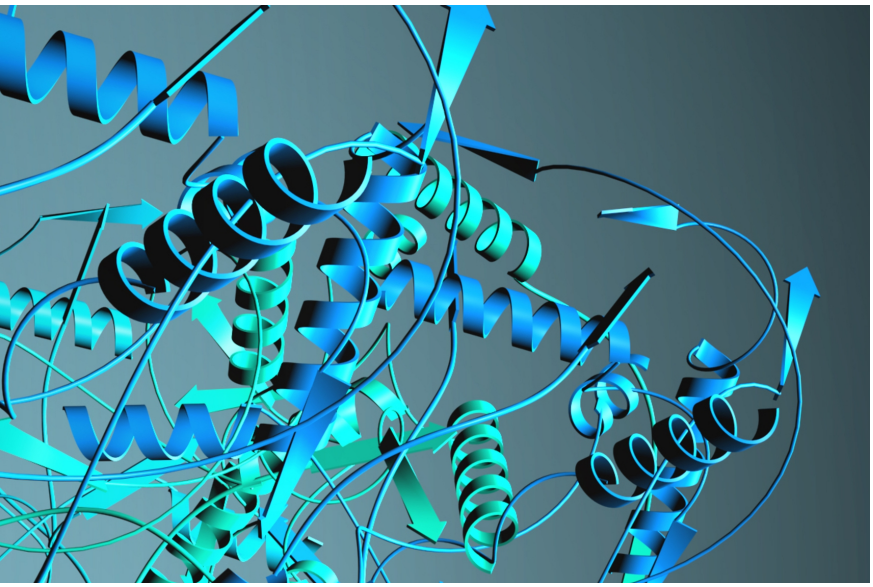


PROTEOMICS

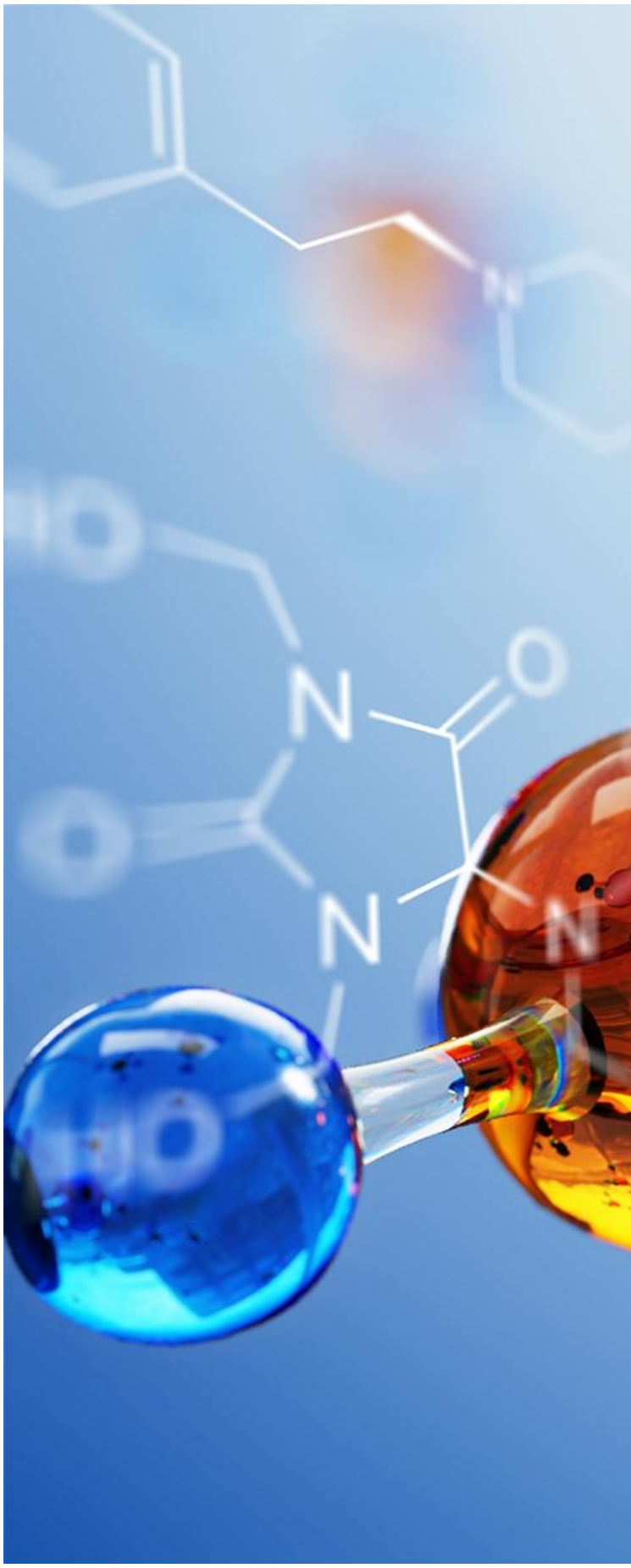
Protein-based biomarkers in urine include cell surface receptors, tumor antigens, protein phosphorylation states, carbohydrate determinants and peptides. Many proteins have been reported as candidate biomarkers, but no protein biomarkers have entered clinical use yet.

Some of the proteins evaluated in pilot studies are alpha-2-glycoprotein 1, AMACR, Annexin A3 (*ANXA3*), apolipoprotein D, b2M, delta-catenin, engrailed-2, heptatocyte growth factor (c- *met*) , I L- 1 8Bpa , in testin a l m u cin (*MUC3*) , matrix metalloproteinases (*MMPs*) , Pepsinogen 3 group 1 (*PGA3*) , thymosin beta-15, and uromodulin (*THP*)[14,15].

Protein marker	Full name
AZGP1/ZAG	Alpha-2-glycoprotein 1
AMACR	Alpha-methylacyl coenzyme A racemase
ANXA3	Annexin A3
APOD	Apolipoprotein D
b2M	Beta-2-Microglobulin
CTNND	Delta-Catenin
En2	Engrailed-2
c-met	Hepatocyte growth factor
IL-18Bpa	Interleukin-18 binding protein
MUC3	Intestinal mucin
MMPs	Matrix metalloproteinases
PGA3	Pepsinogen 3 group 1
TMSB15A	Thymosin beta-15
THP	Uromodulin (Tamm-Horsfall glycoprotein)

METABOLOMICS

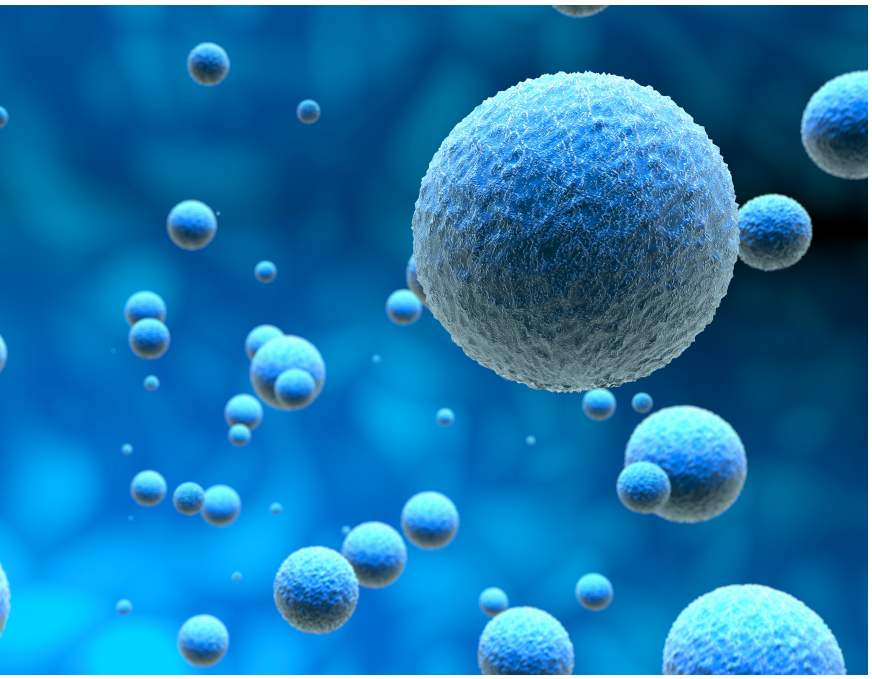
About 87 metabolites for prostate cancer were profiled from various clinical samples including tissues, urine, and plasma through Liquid Chromatography Mass Spectrometry (*LC- MS*) and Gas Chromatography Mass Spectrometry (*GC-MS*)methods. These biomarkers were able to distinguish prostate cancer patients from normal subjects[17]. A follow-up nested case-control study showed that urinary sarcosine (and cysteine) levels were significantly higher in 54 patients who had prostate cancer recurrence after treatment[18].



Examples of urinary metabolites

- Succinate
- Pyruvate
- Oxoglutarate
- Carnitine
- Phosphoenolpyruvate
- Trimethyllysine
- Melatonin
- Isovalerylcarnitine
- Gluracylcarnitine
- Octenoylcarnitine
- Decanoylcarnitine
- Acetyl-CoA
- Palmitoyl Sphingomyelin
- Lactate
- Gluconate
- Adenosine
- 2-methylbutyrylglycine
- Guandinoacetate

URINE AS AN EMERGING LIQUID BIOPSY FOR PROSTATE CANCER BIOMARKERS



EXTRACELLULAR VESICLES/ EXOSOMES

Extracellular vesicles (EVs) are small vesicles secreted by various cell types, including cancer cells. EVs in urine after DRE include exosomes and prostasomes[14]. Exosomes are highly heterogeneous and probably reflect the phenotypic state of the cell that generates them[19].

Urinary exosomes have recently been described as treasure chests. Analyzing urinary exosomes has several advantages in prostate cancer detection[12,14].

Several studies have examined panels of urinary biomarkers for the detection of prostate cancer. Results show that combining different urinary biomarkers markedly increased the sensitivity[6,12,15].

To date, four urinary tests for Pca are commercially available.

Test	Type biomarker	Target Molecules	Available as
Progensa PCA3	lncRNA	PCA3	FDA approved
ExoDx Prostate Intelliscore	Exosomal RNA	ERG, PCA3, SPDEF	CLIA-approved LDT
SelectMDx	mRNA	HOXC6, DLX1, PSA (KLK3)	CLIA-approved LDT
Mi-Prostate Score (MiPS)	RNA	TMPRSS2-ERG, PCA3 Serum PSA	CLIA-approved LDT

**explaining of abbreviations in table*

COLLI-PEE® AS A URINE COLLECTION DEVICE

Since the potential of urine as a liquid biopsy for prostate cancer is more than compelling, urine collection is becoming increasingly important in clinical practice. The content of urine can change at different points of the day, and can be affected by various activities including exercise, diet, medication and lifestyle[20]. Furthermore, urine components, such as human DNA, are not the same in all fractions[21].

For example, one study concluded that first-void/first-catch urine after DRE (Digital rectal exam) resulted in a clear increase in prostate cancer biomarker levels of both cell pellets and exosomes[22].

To use urine for clinical applications, the pre-analytical variation (collection, transport and storage) must be kept to a minimum. Collection through a standard urine cup can be awkward, messy and inconvenient for the user. These can be overcome with **Colli-Pee®**, a urine collection device for **standardized and volumetric collection of urine** which allows **immediate mixing with preservative**. Colli-Pee® variants provide easy collection of first-void urine.

Currently, Colli-Pee® can be prefilled with Novosanis' UCM, to allow preservation of DNA in urine (such as HPV DNA).

To extend the preservation coverage to other analytes (RNA, EV, cfDNA) a new preservative, UAS is currently in development by Novosanis in collaboration with its sister company DNA Genotek.

CONCLUSION

Recent advancements in omics technologies have improved our understanding of the molecular landscape causing cancers. Given the wide array of biomarkers, urine is a promising sample type that can change the way prostate cancer is detected and monitored in the future.

However, for effective clinical applications, standardization of preanalytical conditions for the handling of urine specimens is required[3].

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