Urine as a sample type for multi-omic cancer research

Anya Mehta, Stephanie Jordaens, Danielle Pasmans, Sanne Bruyninckx, Koen Buyers, Vanessa Vanekerkhoven
Novosanis, Belgium
June 2021

INTRODUCTION

What is urinomics
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. Different omics data types exist, which when studied independently, or together, can give insights into various disease states.

This white paper focuses on urinomics, a field which includes all the omics in urine as a sample type. Several biomarker candidates have been identified in urine, including DNA, RNA, proteins, exosomes and metabolites. For different cancer types, some biomarkers are explained in more detail.

URINE AS A SAMPLE TYPE

Urine is an exciting sample type that contains relevant biomarkers for detection of several infectious diseases including sexually transmitted infections (STIs) and Human Papillomavirus (HPV). Additionally, urine has shown potential as a liquid biopsy for detection and monitoring of several cancer types. Urine sampling is also beneficial as it is easy, quick and non-invasive.

URINOMICS AND CANCER RESEARCH

A high number of potentially informative cancer biomarkers have been found in urine. Below are some highlights of the omics work in urine for cancer research.

Genomics/Epigenomics
DNA and DNA modifications, including DNA methylation have shown to be useful biomarkers for several cancer types. Changes in DNA methylation are among the most frequent molecular alterations in human cancer.

Bladder cancer
Telomerase reverse transcriptase (TERT) promoter mutations are extremely specific to bladder cancer. In low-grade non-muscle invasive bladder cancer (NMIBC) tumors, mutations in the fibroblast growth factor receptor 3 (FGFR3) oncogene are frequent. In high-grade NMIBC tumors, mutations in p53 genes, which can cause dysregulation of the RAS-MAPK (mitogen-activated protein kinase) pathway are seen more often. Mutations in RAS (Rat sarcoma) oncogenes occur in 13% of all bladder cancer tumors, providing valuable urinary biomarker candidates. DNA methylation markers commonly altered in the cancer type are also being investigated in urine.

Cervical cancer
Human Papillomavirus (HPV), a common sexually transmitted infection, is the primary cause of cervical cancer. DNA methylation can be used as biomarkers to distinguish productive from transforming high-risk HPV infections, which have a risk of progressing to cancer. Methylation levels have also been found to rise with increased severity and duration of disease.

Prostate cancer
The most common (>90%) genetic alteration in prostate cancer currently is the epigenetic silencing of the glutathione-S transferase P1 (GSTP1) gene caused by promoter hypermethylation. Other epigenetic alterations are also being investigated as biomarkers for the cancer type.

Transcriptomics
Transcriptomics, the study of RNA transcripts, including post-transcriptional regulation of gene expression microRNAs (miRNAs), are an emerging source of biomarkers for many cancer types. Urine based RNA-based biomarkers, including coding and non-coding transcripts and regulatory RNAs, such as miRNAs, are promising in cancer research.

Bladder cancer
Several studies have shown the potential of miRNAs in the detection of bladder cancer. A recent meta-analysis concluded long non-coding RNAs in urine may serve as non-invasive diagnostic biomarkers for the cancer type, but more work is needed in this space.

Prostate cancer
Prostate cancer antigen 3 (PCA3), a prostate-specific long non-coding mRNA, is overexpressed in 95% of all primary prostate cancer specimens and absent in benign prostate tissue and other tumor types, making it a relatively specific biomarker for the caner type.

TMPRSS2-ERG (transmembrane protease, serine 2 – E26 transformation specific (ETS) related oncogene ERG) fusion gene is another highly specific RNA-based urinary biomarker for prostate cancer.

Proteomics
It is well-established that in many cancers several proteins are significantly mis-, up- or down-regulated, and could be taken as signatures for diagnostic confirmation. From a proteomics view, urine can be divided into three major fractions: soluble proteins, exosome-associated proteins and endogenous peptides.

Cervical cancer
The presence of the E6 oncoprotein is necessary for oncogenic transformation. Detection of HPV16/18 E6 oncoprotein in urine can be an attractive alternative to increase screening coverage for cervical cancer especially in low and middle income countries (LMICs).

Bladder cancer
Urinary calprotectin has shown to detect bladder cancer with high sensitivity and specificity. One study showed that the median calprotectin level was 10-fold higher in bladder cancer patients than healthy controls. Two other urinary proteins, stathmin-1 and CD147 also have potential in bladder cancer detection.

Kidney (Renal) cancer
Levels of aquaporin-1, and adipophilin (since renamed perilipin-2, PLIN2) in urine can indicate renal cell carcinoma (RCC). Additionally, kidney injury molecule-1 (KIM-1) may serve as a surrogate biomarker for kidney cancer and a non-invasive pre-operative
Ovarian cancer
Among a wide spectrum of biomarkers, human epidermis protein 4 (HE4) has shown to be the most promising for monitoring patients with ovarian cancer. Unlike CA125, a biomarker found in blood, HE4 is not overexpressed in normal ovarian tissue, benign ovarian disease, or tumors with low malignant potential. Other ovarian cancer biomarkers, including fibrinogen a fragment, collagen a 1 (III) fragment and fibrinogen β NT fragment can also be found in urine.

However, there is yet some debate in the field which suggests that urinary biomarkers may be insufficient for the effective detection of ovarian cancer early stages but may be superior when used alongside other non-urinary biomarkers and transvaginal ultrasonography (TVUS).

Prostate cancer
A study identified and validated 12 novel urinary biomarkers and showed that first-void urine was able to identify patients with prostate cancer with 91% sensitivity. Annexin A3, a calcium-binding protein has also shown to be a novel urine-based biomarker for early prostate cancer detection when used in conjunction with PSA (Prostate Specific Antigen) testing.

Examples of urinary proteins for several cancers

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Source</th>
<th>Detection Method</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSA</td>
<td>PSA testing</td>
<td>Liquid Chromatography Mass Spectrometry (LC-MS) and Gas Chromatography Mass Spectrometry (GC-MS) methods.</td>
<td></td>
</tr>
<tr>
<td>Sarcosine (N-methylglycine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succinate, Pyruvate, Oasglutamate, Carnitine, Phosphaenopyruvatic, Thymine, Methanalin, Isodurvalylcarnitine, Gluanyrcarnitine, Octenoylcarnitine, Decanoylcarnitine, Acetyl-CoA (palmitoyl)</td>
<td>Mass Spectrometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomyelin, Lactate, Glucosone, Adenosine, 2-methylbutyrylglycine and Guanidinoacetate</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

COLLI-Pee® as a Urine Collection Device
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 several cancer types are detected and monitored in the future.

However, for effective clinical applications, standardization of pre-analytical conditions for the handling of urine specimens is required. More work needs to be done to better understand if and how variables such as urine collection, urine fractions, storage, as well as shipping conditions can influence sample quality and impact biomarker detection.

This is where Novosanis’ urine collection device, Colli-Pee® fits in. Urine collected with Colli-Pee® offers improved diagnostic sampling accuracy and patient comfort compared to a regular urine cup. The platform consists of variants, which can collect different urine volumes for various application purposes. Colli-Pee® can be prefilled with stabilization chemicals, allowing for increased sample stability.

References: