Urine as a sample type for *multi-omic* cancer research

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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[1].

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.

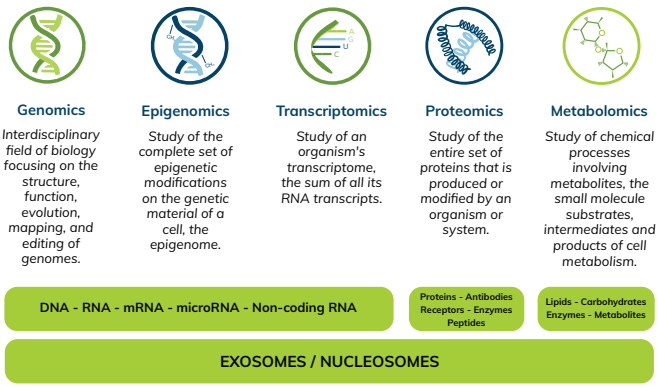

Figure 1: Definitions of the various omics

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) promotor 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[2].

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[3].

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 promotor hypermethylation. Other epigenetic alterations are also being investigated as biomarkers for the cancer type[4].

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[2].

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[4].

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[4].

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[4].

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)[5].

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[2].

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[7]. Other ovarian cancer biomarkers, including fibrinogen α fragment, collagen α 1 (III) fragment and fibrinogen β NT fragment can also be found in urine[8].

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)[7].

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[9]. 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[4].

Examples of urinary proteins for several cancers

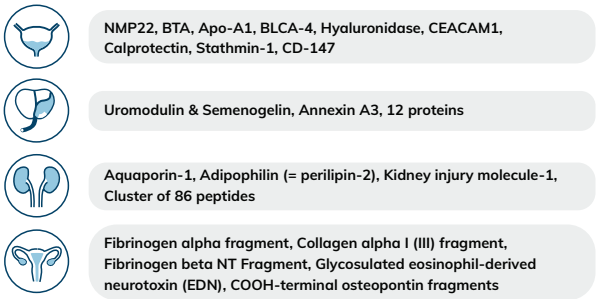

Figure 3: Examples of urinary proteins for several cancers

Metabolomics

Metabolomics provide a global chemical fingerprint of the metabolism of cells, which can indicate physiological and pathological states of biological samples.

Bladder cancer

A study profiled urine metabolites of bladder cancer patients and controls, which found 12 differential metabolites which distinguished bladder cancer and control groups with a sensitivity of 91.3% and specificity of 92.5%. Additionally, a set of candidate biomarkers for bladder cancer including palmitoyl sphingomyelin, lactate, gluconate, adenosine, 2-methylbutyrylglycine and guandinoacetate were also suggested in another study[2].

Breast cancer

The urinary metabolome of breast cancer patients showed an overall superior performance for detection of the cancer type. A sensitivity of 93.5% and specificity of 86.2% were observed for the combined detection of succinic acid & dimethyl-heptanoylcarnitine[10].

Colorectal cancer

Urinary nucleosides, which are modified metabolic products created by the degradation of RNA and DNA can be used for the detection of colorectal cancer. Researchers found that cytidine, 1-methyladenosine and adenosine were significantly elevated in cancer patients. These biomarkers were able to identify patients with colorectal cancer with a sensitivity of 69-97% and specificity of 75-99%[11].

Prostate cancer

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 from normal subjects[12]. 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[13].

Examples of urinary metabolites for several cancers

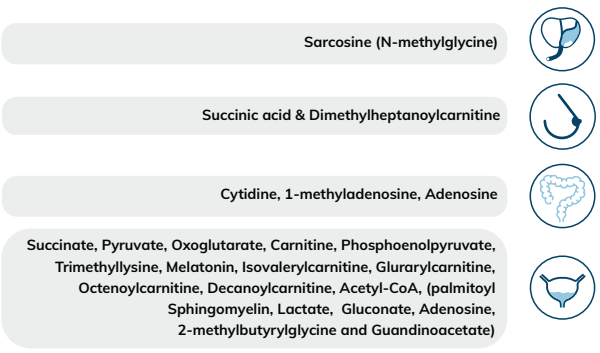

Figure 4: Examples of urinary metabolites for several cancers

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 chemistries, allowing for increased sample stability.

References:

(1) Hasin Y, Seldin M, Lusis A. Multi-omics approaches to disease. Genome Biol. 2017 May 5;18(1):83. doi: 10.1186/s13059-017-1215-1. PMID: 28476144; PMCID: PMC5418815.

(2) Mehta et. al, Urine as an emerging liquid biopsy for bladder cancer biomarkers - April 2021 - Novosanis White Paper

(3) Van Keer S, Pattyn J, Tjalma WAA, Van Ostade X, Ieven M, Van Damme P, Vorsters A. First-void urine: A potential biomarker source for triage of high-risk human papillomavirus infected women. Eur J Obstet Gynecol Reprod Biol. 2017 Sep;216:1-11. doi: 10.1016/j.ejogrb.2017.06.036. Epub 2017 Jun 27. PMID: 28689156.

(4) Jordaens et. al, Urine as an emerging liquid biopsy for prostate cancer biomarkers – May 2021 – Novosanis White Paper https://novosanis.com/sites/default/files/poster/pdf/White%20Paper%20PCa-version2020-06A_EN.pdf

(5) Oliveira CM, Musselwhite LW, de Paula Pantano N, Vazquez FL, Smith JS, Schweizer J, Belmares M, Possati-Resende JC, Vieira MA, Longatto-Filho A, Fregnani JHTG. Detection of HPV E6 oncoprotein from urine via a novel immunochromatographic assay. PLoS One. 2020 Apr 22;15(4):e0232105. doi: 10.1371/journal.pone.0232105. PMID: 32320451; PMCID: PMC7176116.

(6) Zhang, P.L., et al., Urine kidney injury molecule-1: a potential non-invasive biomarker for patients with renal cell carcinoma. Int Urol Nephrol, 2014. 46(2): p. 379-88.

(7) https://novosanis.com/blog/urine-sampling-non-invasive-way-improve-diagnostic-efficiency-ovarian-cancer

(8) Boschetti, E., M.C. Chung, and P.G. Righetti, "The quest for biomarkers": are we on the right technical track? Proteomics Clin Appl, 2012. 6(1-2): p. 22-41.

(9) Jedinak, A., et al., Novel non-invasive biomarkers that distinguish between benign prostate hyperplasia and prostate cancer. BMC Cancer, 2015. 15: p. 259.

(10) Cala, M., et al., Urinary metabolite and lipid alterations in Colombian Hispanic women with breast cancer: A pilot study. J Pharm Biomed Anal, 2018. 152: p. 234-241.

(11) Hsu, W.Y., et al., Urinary Nucleosides as Biomarkers of Breast, Colon, Lung, and Gastric Cancer in Taiwanese. Plos One, 2013. 8(12).

(12) Sreekumar, A., et al., Metabolomic profiles delineate potential role for sarcosine in prostate cancer progression. Nature, 2009. 457(7231): p. 910-4.

(13) Stabler, S., et al., Serum methionine metabolites are risk factors for metastatic prostate cancer progression. PLoS One, 2011. 6(8): p. e22486. factors for metastatic prostate cancer progression. PLoS One, 2011. 6(8): p. e22486.