ADVANCED EXTRACTION OF URINARY MICRORNA FOR EARLY CANCER DETECTION

Microfluidics are improving **EARLY CANCER DETECTION FROM URINE-BASED LIQUID BIOPSIES**. What could this mean for non-invasive screening, treatment selection and response monitoring?

Unlike expensive, invasive and risky tissue biopsies, the biomarkers found in body fluids, such as blood, urine or cerebral spinal fluid, offer an easier way to spot the first signs of cancer or relapse after treatment. But the scarcity of biomarkers released into body fluids during early-stage cancer has remained a challenge.

To date, most liquid biopsy analyses are performed on blood samples and look for a wide range of circulating cancer biomarkers, from fragments of DNA and RNA shed from dying cells, to whole cancer cells and exosomes containing tumour material.

However, circulating tumour cells are scarce and mostly shed from late-stage cancers. Similarly, circulating tumour DNA makes up only a small fraction of total cell-free DNA, and early stage cancers are not likely to release enough to be detectable in a typical blood draw of 10 ml[1].

Enough exosomes

Recently, membrane-bound extracellular vesicles known as exosomes, which are released from cancer cells, have emerged as promising early cancer biomarkers. These contain microRNAs that can modulate gene expression in recipient cells in a tumour's surrounding microenvironment, and they have been associated with the development and progression of the disease[2].

"Circulating tumour DNA and cancer-driving DNA mutations are particularly difficult to detect in early-stage cancer, but exosomes are released by cancer cells pro-actively to communicate with other cells, even at the early stages," explains Yuki Ichikawa, Chief Technology Officer of the Japanese biotechnology company Craif. Furthermore, exosomal microRNAs are much more stable than free-floating RNAs, and are providing new insights into how cancer cells modulate their microenvironment to grow and evade the immune response.

"EXOSOMES ARE RELEASED BY CANCER CELLS PRO-ACTIVELY TO COMMUNICATE WITH OTHER CELLS, EVEN AT THE EARLY STAGES."

Extracting exosomes efficiently has been the main bottleneck, says Ichikawa.

A device developed by Craif consisting of zinc oxide nanowires embedded in a microfluidic channel is designed to more effectivly extract exosomes from urine.

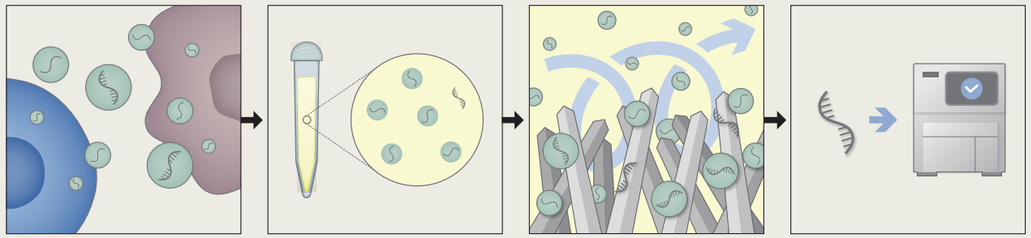
Exosomes are very small, he explains, and conventional centrifugation methods, which take at least four hours, can damage their contents.

Craif has developed a device that consists of zinc oxide nanowires embedded in a microfluidic channel to effectively extract exosomes from urine and collect encapsulated microRNAs[3].

Through a simple 40-minute procedure that requires just 1 ml of urine, the device can isolate a much larger variety of species of microRNA than conventional ultracentrifugation methods. "By combining the technology with machine-learning algorithms we can also detect cancer-associated microRNAs with a high degree of accuracy," Ichikawa adds.

HOW CANCER BIOMARKERS COULD BE IDENTIFIED FROM URINE

If microRNAs can be extracted from urine efficiently and analysed effectively, they could provide the basis for non-invasive early cancer screenings.



Exosomes and microRNA regulate cancer pathology

Exosomes carrying microRNA move between tumour cells and their surrounding microenvironment. They are reflected in cancer pathology from an early stage.

Urine contains microRNA from tumours

Exosomes and microRNA secreted from a tumour site are found in various body fluids, such as blood and urine.

Nanowire device captures and enriches biomarkers

Craif's nanowire microfluidic device captures and enriches exosomal and free nucleic acids in urine.

Measuring microRNA profiles for early cancer detection

Early-stage cancer is detected by analysing microRNA signatures.

Cancer signals in urine

Ichikawa says that based on the expression of the selected microRNAs in urine they are able to differentiate cancer patients from healthy individuals, even in those that have non-urologic malignancies.

In 2017, Takao Yasui, co-founder and Nanodevice Director of Craif, and colleagues showed that urinary microRNAs could serve as biomarkers for five different types of cancer: bladder, prostate, lung, pancreas, and liver[4].

In April 2021, they showed that their device can also detect urinary microRNA profiles that are indicative of early-stage central nervous system tumours with 100% sensitivity and 97% specificity[5].

Although the most promising application of the device to date is in early cancer and minimal residual disease detection after treatment, further research into the function of urinary microRNAs in cancer and their target genes could help predict patient responses to targeted therapies and immunotherapeutics.

The detection of genetic alterations in circulating cell-free DNA biomarkers for instance could be used to match patients with drugs that target particular mutations, and since liquid biopsies can be serially repeated, they would allow dynamic tumour monitoring.

"Pathway analyses of microRNAs reveal that many contribute to cancer-related pathways such as sustained cell division, metastasis and the development of new blood vessels," Ichikawa explains. Understanding more about the roles of microRNAs in cancer could also lead to the development of new treatment options.

What next for urine-based liquid biopsy testing?

Craif is aiming to launch its first urine-based liquid test in 2022. These tests will not replace standard screening programmes, but they could greatly aid the detection of cancers that are not routinely screened for or that don't have very sensitive blood biomarkers.

Among gynecological cancers, for example, ovarian cancer has one of the worst prognoses because it is often not detected until it has progressed to an advanced stage[6]. "We are developing an early detection test for ovarian cancer, as well as a test that can distinguish between benign and malignant brain tumours that will help physicians stratify patients," Ichikawa says.

Carrying out tests on urine that address important needs is particularly attractive, adds Ichikawa. "The patient-friendly method of urine-based biopsies, and the possibility of exploiting the therapeutic potential of microRNA signals found in it, makes them a promising alternative to blood-based biopsies for mass screening." ■

REFERENCES

1. Fiala, C. *et al. BMC Med.* **16**: 166 (2018).
2. Peng, Y., & Croce, C. *Sig. Transduct. Target Ther.* **1**: 15004 (2016).
3. Yasui, T. *et al. Biosens. Bioelectron.* **194**: 113589 (2021)
4. Yasui, T. *et al. Sci. Adv.* **3**: e1701133 (2017).
5. Kitano, Y. *et al. ACS Appl. Mater. Interfaces* **13**: 17316-17329 (2021).
6. Momenimovahed, Z. *et al. Int. J. Womens Health.* **11**: 287-299 (2019).

CRAIF

craif.com/en

ADVERTISEMENT FEATURE

Advertiser retains sole responsibility for content