



A Non-invasive, Low-cost Procedure to Detect Cancer Biomarkers from Biological Fluids

Dr Anton Iliuk, PhD

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Liquid biopsies have recently gained attention as sources of non-invasive diagnostic cancer biomarkers. Traditional biofluid sampling methods, however, are onerous and time-consuming and present many limitations including poor sensitivity for biomarkers in low concentrations. Dr Anton Iliuk and his team at Tymora Analytical Operations, West Lafayette, USA, have developed a way to isolate extracellular vesicles from plasma, urine and other biofluids. They now aim to grow their network of collaborators to use their platform for the discovery of protein biomarkers from several cancers, neurodegenerative diseases, and other conditions.

The Case for 'Liquid Biopsies'

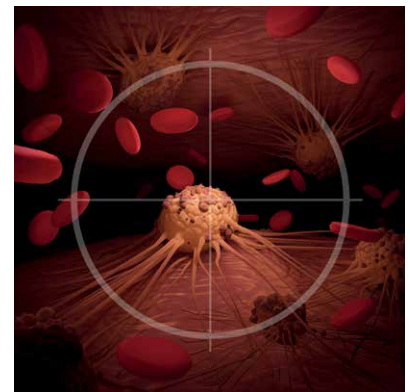
The analysis of biological fluids such as plasma, serum, urine, tears, or saliva, have recently emerged as a source of diagnostic biomarkers. Traditionally, the analysis of altered tissue in cancer and other diseases relies on invasive and painful procedures, such as tumour biopsies, which involve the removal of solid tissue samples that are processed for further testing. The use of 'liquid biopsies' offers several advantages, including low costs, faster processing times, their non-invasive nature and the ability to aid early diagnosis. However, they also present some limitations in terms of sensitivity towards the lower biomarker concentrations in biofluids.

Dr Anton Iliuk and his team at Tymora Analytical Operations, West Lafayette, USA, have optimised a method that allows the isolation of extracellular vesicles (EVs) from plasma, urine, saliva and other biological fluids. Their

procedure involves subsequent analysis of the enriched EV proteins by mass spectrometry, allowing identification of the disease markers that were previously undetectable. Their method will allow clinical scientists around the world to discover and validate protein biomarkers for diagnostic and monitoring purposes. Dr Iliuk's team has successfully used the platform to discover new EV protein markers from several cancers and other conditions, such as neurodegenerative diseases and diabetes.

Overcoming the Limitations of Biofluid Analyses

A major limitation of methods relying on the handling of biofluids is their poor sensitivity towards the relatively low abundance of biomarkers in the blood, especially at the early stages of the disease. Many techniques currently focus on genetic information, especially gene mutations. While



genome information can be helpful as a diagnostic tool, its interpretation is often complex, as there are many layers of regulation that exist between DNA, RNA and the expression of proteins in health and disease. Protein analysis, on the other hand, provides direct, real-time information about the physiological status of the organism and disease progression. Compared to gene testing, protein analysis also offers a simpler setup and is relatively inexpensive.



Dr Iliuk and his collaborators have recently developed a separation technique that is especially adapted for the identification and detection of new protein biomarkers. By using cell-secreted EVs, also called exosomes, they have found a way to bypass the challenges typically associated with the analysis of biological fluids. EVs formation is initiated by cellular membranes, which shed the vesicles into every biological fluid. The EV membranes protect the proteins inside from degradation by external enzymes, preserving the integrity of the biomarkers that could be detected in diagnostic tests. Interestingly, EV-associated proteins and peptides can be identified well before the onset of symptoms, making them promising disease biomarker candidates for detecting early-stage cancer and other pathological conditions.

Tracking Protein Phosphorylation as a Diagnostic Strategy

EVs provide scientists with a quick snapshot and a good representation of the protein and nucleic acid composition of their parent cells. Among others, one very effective way to monitor disease progression through EV sample testing would involve the analysis of protein phosphorylation, as a direct marker of cellular signalling during disease.

The physiological process of phosphorylation involves modification of proteins by enzymes that aid the attachment of a phosphate group onto the protein structure, also resulting in a modification of its functions. Protein phosphorylation is a key control mechanism for the regulation of cellular pathways. The detection of changes in phosphorylation is of fundamental importance in the understanding of how signalling networks interact and how they are misregulated as a result of disease.

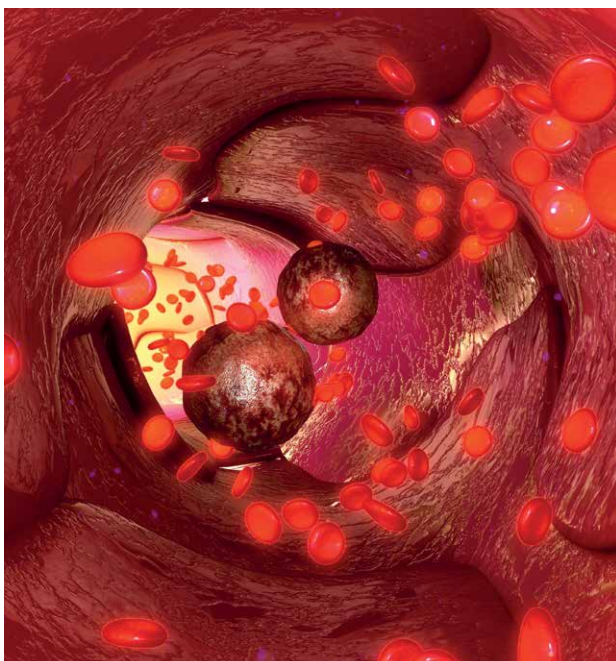
Dr Iliuk proposes that phosphorylation analysis in EVs can be used in two ways: either to detect changes in cancer-induced phosphorylation in EV proteins; or as a tag, whereby phosphorylation acts as an enrichment marker of low abundant proteins that otherwise would not be detectable by traditional methods.

EVtrap: A Novel Method to Detect Multiple Disease Biomarkers

Dr Iliuk and his team have been focusing on the development of a non-invasive and inexpensive approach in the detection and monitoring of bladder cancer, a disease that affects more than 530,000 patients in the USA alone. Their initial goal was to develop an effective disease-monitoring urine test that can

be used to examine bladder cancer patients who underwent treatment and require monitoring for cancer recurrence. The monitoring would look at the direct output of cancer cells through their EV shedding in the urine, with the aim of detecting any recurrence in malignancy at an early stage, allowing all necessary follow-up interventions to be carried out rapidly to stop the disease progression.

After years of hard work and determination, Dr Iliuk and his colleagues recently published a study introducing a rapid EV isolation method called EVtrap (extracellular vesicle total recovery and purification). The technique uses magnetic beads that are able to capture the vesicles and allow their separation by the action of a magnetic field. The EVtrap method enables the full screening of significantly higher levels of EV markers, compared to other common approaches of vesicle sampling. Given the high recovery rate of the technique, the isolated vesicles can be used for several types of follow-up analyses. Additionally, the EVtrap approach is fast and simple, allowing EV capture in 10 to 30 minutes, instead of the 6 to 22 hours needed for traditional EV separation methods based on centrifugation.



Direct Biomarker Detection from Urine and Plasma

The team believes that the EVtrap method could soon be widely used by medical practitioners worldwide for the efficient capture of extracellular vesicles from unfiltered human urine samples. The data in the study showed that close to 2,000 unique phosphopeptides could be identified from more than 860 unique phosphorylated proteins using just a 10 mL sample of urine. These data offer hope that urine EV phosphorylated proteins could be used not only for early stage cancer detection, but also as molecular targets in companion diagnostic procedures for the targeted treatment of several types of cancer.

The diagnostic potential of the technique is not limited to the analysis of urine. Dr Iliuk and his collaborators successfully adapted the EVtrap method for the protein phosphorylation analysis of EVs from human plasma. Strikingly, by using EVtrap, they reported being able to identify over 5,500 unique phosphopeptides representing almost 1,600 phosphorylated proteins by using samples of only 1 mL of plasma. This allowed them to analyse plasma samples from patients diagnosed with chronic kidney disease or kidney cancer, identifying dozens of phosphoproteins capable of distinguishing disease states from healthy controls.

The team believes that the EVtrap method can similarly be adapted for DNA/RNA examination. The approach can also be easily automated for high-throughput screening assays and hands-off analyses. EVtrap will allow clinical practitioners to be able to uncover plasma biomarkers even at very low levels. The study paves the way for the development of non-invasive detection of renal cell carcinoma from plasma, even at early stages of the disease.



Financial Considerations and Future Plans

In addition to the advantages outlined above, the EV capturing method is set to offer several steps forward also in terms of financial considerations. Taking bladder cancer as an example, due to the requirements for ongoing monitoring and a large rate of re-occurrence of the disease, more than a million diagnostic biopsies are booked every year in the USA alone, putting a significant financial strain on the healthcare system. Dr Iliuk's EVtrap method offers a new cost-effective approach for the early diagnosis and follow-up monitoring of cancer and other diseases.

The team hopes to scale up the production of multi-biomarker tests, based on the use of 2 to 6 protein biomarkers detectable in the urine EVs of bladder cancer patients. The team argues that using multiple markers in a single test offers a better strategy for disease diagnostics, especially in cancer, given the complexity of the cellular pathways occurring in the disease. The multi-marker approach is expected to increase the sensitivity of disease detection over other traditional approaches. By using a high-sensitivity approach, clinicians can further refer positive results for further biopsy analysis, while using a negative test result as the point of elimination.

Urinary EV biomarkers can also be used for the successful diagnosis of kidney and prostate cancers. Dr Iliuk now aims to extend the scope of the EVtrap technique to isolate vesicles from other biological fluids, such as plasma, tears and saliva. The platform offers the capability of discovering protein biomarkers from several other diseases aside from cancer, including diabetes and neurodegenerative conditions. The team hopes to expand their network of collaborators to include clinicians and researchers that wish to discover novel biological markers for their disease of interest.



Meet the researcher

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Dr Anton Iliuk is the co-founder, president and chief technology officer of Tymora Analytical Operations, West Lafayette, USA. He obtained his PhD in biochemistry in 2011 from Purdue University, Indiana. His research has focused on the development of novel techniques for non-invasive biomarker discovery, proteomics and phosphorylation analysis. Through these efforts, he published over 35 manuscripts and book chapters and submitted 6 patent applications. Dr Iliuk has given oral presentations, showcasing his research, at multiple international conferences. At Tymora, he is the co-inventor of several technologies and applications, including PolyMAC, pIMAGO, EVCISE, and EVtrap.

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KEY COLLABORATORS

Multiple academic institutions, cancer centres, hospital systems, pharmaceutical and diagnostic companies.

FUNDING

Funding from NIH and NSF (SBIR and STTR grants), the Michael J. Fox Foundation and Indiana Matching Fund.

FURTHER READING

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