Advanced technologies in the pursuit of cancer eradication

Associate Professor Iulia Lazar discusses the advancement of microfluidics technology and shares her vision for the future of proteomics and biomarker discovery

Could you begin by explaining why you chose to pursue a career in cancer research? What motivates your work?

Cancer is a systemic disease. It affects the entire body and, at the molecular level, the functionality of the whole cell. To understand, fight and defeat this disease we need to adopt analysis and screening strategies that match this complex challenge. Inherently, this involves the development of technologies that enable a systems-level exploration of cancer onset and progression.

I started my career by building mass spectrometry and microfluidics instrumentation, but technologies evolve fast. From assaying a single gene or protein to profiling whole genomes, transcriptomes or proteomes, we are today able to generate comprehensive panels of biomolecules that are characteristic of a disease or its stage of development. The advance of mass spectrometry instrumentation has brought superior capabilities to deciphering the information encoded by the protein complement of a cell. Merging mass spectrometry with high-throughput front-end technologies such as microfluidics, and using this combination for the comprehensive analysis of altered cell states, seemed to be a natural evolution of my research interests.

As for my motivation, I'm driven by curiosity, love of research and the desire to contribute to the resolution of difficult problems.

How are proteomic and microfluidic technologies enabling you to gain further insight into the breast cancer cell cycle and identify biomarkers as drug targets?

My laboratory has a longstanding interest in exploring cell cycle-related changes that lead to the aberrant proliferation of cancer cells, as well as in developing microfluidic and proteomic technologies that can advance this effort. A question of interest that we are pursuing with proteomic technologies is whether the misregulation of gene and protein expression in various stages of the cell cycle can lead to the identification of upstream drug targets or downstream putative biomarkers. Once proteins of interest are identified, we want to develop microfluidic platforms that enable targeted, and therefore more sensitive, detection of such proteins in a high-throughput manner.

Could you introduce the lab-on-a-chip technology you are creating?

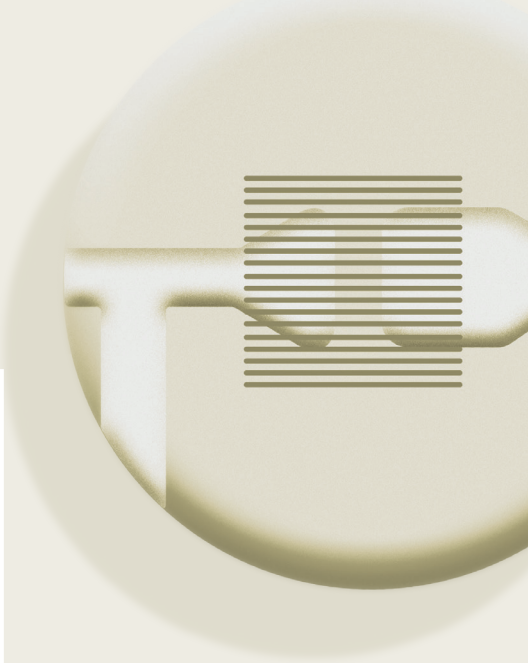
The lab-on-a-chip technology developed in my laboratory is directed toward creating miniaturised platforms for complex sample analysis as a front end to mass spectrometry detection. We develop functional elements for fluidic propulsion, valving, mixing, fast enzymatic reactions and sample separations. Most importantly, we are interested in developing the interface between the two technologies: microfluidics and mass spectrometry. Ultimately, we envision a fully integrated, stand-alone platform that facilitates the detection process. The miniaturised format enables the manipulation of small sample amounts, and fast, low-cost and high-throughput analysis. Mass spectrometry, on the other hand, enables the unambiguous detection of the components of interest.

Within the next five years, what would you most like to accomplish in your cancer research?

I would like to be at the stage where we can make correlations between certain defective processes in cancer cells, the upstream causes and downstream effects. Properly designed experiments will enable us to monitor changes in the protein complement of the cell, and develop mathematical models that establish cause-effect relationships between the origins and the products of the disease. The integration of such findings with clinical research will allow us to predict putative drug targets and biomarkers of disease. This work will be performed in collaboration with colleagues that use computational systems biology approaches for analysing large data sets. As for our microfluidic technology, I would like to see it implemented in routine laboratory research.

Microfluidic technologies and mass spectrometry

Researchers at **Virginia Tech**, USA, have combined groundbreaking microfluidics technology with advanced mass spectrometry to rapidly detect molecular changes in cancer cells and provide a powerful tool for future biological research



CELLULAR REPLICATION IS a tightly controlled process with strict molecular checkpoints before both DNA replication and cellular division can occur. When these regulatory controls break down, cells with damaged DNA can proliferate without constraint, leading to cancerous growth.

There are thousands of proteins involved in regulating a cell's passage into the DNA replication phase, as Associate Professor Iulia Lazar's research into the cell cycle has revealed. Her systems-level approach at Virginia Tech involves identifying cell cycle proteins responsible for cancer initiation and progression, resulting in databases of proteins that can be queried for potential markers of disease.

LAB-ON-A-CHIP

Lazar's team utilises microfluidic technologies, where tiny quantities of cellular extracts can be processed and analysed in a sensitive and high-throughput manner. The micro samples are assessed using mass spectrometry, which characterises the chemical composition of a sample and provides comprehensive protein-level information to pinpoint protein entities with therapeutic target or diagnostic potential.

Advances in microfluidics and mass spectrometry instrumentation have enabled Lazar to develop innovative proteomic 'lab-on-a-chip' technologies, which speed up the analysis process to allow rapid changes in protein expression and structure to be detected. Lazar's lab-on-a-chip integrates several processes into a single chip, including proteolytic digestion, sample enrichment and separation by liquid chromatography to enable the constituent compounds to be assessed by mass spectrometry. These miniaturised platforms generally provide better results than conventional lab-bench approaches, and significantly reduce analysis times and reagent costs.

QUANTIFYING PROTEIN PHOSPHORYLATION

One of Lazar's most exciting projects will enable the rapid quantification of protein phosphorylation in a cell. "Reversible protein phosphorylation of certain amino acids represents a key signal transduction mechanism that is involved in controlling fundamental biological processes such as gene expression, cell cycle progression, growth, proliferation, differentiation and death," she explains. "Early phospho-signalling events, in response to external stimuli, unfold within a short timeframe and therefore are difficult to detect and monitor with existing technologies." Abnormal protein phosphorylation is often involved in diseases, such as cancer, inflammation and metabolic disorders; as it is not easily predictable with bioinformatics approaches it must therefore be detected in the lab.

By expanding the capabilities of her lab-on-a-chip, Lazar can meet the challenge of assessing phospho-signalling events on the short timescale required. "In this project, we want to advance our microfluidic technology to a new level, and build a platform that integrates cell handling ability with the power of mass spectrometry detection to elucidate molecular structure," she elaborates. "The fast cell handling and sample processing capabilities of microfluidics will enable us to capture changes in phosphorylation that are involved in the initiation of signalling processes in cells."

Lazar and her team have already identified 3000 phosphorylated peptides that can be used as targets for detection on the chip, and have developed microfluidic components for processing cell extracts. Now, all that remains is to integrate these parts, says Lazar: "To begin, we will target proteins involved in phosphorylation signalling in SKBR3 breast cancer cells. The use of specialised mass spectrometry techniques will enable the detection of low-abundance target peptides and the quantitation of their phosphorylated states by using stable isotope-labelled peptide counterparts". The chip will provide novel insights into the events that lead to the initiation and propagation of the phosphorylation signalling cascades that regulate many cellular processes.

FUTURE POTENTIAL

Advances in microfluidics and proteomics by Lazar and others are providing us with unrivalled insight into the molecular mechanisms that control cellular function. Lazar encourages everyone to make the most of this: "Mastering these technologies will empower us with unprecedented control over the processes that alter or restore normal cell and biological function, and that ultimately determine the status of our health. Capitalising on this power should become a major thrust in every research laboratory".

IDENTIFICATION AND QUANTITATION OF CELL PHOSPHORYLATION

OBJECTIVE

To identify cell cycle proteins responsible for cancer cell proliferation through the use of microfluidic and mass spectrometry technologies, that can lead to improved approaches for the treatment of disease.

KEY COLLABORATORS

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