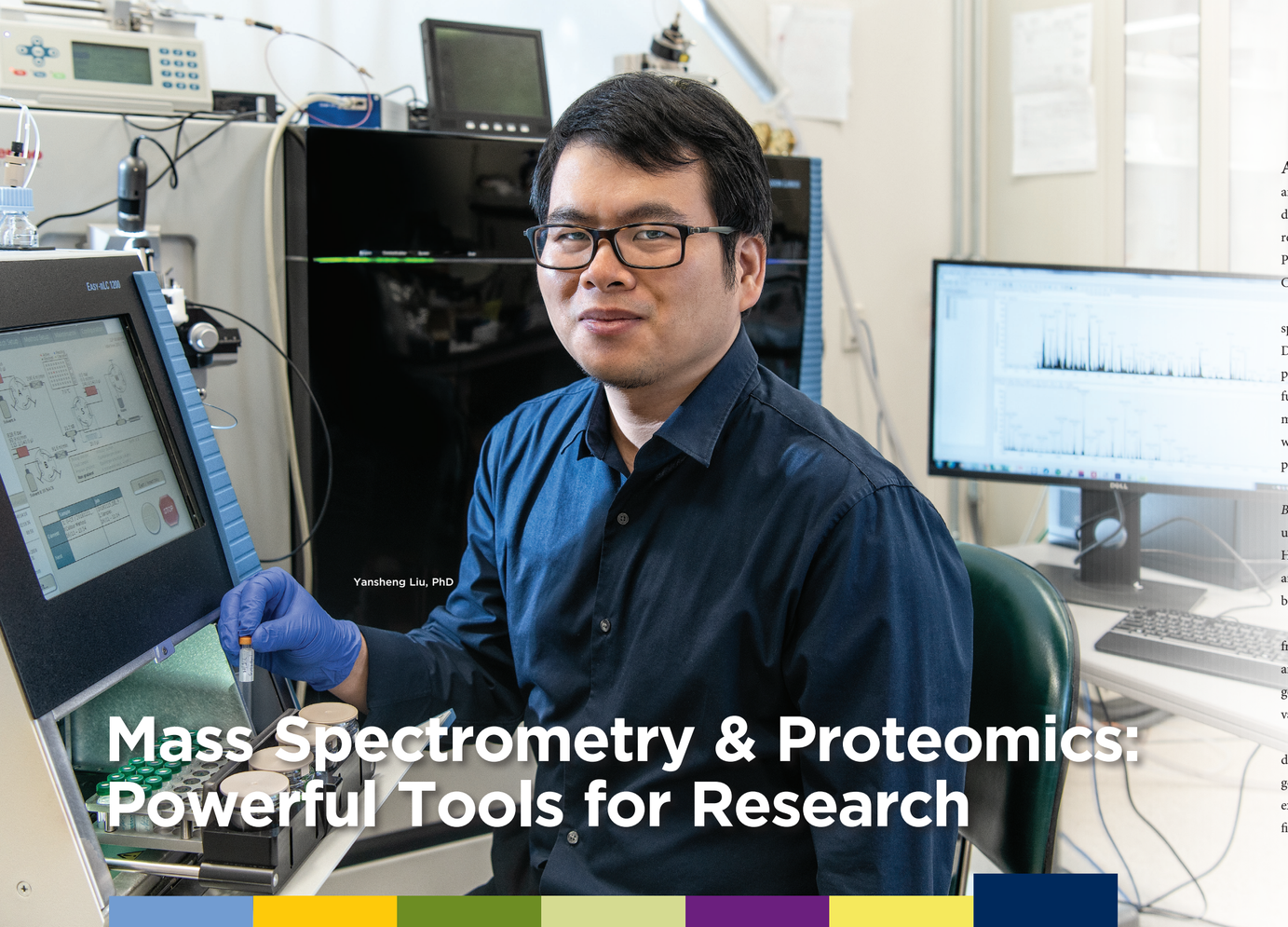

Mass Spectrometry & Proteomics: Powerful Tools for Research

Advances in mass spectrometry and proteomics are giving researchers new ways to better understand, detect, diagnose, and treat cancer. A year ago, Yale recruited Yansheng Liu, PhD, Assistant Professor of Pharmacology, to bring these innovative tools to the Cancer Biology Institute.

Dr. Liu arrived from Zurich, Switzerland, where he spent more than six years in the Proteomics Lab of Dr. Ruedi Aebersold, a world-renowned pioneer in proteomics. He was lured here in part by Yale's offer to furnish his laboratory with the fastest and most versatile mass spectrometer available, an Orbitrap Fusion Lumos, which Dr. Liu calls essential for his next-generation proteomics research.

One example of that research is now in press at *Nature Biotechnology*. "The paper presents something quite unexpected and surprising about HeLa cells," said Dr. Liu. HeLa is a line of human cancer cells that can be cloned and cultured, and may be the most widely-used cell line in biological and biomedical research.

Dr. Liu and his colleagues collected 14 HeLa samples from 13 labs in six countries, cultured them, and then analyzed them using mass spectrometry (MS), proteomics, genomics, and transcriptomics. They found significant variation between HeLa variants.

Equally surprising, the scientists often found progressive divergence even within a specific variant. "After just 50 generations," explained Dr. Liu, "if we compare the gene expression of one HeLa cell line from beginning to end, we find six percent of the genome is significantly different."

The implications are important, he added. Researchers assume that their HeLa cell lines are homogenous and that research based on them can be independently verified—a crucial aspect of science. But if the HeLa cells vary across and even within strains, that can change results and thwart verification. Dr. Liu's paper cites a survey conducted by *Nature* in 2016 in which more than half of the participating researchers agreed that there is a "reproducibility crisis" in the life sciences, which has been blamed on factors such as contamination, statistical error, incompetence, fraud, and misidentification of cell lines. Dr. Liu's research suggests that another reason might be genomic volatility among supposedly homogenous cell lines. He believes that MS and proteomics can help solve the reproducibility crisis by providing another way to do cell line authentication, measuring steady-state gene expression at the transcript and proteome levels.

He is certain that MS and proteomics are even more valuable when applied broadly in cancer research. These tools and experimental strategies can capture and characterize not only protein expression but protein modifications such as phosphorylation and ubiquitination, protein turnover, and protein localization. "All of these are quite relevant for cancer research," he said.

For instance, MS and proteomics are incredibly powerful for identifying and characterizing molecular elements. With a new MS method called Data-Independent Acquisition (DIA), Dr. Liu can quantify almost 800 proteins in plasma in just two hours. In one microgram of cancer tissue, he can quantify 5,000 proteins. In a cancer cell line, 6,000 - 8,000 proteins. "DIA can provide unprecedented reproducibility among 100-1000s samples. This gives us bigger opportunities to understand more deeply what is going on at the proteome level," said Dr. Liu.

Dr. Liu is also enthusiastic about using MS and proteomics to study protein localization. If proteins get localized aberrantly—put into the wrong cellular compartments—disease can result, including cancer. "We have a very cool technique," explained Dr. Liu, "where we can assign a protein or a modified protein into an organelle."

He is eager to use these technologies to advance research across Yale Cancer Center, and has some collaborations underway. With Andre Levchenko, PhD, John C. Malone Professor of Biomedical Engineering and Director of the Systems Biology Institute, Dr. Liu is looking at the metastatic features of melanoma in patients and cell lines, in particular protein modification and turnover.

He is also assisted by Anatoly Kiyatkin, PhD, a postdoc at the Cancer Biology Institute, to perform a study that is monitoring cell signaling stimulated by EGF (epidermal growth factor) or NGF (nerve growth factor) ligand, a process implicated in many cancers. Using DIA-MS, Dr. Liu can measure changes instantaneously and periodically in both the protein's abundance and its phosphorylation, and their respective lifetime, to provide a much better understanding of EGF receptor signaling in cancer.

"I look forward to more clinically-related collaborations with physicians in the Cancer Center," he said. "We can definitely work together to bring better proteomic measurement to particular questions in clinical cancer research."