SPOT LIGHT

High-dimensional single-cell analysis enhances precision medicine

XIANTING DING, PHD

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How a dual-mode technology enables better insight

Xianting Ding, PhD, is a professor in the School of Biomedical Engineering and deputy director of the Institute for Personalized Medicine at Shanghai Jiao Tong University. The Ding lab focuses on two main objectives: detecting disease-correlated biomarkers to enable early diagnoses and manipulating these targets to develop effective and personalized combinatorial drug therapies. “We believe that the exploration of immunity, metabolism and cell activity in clinical specimens is important for optimizing treatment response and improving patients’ survival time,” he says.

Biological systems are made up of complex layers of molecules interacting to generate a healthy homeostasis with the ability to respond to infiltrating disease. A dysfunctional system can cause aberrant expression and other signaling errors. Ding points out that research on SARS-CoV-2, for example, shows evidence that immune system abnormalities are highly correlated with disease progression. Likewise, he and his team aim to correlate abnormal immune profiles with clinical phenomics of other diseases by applying high-dimensional single-cell analysis.

Given that available samples, typically blood or tissue, tend to be small or acquired sparingly, Ding must apply various techniques to comprehensively gather enough data to formulate solutions. The group uses a combination of CyTOF® in mass cytometry and Imaging Mass Cytometry™ (IMC™) and fluorescence-activated cell sorting (FACS), Orbitrap™ for mass spectrometry and liquid chromatography-mass spectrometry (LC-MS) to study the different aspects of a disease.

The lab recently published work on colon cancer, where IMC allowed them to map more complex characteristics and related immune cell networks. These types of studies require labeling more markers than allowed by conventional techniques. With IMC, the team can increase its marker count to meet this demand using as many as 37 markers in one panel.

“IMC helps us understand the cell population distribution in a diseased area in contrast to a non-diseased area,” he explains. “Compared to other high-multiplex technologies, IMC excels at providing both spatial information and high multiplexity. It allows us to see spatial context more intuitively and enables accurate detection, treatment and prognosis of the disease by analyzing the immune system in the peripheral blood.”

Taking advantage of dual mode

One successful approach in generating an in-depth view of tissue architecture and spatial distribution of cells in addition to an overall immune cell profile is the dual-mode capability of the Hyperion™ Imaging System, which enables analysis of both suspension and tissue. Ding discussed his use of mass cytometry and IMC for an in-depth characterization of the immune cell subsets and protein profiles involved in signaling

pathways in the peripheral blood and skin of patients with psoriasis (PS).

Based on the behavior of the immune cell subsets as seen by CyTOF, Ding was able to hypothesize how cell migration affects the progression of the disease.

The group analyzed peripheral blood mononuclear cell (PBMC) samples isolated from four patients who were newly diagnosed with graft-versus-host disease (GVHD) and four samples from healthy controls. Based on a combination of surface markers, they used CyTOF to simultaneously analyze T cell and B cell subsets using mass cytometry. Results showed that the frequencies of total T and B cells were statistically significantly decreased in GVHD samples, while frequencies of specific CD8+ T effector memory cells were increased.

Following CyTOF mass cytometry analysis, two samples of PS lesions were obtained and stained for 31 immune markers for Imaging Mass Cytometry. In the IMC images, the team observed colocalization of immune cell subsets, validating their expected cell distribution and indicating the stability and reliability of the technique.

Using dual-mode capability on the Hyperion Imaging System, the team identified 15 major immune cell populations in T cell lineages and characterized various other T cell populations simultaneously.

Based on the behavior of the immune cell subsets as seen by CyTOF, Ding was able to hypothesize how cell migration affects the progression of the disease. For example, a reduction of lymphocytes in peripheral blood suggests that they may migrate to the lesion area. That hypothesis can be verified with IMC data. Ding adds that the use of single-cell mass cytometry allows systemic-level characterization of lymphocyte subpopulations and dysregulated signaling pathways in the blood, allowing him to identify abnormalities of different immune cell subsets.

The use of both suspension and imaging modes on the Hyperion Imaging System has the potential to benefit many research areas, including oncology, immunology, immunophenotyping and more. Mass cytometry data can be used to predict the occurrence and progression of diseases, and IMC data represents the mechanism in lesions or other diseased tissue. Further study using these technologies has prompted Ding to develop novel probes for IMC to support successful imaging acquisition and offer new opportunities for diagnosing malignancies in the future.

> “Compared to other high-multiplex technologies, IMC excels at providing both spatial information and high-multiplexity.

References:

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