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Cancer Systems Biology in the Era of Single-Cell Multi-Omics

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Tumor tissue is a multifaceted ecosystem in which tumors cells are surrounded and influenced by a myriad of non-cancerous cells including immune, stromal, vascular, and other cell types.[1] Driven by stochastic genetic mutations, epigenetic modifications, and aberrant gene expression profiles, tumor cells themselves also exhibit extraordinary intratumoral heterogeneity that gives rise to malfunctioning of signaling networks and plays important roles in tumor invasion, proliferation, metastasis, as well as stromal remodeling and immune system suppression.[2,3] This pronounced cell-to-cell variations make traditional bulk-level profiling far away from an accurate representation of the tumor ecosystem. In this regard, single-cell multi-omics tools provide a great opportunity for researchers to improve the understanding of molecular roles of tumor heterogeneity, thanks to their high spatiotemporal resolutions down to the level of single cells as well as their analytical capacity at the systems scale.[4-6]

To date, a panoply of mono-omics technologies have been developed to effectively profile different molecular layers of single cells including genome, epigenome, transcriptome, proteome, metabolome, and so forth.[7-9] The quantification of these molecular signatures of cellular processes at single-cell resolution enables us to ask questions from perspectives previously unattainable and thereby facilitates our understanding of the cause and consequence of tumor heterogeneity in tumorigenesis, metastasis, and immune response. In addition, building on the development of these mono-omics technologies, tools for simultaneous measurement of multiple omic layers from the same single cells have emerged in recent years through the rational design of bio-recognition interface and the leverage of advanced biotechnologies.[10-13] Single-cell multi-omics tools allow for interrogating the links between different classes of biomolecules to resolve the interplays between distinct molecular landscapes. These integrated measurements not only offer a holistic view of cellular compositions and phenotypic states of a given population, but also enable detailed investigations into the developmental history, inter- and intracellular signal transduction, as well as the roles of significant subpopulations or rare cell types in specific physiological or pathological processes across multiple modalities. In the meantime, such measurements pose new challenges in data analysis and interpretation, as different omic layers require different suites of analytical approaches that are not always compatible. While each single cell can provide an anchor to connect different data modalities, computational frameworks that can integrate diverse sets of information from different molecular layers into harmonized atlases for effective visualization, hypothesis generation, and data interpretation are still a pressing need in the field.[14]

In this special issue, we have collected current efforts that have been made in the development of novel single-cell technologies as well as their applications in immuno-oncology and cancer systems biology. Liu et al. reported a multiplexed single-cell analytical platform to quantify secreted cytokines from single cells.[15] Secreted cytokines play important roles in mediating cell-cell communications in various physiological and pathological processes. Conventional single-cell cytokine secretion assays are mainly based on the adaption of an enzyme-linked immunosorbent assay which measures single-cell secretion footprint of a given cytokine through transforming the bio-recognitions between the antibodies and cytokines into colorimetric or fluorescence signal readouts. Nevertheless, they are normally limited to three less than five cytokines that can be simultaneously detected due to the fluorescence spectral overlap. To improve the assay multiplexity without the adaption of sophisticated experiment handling or bulky equipment, a high-density polydimethylsiloxane microwell stencil was sandwiched between two antibody-coated glass slides to form periodic compartments for single-cell trapping, culturing, and cytokine secretion profiling. With 5-plexed and 3-fluorescence colors designed for detection antibodies, five or more secreted cytokines from more than 1000 single cells could be simultaneously profiled without the adaption of sophisticated fluid handling system or bulky equipment. Moreover, the authors demonstrated the utility of this single-cell technology through an investigation of secretome heterogeneity of human monocytic U937 cells in response to lipopolysaccharide and phorbol myristate acetate stimulations. The technical simplicity and high throughput make this single-cell secretion assay a unique and informative tool in dissecting cellular heterogeneity in secretome signatures.

As an attempt to move forward from basic research to translational and clinical applications, Bowman et al. adapted a highly-multiplexed single-cell proteomic assay (32-plex, IsoLight automation system) to characterize the polyfunctionality of pre-infusion anti-CD19 chimeric antigen receptor (CAR)-T cell products from a cohort of patients with Non-Hodgkin's Lymphoma via quantitatively profiling 30+ cytokines secreted from CD4+ and CD8+ T cells in response to CD19 antigen-specific

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stimulation.[16] To better resolve the clinical relevance of the CAR-T cell polyfunctionality, a comprehensive visualization toolkit was developed by integrating 3D Uniform Manifold Approximation and Projection and t-distributed stochastic neighbor embedding into a proteomic analysis pipeline which could be further built into the IsoLight system. The combined use of commercial single-cell proteomic assays and the new bioinformatics pipeline developed in this work was envisaged to promote the understanding of underlying mechanisms in cell-based immunotherapies for improved personalized cancer medicine.

Single-cell RNA-sequencing (scRNA-seq) has become one of the most widely used single-cell analytical approaches due to its high-throughput, robust performance, and good compatibility to be coupled with other single-cell profiling technologies. Dong et al. recently incorporated scRNA-seq with a rare cell enrichment approach to decipher the intratumoral heterogeneity of disseminated tumor cells (DTCs) derived from liquid biopsy samples.[17] DTCs are tumor cells spreading from primary sites to body fluids, which are considered as an important biomarker for prognostic evaluation of cancer patients because of their critical roles in cancer metastasis. However, the rarity and low viability of DTCs, as well as the co-existence of large amounts of non-cancerous cells, imposes great challenges in transcriptomic profiling of DTCs through scRNA-seq technology. To overcome this obstacle, a CD45 depletion kit was employed to remove the leukocytes in liquid biopsies sampled from malignant pleural effusions (MPE) and resultant cell samples were subsequently processed for scRNA-seq. Five main cell populations including tumor, mesothelial, monocyte, T, and B cells were identified while the DTCs could be further clustered into four subgroups with their distinct functional features characterized. These results demonstrated that the rational combination of rare cell enrichment methods with scRNA-seq technology paved a new avenue to molecular profiling of rare cell types.

Most of the single-cell multi-omics tools mainly focus on the measurements of cellular biomolecules which only contain the chemical essence of cellular functions, but overlook important cellular physical information (e.g., cell mass, size, and motility). Recently, Han et al. developed a microfluidic cell trap array that can monitor the motility behavior of single hematopoietic stem/progenitor cells (HSPCs)—a clinically relevant parameter for peripheral blood stem cell transplantation.[18] Following on-chip sorting and selection, collected cells were subjected to RNA-seq to build the links between HSPC motility and stem-cell maintenance. This approach not only offers a novel strategy to decipher motility heterogeneity in HSPCs but facilitates the screening of HSPC mobilization compounds as well.

Meanwhile, several reviews in this special issue attempted to portray the field of single-cell multi-omics from different perspectives. Yang et al. comprehensively summarized the principles, developments, advantages, and limitations of recently emerged single-cell proteomic technologies, along with their applications in dissecting cellular heterogeneity.[19] In parallel, Kravchenko-Balasha highlighted the implications of bulk- and single-cell proteomic assays as well as associated computational tools in unveiling and constructing the inter- and intratumoral signaling networks for personalized medicine.[20] Zhu et al. focused on the elaboration of scRNA-seq and associated technologies adapted to the studies of hematological diseases, revealing the history of single-cell omics from basic research to translational and clinical applications.[21] Peng et al. comprehensively reviewed recently developed single-cell multi-omics tools with detailed comparisons of their properties from different perspectives. Meanwhile, their applications in tumor-immune interactions were also highlighted.[22]

Single-cell multi-omics is a rapidly growing field that calls for cross-disciplinary efforts ranging from chemistry, physics, biology, engineering, computational science to medicine. We greatly appreciate all contributors to this special issue and their achievements in this exciting field. We encourage more scientists to join us to leverage these powerful single-cell toolkits to gain a systems-level understanding of cellular states, interactions, and behavior in human cancers and transform the development of diagnostic and therapeutic approaches in oncology.

Conflict of Interest

The authors declare no conflict of interest.

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[1] A. Marusyk, K. Polyak, *Bioch. Biophys. Acta, Rev. Cancer* **2010**, *1805*, 105.
[2] M. J. Lee, S. Y. Albert, A. K. Gardino, A. M. Heijink, P. K. Sorger, G. MacBeath, M. B. Yaffe, *Cell* **2012**, *149*, 780.
[3] T. Pawson, N. Warner, *Oncogene* **2007**, *26*, 1268.
[4] F. S. Fritzsch, C. Dusny, O. Frick, A. Schmid, *Annu. Rev. Chem. Biomol.* **2012**, *3*, 129.
[5] I. Tirosh, M. L. Suvà, *Annu. Rev. Cancer Biol.* **2019**, *3*, 151.
[6] L. Mincarelli, A. Lister, J. Lipscombe, I. C. Macaulay, *Proteomics* **2018**, *18*, 1700312.
[7] D. Wang, S. Bodovitz, *Trends Biotechnol.* **2010**, *28*, 281.
[8] S. M. Prakadan, A. K. Shalek, D. A. Weitz, *Nat. Rev. Genet.* **2017**, *18*, 345.
[9] X. Xu, J. Wang, L. Wu, J. Guo, Y. Song, T. Tian, W. Wang, Z. Zhu, C. Yang, *Small* **2020**, *16*, 1903905.
[10] L. Chappell, A. J. Russell, T. Voet, *Annu. Rev. Genomics Hum. Genet.* **2018**, *19*, 15.
[11] Y. Su, Q. Shi, W. Wei, *Proteomics* **2017**, *17*, 1600267.
[12] Y. Hu, Q. An, K. Sheu, B. Trejo, S. Fan, Y. Guo, *Front. Cell Dev. Biol.* **2018**, *6*, 28.
[13] T. Stuart, R. Satija, *Nat. Rev. Genet.* **2019**, *20*, 257.
[14] F. Finotello, D. Rieder, H. Hackl, Z. Trajanoski, *Nat. Rev. Genet.* **2019**, *20*, 724.
[15] M. Liu, M. Jin, L. Li, Y. Ji, F. Zhu, Y. Luo, T. Liu, B. Lin, Y. Lu, *Proteomics* **2020**, *20*, 1900231.
[16] N. Bowman, D. Liu, P. Paczkowski, J. Chen, J. Rossi, S. Mackay, A. Bot, J. Zhou, *Proteomics* **2020**, *20*, 1900270.
[17] Y. Dong, Z. Wang, Q. Shi, *Proteomics* **2020**, *20*, 1900224.
[18] X. Han, Y. Ma, K. Zhang, P. Zhang, N. Shao, L. Qin, *Proteomics* **2020**, *20*, 1900223.
[19] L. Yang, J. George, J. Wang, *Proteomics* **2020**, *20*, 1900226.
[20] N. Kravchenko-Balasha, *Proteomics* **2020**, *20*, 1900227.
[21] Y. Zhu, Y. Huang, Y. Tan, W. Zhao, Q. Tian, *Proteomics* **2020**, *20*, 1900228.
[22] A. Peng, X. Mao, J. Zhong, S. Fan, Y. Hu, *Proteomics* **2020**, *20*, 1900271.