Commentary: “Zooming in” on Glioblastoma: Understanding Tumor Heterogeneity and Its Clinical Implications in the Era of Single-Cell Ribonucleic Acid Sequencing

Glioblastomas (GBMs) are notorious for their intratumoral heterogeneity and their diverse and constantly changing tumor microenvironment (TME). Recent advances in single-cell genomics have enabled widespread implementation of the technology for a detailed understanding of not only the cellular and genomic composition of GBM but also their TME. One particular technique that has received significant attention and translation is single-cell RNA sequencing (scRNA-seq).1

In the recent, comprehensive, and elegant review by Khalafallah et al.2 the authors focus on the clinical implications of scRNA-seq in the management of GBM. The authors describe the evolution of research techniques, beginning at the whole tumor level, using bulk RNA-seq, progressing to multiregional, tumor bulk RNA-seq, and “zooming in” on to scRNA-seq. Each new step progressively increases spatial resolution, enabling a better delineation of the spatial distribution of diverse, intratumoral cellular populations, and the TME. As the authors note, whole tumor bulk RNA-seq offers a limited view because it provides only an average, composite genomic profile of the tumor, without spatial resolution. However, the initial technology led to precision genomics that identify genetically discrete subtypes showing distinct responses to therapy and unique genetic signatures.3 The next advance that increased the spatial resolution was bulk RNA-seq of multiple regions of the individual tumor.4,5 Ultimately, scRNA-seq provided the highest spatial resolution because RNA-seq of individual cells yields a complete transcriptomic representation of all the cells within the tumor. As the authors note, scRNA-seq studies of GBM validated the conclusions of earlier approaches, and extended our understanding to the cellular level. It became clear that the cellular subpopulation of a single tumor could belong to more than one of the previously described GBM subtypes6,7; importantly, increased tumor heterogeneity was linked to decreased survival.6 Another discovery was the presence of a stem-cell-like subpopulation of cells within the tumors,6 underlying treatment-resistance and clinical recurrence.

The published review by Khalafallah et al.2 clearly records the timeline of technological advances to enhance the spatial resolution of intratumoral topography using scRNA-seq. However, from a clinical perspective, further progress will come from studying individual tumors on a single-cell level along the trajectory of a patient’s illness and noting the impact of time and therapies on the molecular heterogeneity.1 The longitudinal approach could also be used to serially monitor the immune landscape of the tumor as it evolves, thereby informing optimal therapeutic targeting of the immune TME at a specific point in time. For example, there is keen interest in neuro-oncology currently to convert an immunologically suppressive TME, dominated by M2-macrophages, into an M1-immunostimulatory state.8-10 In this context, single-cell profiling of myeloid cells in GBM across disease stages reveals that beyond the linear M1/M2 activation paradigm, subsets of glioma-associated macrophages could serve as biomarkers to measure and potentially predict response to therapy,11 or therapeutic targets to alter the outcomes of patients with gliomas,11,12 as demonstrated in patients with recurrent lung cancer.13 For patients with recurrent GBM, we (S.J.B. and S.B.) will be targeting IL-6, which has dual roles in promoting immunosuppressive macrophages populations’ and maintaining intratumoral heterogeneity,14 in a phase 2 clinical trial sponsored by NRG Oncology (BN010; NCT04729959).

With broad clinical implications apparent, Khalafallah et al.2 highlight the many aspects of scRNA-seq that impact the way we view glioma heterogeneity. To delve further into preclinical
work, a few experimental caveats are in order: (1) the number of detected RNAs in a cell; (2) the static nature of the scRNA-seq; and (3) RNA-seq in the nucleus may not be identical to the cytoplasmic RNA complement. When an scRNA-seq method depends on conversion of RNA into cDNA, the efficiency of this conversion is low, ~10%, meaning that many of the cellular RNAs will not be part of the cDNA library. Furthermore, most high-throughput scRNA-seq methodologies sequence an individual cell to a depth of 50 to 100,000 reads or sequences within a cell (Drop-seq, 10xGenomics). As there are ~200 to 300,000 mRNAs in a single cell, this read depth does not provide full representation of the cell’s transcriptome. Some of the presumed cellular heterogeneity can result in the technology not detecting all of the cell’s RNA; the lower abundance RNA would only be stochastically detected. Also, the transcriptome is dynamically changing with different tumor influences, and would only be stochastically detected. Also, the transcriptome not detecting all of the cell’s RNA; the lower abundance RNA the presumed cellular heterogeneity can result in the technology provide full representation of the cell’s transcriptome. Some of the 300,000 mRNAs in a single cell, this read depth does not produce data appropriate to a specific cell at the time of harvest, but could be different at a later time. Finally, many high-throughput studies utilize isolated nuclei as the source of RNA rather than the cellular cytoplasm—which could have a different RNA landscape. Nuclear RNA is transient in nature. Taken together, we agree with the authors that scRNA-seq is an important technology that will surely impact neuro-oncology and has the potential to improve patient outcomes, drug discovery, and clinical trials via personalized medicine.2,15 Spatially resolved single-cell RNA-seq transcriptome analyses of tumor tissues in different regions will provide deeper insight into spatial regulation of tumorigenesis, glioma plasticity, immunity, and tumor heterogeneity.

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REFERENCES