Single-cell RNA Sequencing Charts Heterogeneity in Glioblastomas

Could this heterogeneity help focus future research?

Glioblastoma is one of the most lethal malignancies. Human cancers are complex ecosystems comprised of multiple populations of cells with distinct phenotypes, and glioblastoma is no exception. In fact, it is an archetypal example of a heterogeneous cancer. A study led by a multidisciplinary team at Massachusetts General Hospital and the Broad Institute and published in the June 2014 issue of *Science* provides a deep analysis of the nature of intratumoral heterogeneity in primary glioblastoma, yielding valuable insights as to why this cancer is so lethal.¹

Using single-cell RNA sequencing to analyze five freshly resected primary glioblastoma tumors, the team generated full-length transcriptomes for 430 individual tumor cells that enabled them to characterize the cellular diversity of the tumors. The researchers identified heterogeneity in major cellular regulatory programs, highlighted mosaic expression of signaling receptors that drive proliferation, and characterized chromosomal aberrations in individual cells. Although glioblastoma researchers have long been aware that there were multiple types of cells within a glioblastoma, it had been difficult to quantify the full extent and nature of the diversity.²

These five tumors are also the first whole tumors to be characterized through single-cell RNA sequencing. This analysis has provided the highest level of detail on glioblastoma tumors to date, allowing researchers to refine their understanding of this type of cancer and guiding future investigations into potential therapies for the disease.

The study required close collaboration among the members of a team made up of surgeons and researchers from the Mass General Department of Neurosurgery, Department of Pathology, the Center for Cancer Research, and molecular and computational biologists at the Broad Institute. The team included Mass General pathologists Bradley Bernstein, MD, PhD, and Mario Suvà, MD, PhD; Aviv Regev, PhD, core member of the Broad Institute and director of the Broad’s Klarman Cell Observatory; (continued on page 2)
Deriving Single-Cell Transcriptional Programs in Glioblastoma

This chart shows the methodology by which researchers conducted single-cell RNA sequencing of glioblastoma tumors. The process required collaboration among neurosurgeons, neuropathologists, experimental pathologists, and molecular biologists.

**Primary GBM**

1. **Neurosurgery (1, 2)**
   - Neurosurgeon removes the tumor from the patient and performs tumor resection.

2. **Dissociation**
   - Tumor is dissociated, forming a single-cell suspension.

3. **Remove RBC/Debris**
   - Red blood cells and debris are removed.

4. **CD45 Depletion**
   - CD45-depleted cells are enriched for living tumor cells.

5. **Single Cell Sort**
   - Single cells are sorted for analysis.

6. **SINGLE CELL cDNA LIBRARY**
   - DNA is isolated from single cells and converted into cDNA.

7. **POPULATION cDNA LIBRARY**
   - DNA is isolated from bulk tumor samples.

8. **T=2-3 hrs**
   - Cells are stored frozen at -80 degrees Celsius.

**NEUROPATHOLOGY (3)**
- The neuropathologist evaluates the tumor specimen for quality and takes samples of excess material for the next step.

**EXPERIMENTAL PATHOLOGY (4)**
- The pathologist performs mechanical and enzymatic tissue dissociation, then removes debris, red blood cells, dead cells, and inflammatory cells.

**EXPERIMENTAL PATHOLOGY (5)**
- The pathologist sorts single cells into 96-well plates and stores them, frozen, at ~80 degrees Celsius. A piece of bulk tumor is also banked and stored frozen at ~80 degrees Celsius.

Patient-to-patient differences, with distinct signature genetic events affecting patients of different ages and at different locations in the brain. These tumors also contain cellular niches enriched for specific phenotypic features, most importantly those related to glioblastoma stem cell programs, subpopulations that represent a reservoir for recurrences.

No current model recapitulates the complexity of these tumors; thus, the analysis sought to map the extent and pattern of cellular heterogeneity directly in tumors. The information was also used to determine whether characteristics observed in different models were seen in *in vivo* tumors.


The epidermal growth factor receptor (EGFR) is a drug target that is amplified in some tumors and is often considered to be the driver of tumor growth. Yet the team’s single-cell analysis showed that the mutations affecting EGFR can vary from cell to cell and that not all tumor cells express EGFR. Thus, proliferation can be driven by different EGFR variants or, alternatively, by other pathways. That explains why a drug targeting a specific EGFR mutation probably would not be sufficient on its own to eradicate a glioblastoma tumor.

In addition, the results of the analysis showed that some cells in each tumor were closer to a stem cell state than others were. This confirmed the importance of these alternate “epigenetic” cell states in human tumors. However, their analysis also showed that there was not a single distinct population of cells in the tumors that had stem-cell-like properties, but rather a continuum of cells with stem-cell-like...
These stem-like programs may contribute to tumor regrowth after therapy.

**SUBTYPES IN A SINGLE TUMOR**
Glioblastomas have been classified according to a subtype scheme that analyzes the tumor by averaging the expression of genes across millions of tumor cells in a single sample to determine the dominant subtype. However, this more granular analysis enabled researchers to show a mixed population of subtypes within each tumor. It now appears that tumors in different patients contain most or all of the same states, with only the proportion of each cell type varying. In addition, the team found a correlation between increased intratumoral heterogeneity and decreased survival.

This suggests that research into possible drug therapies should take into account heterogeneity within tumors as well as the limitation of classifying tumors according to subtype. For example, future research might identify therapies that will be more effective against a larger portion of the tumor, or a combination of therapies that together would eradicate most or all of the tumor cells.

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**A New Drug for Treatment-Resistant Non-Small Cell Lung Cancer**

**Does CO-1686 hold promise for treating EGFR-mutation NSCLC?**

Lung cancer is the most common cancer worldwide, with 1.7 million new cases diagnosed annually. Some 85 percent of those diagnoses involve non-small cell lung cancer (NSCLC). Ten years ago, a Massachusetts General Hospital Cancer Center team that included Daniel Haber, MD, PhD, and Thomas Lynch, MD, discovered that a subset of NSCLCs (about 10 to 15 percent of total NSCLCs in Caucasian patients and 30 to 35 percent in East Asian patients) carried a mutation in the epidermal growth factor receptor (EGFR). Tyrosine kinase inhibitors (TKIs), including erlotinib (Tarceva) and gefitinib (Iressa), were developed to treat the cancer by targeting the EGFR mutation. Approximately 60 percent of EGFR-mutation cancers eventually become resistant to tyrosine kinase inhibitors (TKIs) because of the presence of a second, “gatekeeper” mutation: T790M. And while a new category of drugs—second-generation EGFR inhibitors—seemed to dissolve those tumors in vitro, they were unsuccessful in treating patients because, at the required dosage, the side effects—rash and diarrhea—proved too severe.

Now a third-generation EGFR inhibitor, CO-1686, shows promising results for patients whose tumors have become resistant to TKIs. The results of a phase I/II clinical trial of the drug, conducted by lead investigator Lecia Sequist, MD, medical oncologist at Mass General Cancer Center, have yielded very positive data. In addition, CO-1686 does not cause the skin rash and diarrhea seen with second-generation EGFR inhibitors. (continued on page 4)

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**Combating Multiple Mutations**

MRI imaging of an NSCLC patient at baseline (left) and after six weeks of therapy (right). Early clinical studies of CO-1686 have shown promising results for individuals with both EGFR and T790M mutations, with a reduced side-effect profile compared with previous EGFR inhibitors.

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SOURCE: Lecia Sequist, MD