MASSACHUSETTS GENERAL HOSPITAL
CANCER CENTER

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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;

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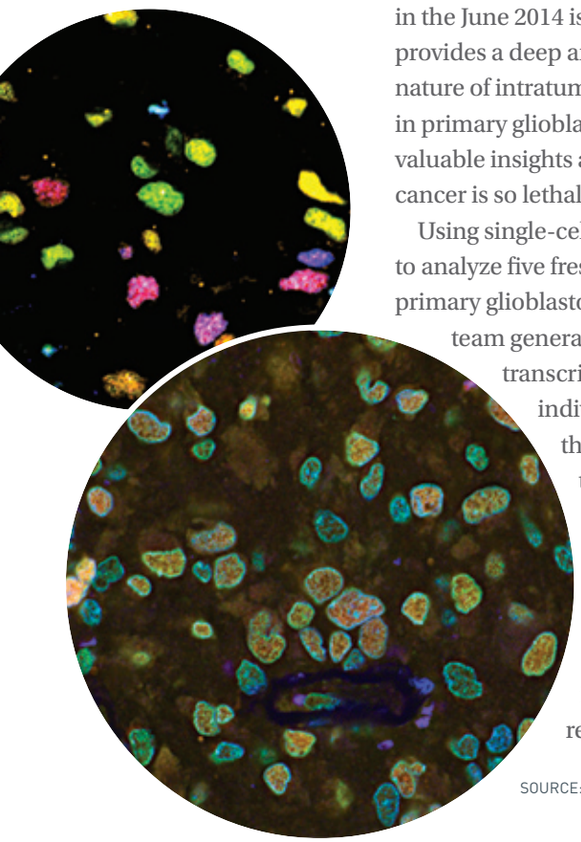


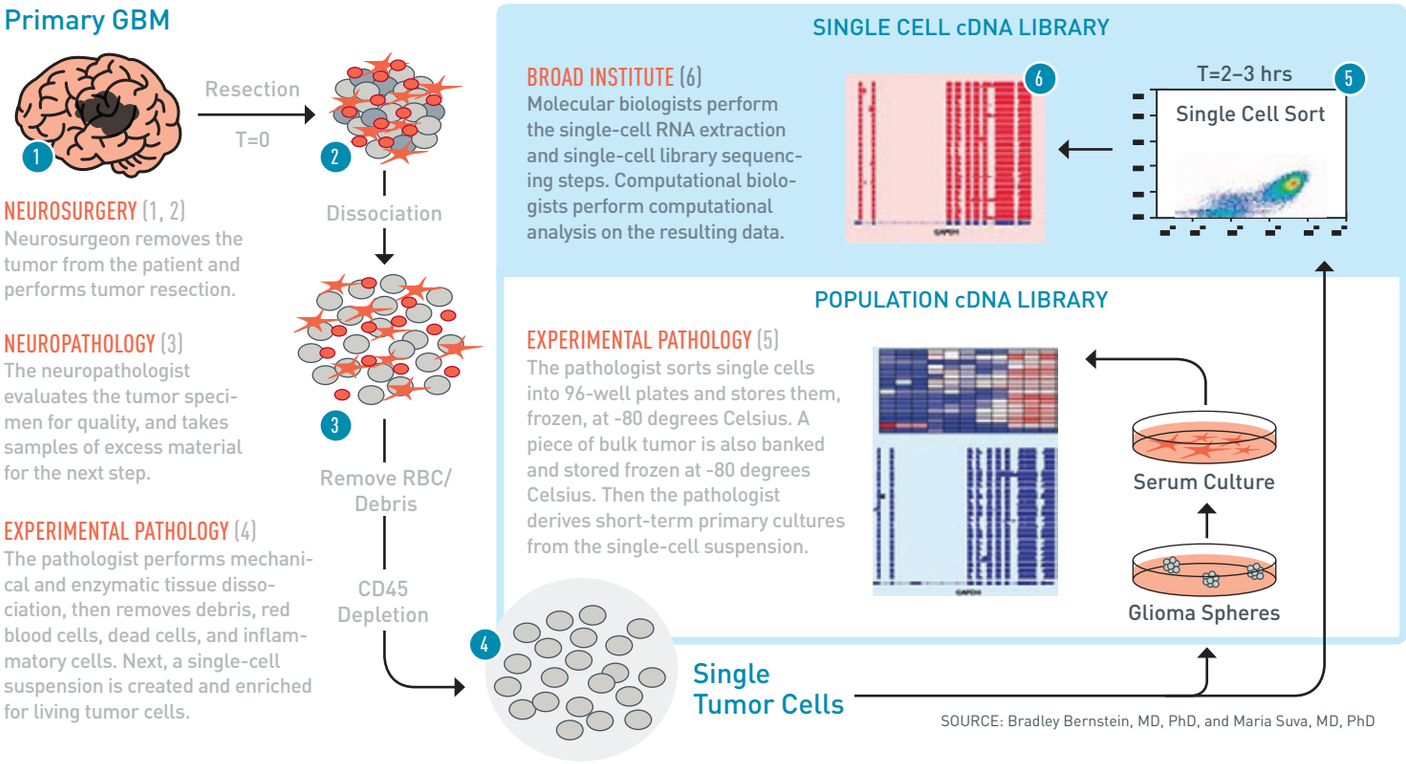
Glioblastoma tumor cells. These immunofluorescent images illustrate a strikingly heterogeneous population of cells present in tumors.

SOURCE: Anoop Patel, MD

INSIDE →

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.



SOURCE: Bradley Bernstein, MD, PhD, and Maria Suva, MD, PhD

(continued from page 1) and Anoop Patel, MD, and Itay Tirosh, postdoctoral fellows in the Bernstein and Regev labs, respectively.

HETEROGENEITY AND STEMNESS

The team's success depended on a great deal of coordination and flexibility by team members, so that the tumor could be prepared and analyzed before tissue degradation occurred. The tumors were first removed surgically and resected, and excess material was sampled. Next, mechanical and enzymatic tissue dissociation was performed. Then the team prepared a single-cell suspension enriched for living tumor cells, sorting the cells into 96-well plates. Short-term primary cultures were derived, and from those the team performed single-cell RNA extraction, sequencing and computational analysis to produce data showing all gene activity in each individual cell (see chart).

Glioblastomas are intrinsically heterogeneous tumors, displaying 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

[1] Patel, Anoop P, Itay Tirosh, John J Trombetta, Alex K Shalek, Shawn M Gillespie, et al. "Single-cell RNA-seq Highlights Intratumoral Heterogeneity in Primary Glioblastoma," *Science* 344, no. 6190 (June 2014), 1396-1401.

[2] Easwaran, Hariharan, Hsing-Chen Tsai, Stephen B Baylin. "Cancer Epigenetics: Tumor Heterogeneity, Plasticity of Stem-like States, and Drug Resistance," *Molecular Cell* 54, no. 5 (June 2014), 716-27.

signatures. 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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