High-resolution brain tumor mapping reveals possible reason why some patients don't respond to new drug

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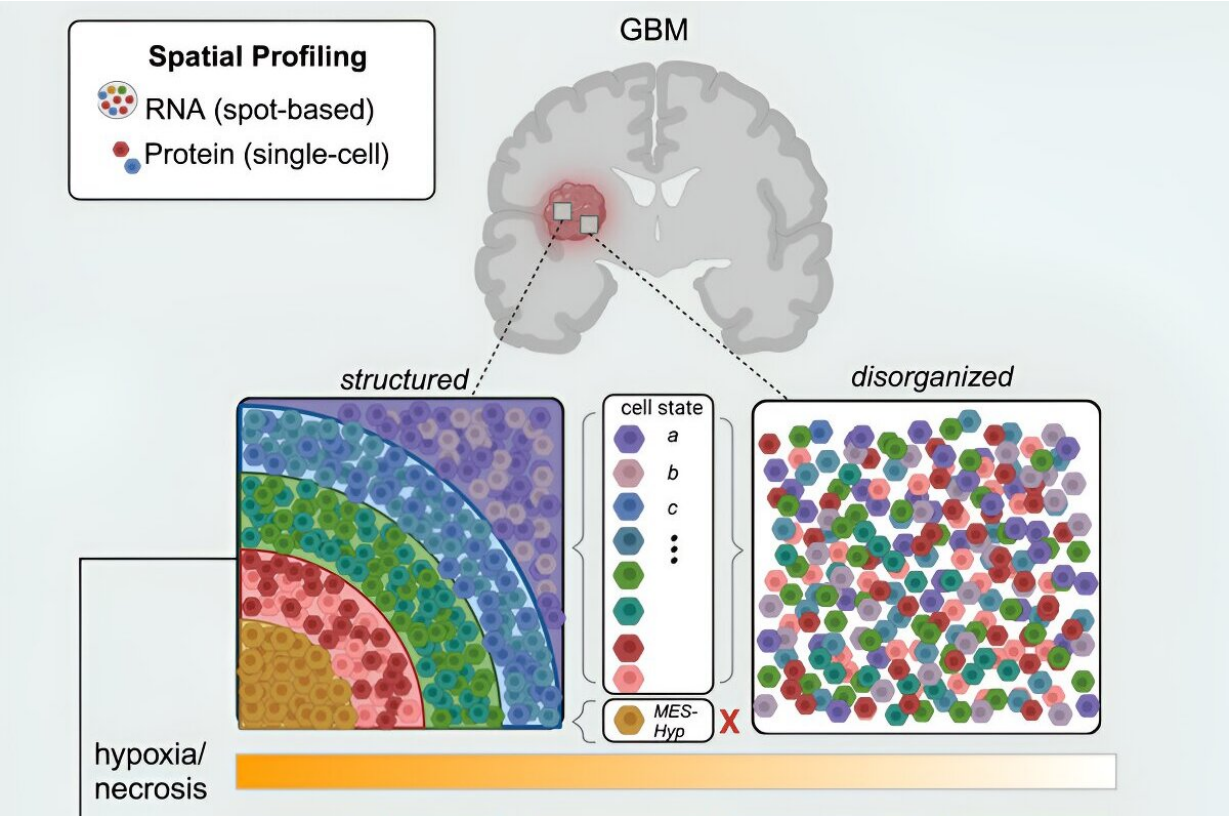


Graphical abstract. Credit: *Cell* (2024). DOI: 10.1016/j.cell.2024.03.029

The cells that make up cancerous brain tumors are extremely varied and sometimes create unique three-dimensional shapes. As far back as 1932,

American neurosurgeon Percival Bailey attempted to label these cells and discovered that they can be divided into several families of cells with similar properties.

More than 90 years later, we still know precious little about the identities of cell groups that make up different kinds of brain tumors, these groups' organization and how they affect the course of the disease and the outcome of treatment. This is why the success rate for treatment of most brain cancers is typically not high.

Over the past decade, genetic sequencing technology that works at the single-cell level has made it possible to examine, in minute detail and in one fell swoop, thousands of cells in the same tissue, to understand which genes they express and to then categorize them and study the role of each group.

Scientists in Dr. Itay Tirosh's research group in the Weizmann Institute of Science's Molecular Cell Biology Department, in collaboration with Prof. Mario L. Suvà's lab at Massachusetts General Hospital, harnessed this technology in order to reexamine some of the unanswered questions in the field of brain tumors.

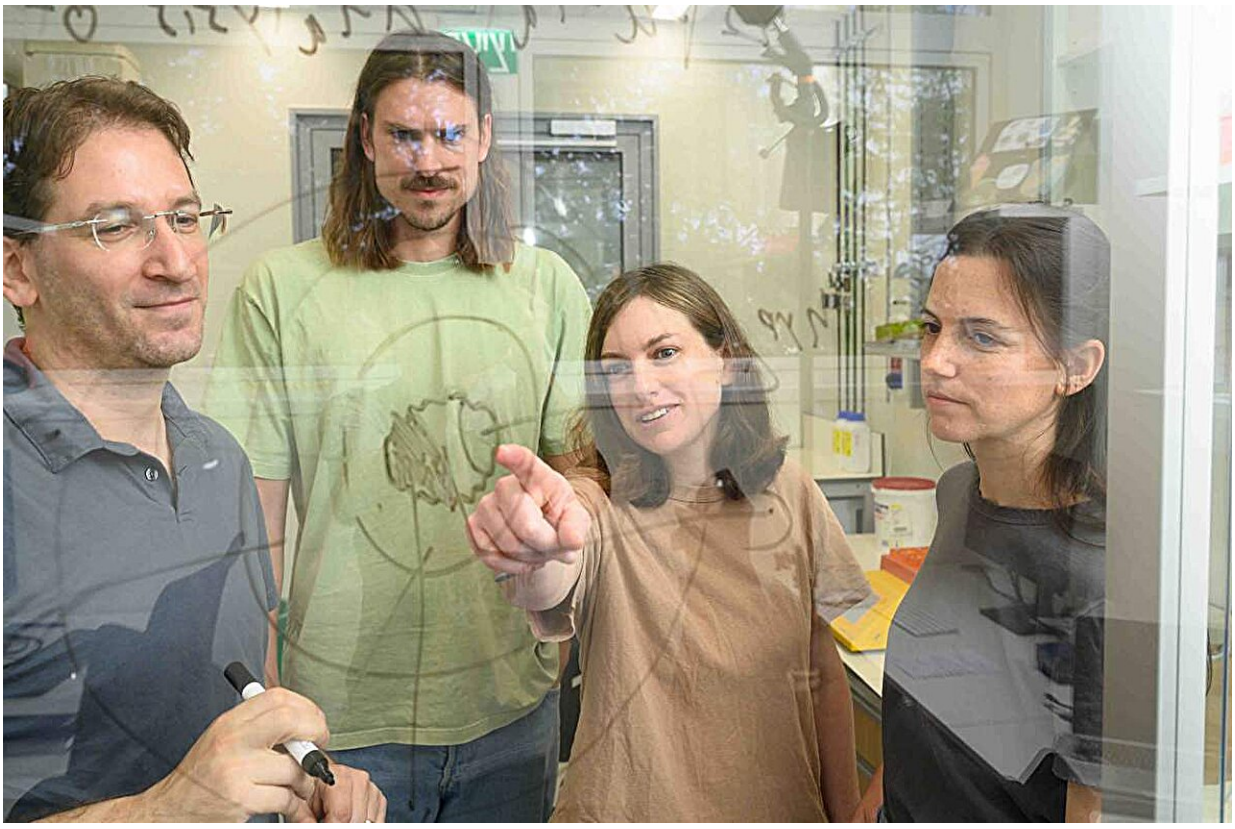
The most common type of primary brain tumor is the glioma, which originates from the support cells that assist our nerve cells. There are two main types of glioma tumors: those that are usually less aggressive and have a mutation in the gene encoding an enzyme called IDH, and those without this mutation, which are highly aggressive and known in medical terminology as glioblastoma.

In the past few years, researchers from Tirosh's lab have been using single-cell RNA sequencing to analyze the cellular composition of both kinds of tumors. They revealed that the tumor cells are divided into groups, each of which expresses a unique genetic program that

determines the biological "state" of the cancer cells in this group.

Among other findings, the researchers discovered groups of cells that use their unique genetic programs to mimic normal brain cells.

In a study published in *Cell*, researchers from Tirosh's lab—led by Dr. Alissa Greenwald, Noam Galili Darnell and Dr. Rouven Hoefflin—harnessed technologies that make it possible to not only sequence the RNA on the single-cell level but also spatially map its expression.



(l-r) Dr. Itay Tirosh, Dr. Rouven Hoefflin, Dr. Alissa Greenwald and Noam Galili Darnell. Credit: Weizmann Institute of Science

This allowed them, for the first time, to identify which genes are uniquely expressed in each of the thousands of areas within a brain tumor. As a result, they were able to precisely map how glioblastoma and glioma tumors are organized. To conduct the study, they took biopsies from 13 patients with glioblastomas and from six patients with gliomas that had the IDH mutation.

The researchers' first discovery was that the groups of various cells within a glioma tumor are not distributed evenly across the tumor; rather, they are concentrated in various environments inside the growth. These microenvironments are not entirely homogenous. Cells from other groups were always found in proximity to other types of cells.

In the next stage of the study, the researchers checked whether there were groups of tumor cells that usually exist in proximity to each other. They discovered that the cells not only had preferred neighbors but also that these good-neighbor couplings were consistent in different patients.

Certain neighboring pairs imitated the natural behavior of brain tissue. For example, cells that imitate the parent cells of the oligodendrocyte support cell were found close to endothelial cells, which line the walls of blood vessels. This coupling also occurs in healthy tissue, since endothelial cells release substances that are vital for the survival and proliferation of oligodendrocyte precursor cells.

Similarly, cells that imitate neuron progenitor cells were found in the parts of the tumor that penetrated healthy brain tissue, just as progenitor cells in healthy tissue migrate when the tissue is regenerated.

Taking an overview to gain a fuller understanding of these couplings, the researchers realized that the cells created five distinct layers by organizing themselves into separate environments within the tumor. The innermost layer—the core of the tumor—is made up of necrotic cells,

which do not receive enough oxygen to survive.

In the layer surrounding the necrotic core, the researchers found cells similar to embryonic connective tissue, as well as additional cells, including immune system cells responsible for causing inflammation. The third layer was primarily made up of blood vessels, endothelial cells forming blood vessel walls and additional immune system cells.

Cells in the two outer layers of the tumor don't suffer from a lack of oxygen. This enables groups of tumor cells that mimic healthy brain tissue—progenitors of neurons and support cells—to develop in the fourth layer.

The fifth, outermost layer contains healthy brain tissue, into which the tumor penetrates. These findings about the different layers of a tumor indicate that the driving force behind the tumor's layered structure is the lack of oxygen, which is exacerbated as the disease progresses and the tumor develops.

Based on these findings, the researchers noticed a much more chaotic structure in less aggressive tumors—which are also usually smaller—and in areas of the tumor with a plentiful supply of oxygen.

In most glioma tumors with the IDH mutation, for example, there was usually no necrotic tissue, and the structure of the tumor was disorganized; in the rare cases when there was necrotic tissue, the biopsies also showed a relatively well-ordered structure.

"We discovered that an organized spatial structure is characteristic of the more aggressive tumors," Tirosh explains.

"The lack of oxygen in the tumor cells' environment influences the gene program that they express and therefore affects their state. As the tumor

grows, distinct layers are formed, some of which may be less accessible to drugs and to cells from the immune system, and these could make the tumor more resilient."

The changing status of cancer cells

Researchers from Tirosh's lab used the information they collected on the cellular composition of glioma tumors to work out how a new, promising drug helped some of the patients with this type of cancer.

To do so, they used biopsies from tumors of three patients who had participated in a clinical trial of the new drug and who had responded to the treatment, as well as biopsies from six patients who had not undergone any treatment. To complete the picture, they also used data from biopsies taken from an additional 23 patients who had taken the drug and 134 patients who had not.

The research team, led by Dr. Avishay Spitzer, found that the drug, which works by inhibiting the mutant IDH enzyme, caused the cells to alter the gene program that they expressed. In fact, the treatment encourages the cancerous stem cells to differentiate into mature cells, thereby undermining their ability to divide rapidly, blocking the disease's progress.

The researchers postulated that if the drug works by causing cancerous cells to differentiate into mature cells, the mutation attacking the gene that is critical to the differentiation process could explain those cases in which the drug does not work.

In the biopsies taken from patients who did not receive the drug, they identified a certain gene that is linked to low levels of mature cancerous cells. When they silenced that gene in a mouse model of cancer, they found, as expected, that the drug did not work.

"This indicates that the gene mutation we identified could be a biological marker allowing us to determine in advance which patients will benefit from the treatment and which will not," Tirosh explains. These new findings could also help find a course of treatment that combines IDH inhibitors with another drug that encourages the differentiation process and increases the treatment's impact on the tumor.

"Our two most recent studies revealed the forces that shape the character of cancerous cells in a tumor, be that in their untouched environment or in one resulting from a therapy that alters the cells' genetic program," Tirosh says.

"These findings pave the way for a new approach to cancer treatment, since once we are familiar with the cell groups that populate every area of the tumor and we know how a cell can move from one state to another, we might be able to develop new targeted treatments that will alter the course of the disease.

"The understanding that both the composition of cells within the tumor and its three-dimensional structure are linked to the level of the tumor's aggressiveness could also lead to new diagnostic methods that do not rely solely on the volume of the tumor and the mutations it contains."

More information: Alissa C. Greenwald et al, Integrative spatial analysis reveals a multi-layered organization of glioblastoma, *Cell* (2024). DOI: 10.1016/j.cell.2024.03.029

Provided by Weizmann Institute of Science

https://medicalxpress.com/news/2024-08-high-resolution-brain-tumor-reveals.html