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Journal

Cancer Discovery, 10(7)

ISSN

2159-8274

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Publication Date

2020-07-01

DOI

10.1158/2159-8290.cd-20-0493

Peer reviewed

HHS Public Access

Author manuscript

Cancer Discov. Author manuscript; available in PMC 2021 July 16.

Published in final edited form as:

Cancer Discov. 2020 July ; 10(7): 907–909. doi:10.1158/2159-8290.CD-20-0493.

Utility of Human-Derived Models for Glioblastoma

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Summary:

In this issue, Pine and colleagues compared single-cell RNA-sequencing data across four distinct types of glioblastoma stem cell–derived tumor models, reinforcing the importance of a three-dimensional microenvironment for accurate recapitulation of cellular states.

Glioblastoma (GBM) is the most aggressive primary malignant brain tumor. Despite decades of research, GBM tumors remain incurable. Long-term survival is under 2 years (1). Glioblastoma tumors may rarely carry neomorphic mutations in isocitrate dehydrogenase correlating with modestly improved survival. Why are these tumors so resistant to therapy? Glioblastoma stem cells (GSC) self-renew and differentiate, placing them at the apex of the tumor's cellular hierarchy, enabling resistance to therapy and driving recurrence. GBM is also highly invasive, a feature that is independent of grade, and has been difficult to recapitulate in xenograft models. Ideally, models for GBM should adequately reflect patient tumor composition and faithfully recapitulate cellular states associated with GSCs.

Preclinical model systems are vital tools for studying tumor biology and testing treatment modalities. Over decades, a wide range of human-derived GBM models were introduced and adopted (Fig. 1). Since their appearance in the 1950s, cancer cell lines have been the traditional standard. Cell lines are relatively easy to maintain, engineer, xenograft, and sequence. However, the establishment of cell line exerts selective pressure to survive on plastic. Further selection also occurs heterogeneously after cell lines are distributed, likely due in part to differences in culture conditions employed in different laboratories (2). Thus, cell lines may poorly represent the tumors from which they were derived. Indeed, when injected orthotopically, GBM cell lines typically do not form infiltrative tumors characteristic of human disease, instead forming encapsulated tumors that grow adjacent to the surrounding brain.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Cell culture also selects against some signature lesions in GBM, like amplification of *EGFR* , while leading to increased methylation of the MGMT promoter, a biomarker that correlates with sensitivity to alkylating chemotherapy. Previous research by the Fine laboratory found that primary GBM cells maintained in classic serum-containing conditions gained an epithelial cell–like morphology that was not reversed following transition to neurobasal media required to maintain GSCs. Cells grown in serum also showed decreased tumorigenic potential and invasiveness, altered gene expression, and loss of stem cell markers when compared with GBMs grown as two-dimensional spheres (2-D) in neurobasal conditions (3).

Efforts to model invasive biology and preserve signature genetic abnormalities by avoiding selective pressures associated with culturing on plastic led to the popularity of patient-derived xenografts (PDX). Attempts to transplant freshly resected surgical material into the brains of immunodeficient mice started in the late 1970s and have evolved subsequently. Human GBM cells can be dissociated and injected orthotopically, or passaged first into the flanks of nude mice, then injected into the brain. Compared with cell lines, the resulting tumors better preserve the morphologic and genetic properties of parental GBM, and also more accurately mirror clinical responses to treatment. These features, together with the fact that PDXs provide an *in vivo* system to assess drug absorption, metabolism, sensitivity, and blood–brain barrier permeability, make them a valuable tool for preclinical testing. Today, large PDX collections recapitulating the genetic diversity of GBM are being built across the globe. PDX models do, however, have limitations. First, not all engrafted tumors grow in mice, introducing selection bias. For tumors that do grow, passaging and scaling up can be very time-consuming, with strong selection for faster-growing, less representative isolates. Second, serial passaging in the murine environment can change PDXs. Human stroma is substituted by murine stroma, and mouse-specific copy-number alterations accumulate. These changes may influence response to therapy (2). Third, the need for an immunodeficient host hampers application in immunotherapy, although advances are being made in humanizing the mouse immune system.

Ex vivo organoid culture systems have gained popularity over the last decade. In a typical organoid, human stem cells are embedded onto a matrix and cultured under serum-free conditions, allowing them to preserve the heterogeneity of the organs from which they were derived. To study GBM using this system, patient-derived cell lines or tumor tissue can be used directly to generate three-dimensional tumor organoids (TO; ref. 4). A recent study showed that TOs recapitulated intratumoral heterogeneity evidenced by similar mRNA and DNA sequencing profiles and aggressive infiltration upon engraftment, resulting in utility as models for targeted therapy and chimeric antigen receptor T-cell immunotherapy (5). Notably, TOs were able to develop hypoxia gradients and partially preserve microvasculature and immune cells, features that are difficult to model *ex vivo*.

In contrast to TOs, GBM cerebral organoids (GLICO), also pioneered by the Fine laboratory, start by growing normal cerebral organoids from pluripotent stem cells, resulting in brain-like structures. GSCs grown in these cerebral organoids can infiltrate the stroma, exhibiting heterogeneous levels of invasiveness that correlated to parental tumors. In addition to preserving key genetic and signaling features, GLICOs can model the network of

tumor microtubes (6). Organoid systems can be derived relatively quickly and can be scaled to facilitate high-throughput screening. Their shortcomings lie in the limitations of cerebral organoids, which lack immune cells and a blood–brain barrier, both of which likely contribute to therapy resistance in GBM. As new methods for modeling tumor immune microenvironment in organoid systems are being developed (7), improved cerebral organoid models are likely forthcoming. In addition, GLICOs to date utilize GSCs grown in culture, rather than using freshly resected tumors as a starting point.

Although all models mentioned have been adopted in GBM research, few comparisons have been made across models. With the development of single-cell RNA-sequencing (scRNA-seq) technology, tumor heterogeneity can be qualified and quantified in unprecedented detail (8). In this issue of *Cancer Discovery*, Pine and colleagues established four models from each of five GBM tumors, including 2-D, PDX, TO, and GLICO, then performed scRNA-seq on GSCs sorted from each model (9).

So how do the four models differ? Unsupervised hierarchical clustering showed clustering of 2-D and TO models built with pure tumor cells; models grown on mouse brain or human organoids, PDX and GLICO, formed a separate cluster. Analysis of cellular composition revealed that about a quarter of the cells in TO, PDX, and GLICO models were in a cell-cycle cluster, whereas a majority of cells in 2-D were cycling. TO had a higher portion in a hypoxia and mesenchymal-like cluster, PDX had nearly half of its cells in the classic astrocyte (AC)-like cluster, whereas GLICO contained a higher percentage of a proneural and neural/oligodendrocyte progenitor cell (NPC/OPC)–like cluster. These findings are in line with recent observations from scRNA-seq of primary GBM, noting heterogeneity in cellular states that recapitulate distinct neural cell types (8, 10).

How well do these models recapitulate cellular states associated with GSC in primary tumors? GSCs in GLICO correlated most closely to the bulk transcriptome from matched primary tumors. When all models were correlated with The Cancer Genome Atlas bulk RNA-sequencing data, GLICO models showed a higher proportion of cells in the proneural subtype and a very low proportion of mesenchymal type, indicating a strong stemness signature (10). When metamodule scores for the four cellular states (NPC, OPC, AC, and mesenchymal-like) were visualized as a 2-D figure, GLICO models showed the broadest spread of data points, suggesting increased cell type diversity. Pine and colleagues did not generate scRNA-seq data from their primary tumors. In comparing the scRNA-seq data from their models to primary tumors, they therefore used published scRNA-seq results from a set of unmatched GBMs. By this analysis, GLICO models best maintained an enriched NPC/OPC-like subpopulation that resembled unmatched primary tumors.

Interestingly, when cells from the GLICO model were replated into 2-D cultures, they developed a dendrite-like network after 7 days, and then at 30 days grew into neuro-spheres. The noncycling NPC-like and AC-like subgroups in GLICO were lost, and the resultant cells closely resembled the 2-D phenotype. Because the GSCs isolated from patient tissue showed the same change, and GLICO was built with initially 2-D–cultured cells, these data suggest that adequate microenvironment is necessary and sufficient for recapitulating some aspects of cellular plasticity in primary GBM.

What model should be used in preclinical studies of GBM? Resemblance to human tumor is essential; however, engraftment rate, flexibility, cost, and scalability represent important considerations. Although Pine and colleagues provide a valuable comparison among models, this analysis reflected a single time-point shortly after model establishment, and did not consider whether genetic differences among primary tumors influenced the accuracy of GLICO models. Cancer research moves quickly, and new models are almost always favored over old ones. In general, the best models most accurately inform clinical practice. Comparing response to therapy in individual patients with GBM with response to the same therapy applied to matched models derived from each patient's tumor would help to validate functional differences among modeling strategies.

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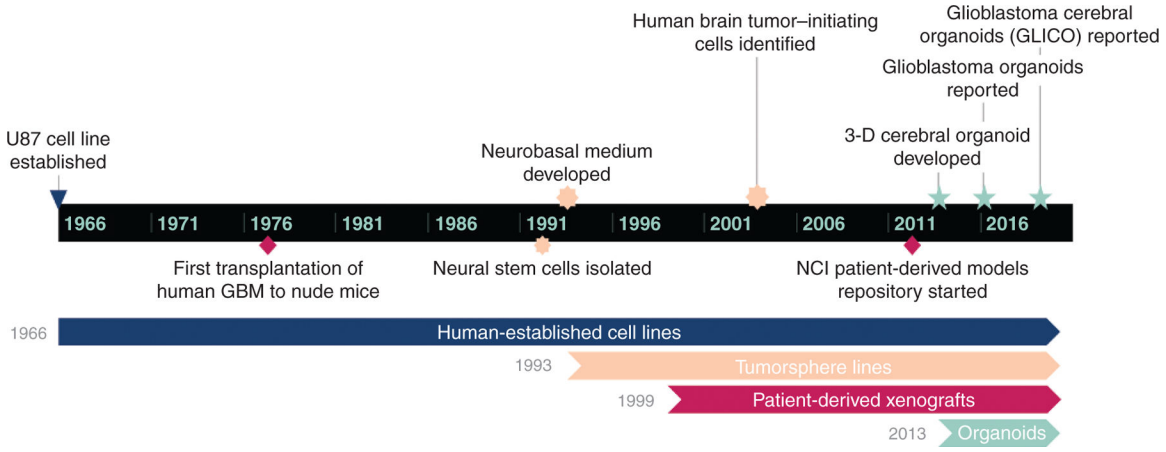

Figure 1.
The timeline and milestones of human-derived GBM models.