OTJHS
Online Turkish Journal of Health Sciences
e-ISSN: 2459-1467
OTSBD
Online Türk Sağlık Bilimleri Dergisi
Online Turkish Journal of Health Sciences 2026;11(2): 196-204
Online Türk Sağlık Bilimleri Dergisi 2026;11(2): 196-204

A Patient-Derived Glioblastoma Organoid Model that Preserves Intra- and Intertumoral Heterogeneity
İntra- ve İntertümöral Heterojeniteyi Koruyan Hasta Kaynaklı Glioblastoma Organoid Modeli

[1]Melis ERCELIK, [1]Cagla TEKIN, [2]Pınar ESER, [2]Ahmet BEKAR, [2]Hasan KOCAELI, [3]Mevlut Ozgur TASKAPILIOGLU, [1]Berrin TUNCA

[1]Department of Medical Biology, Faculty of Medicine, Bursa Uludag University, Bursa, Türkiye.
[2]Department of Neurosurgery, Faculty of Medicine, Bursa Uludag University, Bursa, Türkiye.
[3]Department of Neurosurgery, Faculty of Medicine, Istanbul Yeni Yuzyıl University, Istanbul, Türkiye.

Melis Ercelik: https://orcid.org/0000-0003-0366-2424
Cagla Tekin: https://orcid.org/0000-0002-2568-3667
Pınar Eser: https://orcid.org/0000-0003-0132-9927
Ahmet Bekar: https://orcid.org/0000-0002-2716-1985
Hasan Kocaeli: https://orcid.org/0000-0003-4140-5955
Mevlut Ozgur Taskapilioglu: https://orcid.org/0000-0001-5472-9065
Berrin Tunca: https://orcid.org/0000-0002-1619-6680

Abstract

Objective: Glioblastoma (GB) is an aggressive brain tumor characterized by extensive heterogeneity. We aimed to generate patient-derived GB organoids preserving histological features and to establish a biobank for future studies.

Materials and Methods: Tumor tissues from GB patients were dissected into 0.5–1 mm fragments and cultured for 14 days in organoid medium under orbital shaking. Immunohistochemical analysis was performed to assess GFAP, OLIG2, IDH-1, p53, ATRX, and Ki67 expression before and after organoid formation.

Results: All tumor samples formed spherical organoids within 14 days. Histological analysis showed preservation of GB-specific morphological features, including nuclear atypia and pleomorphism. GFAP, OLIG2, and ATRX expression patterns were concordant between parental tumors and organoids in cases. IDH-1 expression was consistent in three patients. Ki67 and p53 expression levels showed similar patterns between organoids and corresponding tumors.

Conclusions: Patient-derived GB organoids retain key histopathological features and may serve as a platform for GB modeling.

Keywords: Glioblastoma, Organoid culture, Tumor heterogeneityse

Öz

Amaç: Glioblastoma (GB), yaygın heterojenite ile karakterize edilen agresif bir beyin tümörüdür. Amacımız, histolojik özellikleri koruyan hasta kaynaklı GB organoidleri üretmek ve gelecekteki çalışmalar için bir biyobanka oluşturmaktır.

Materyal ve Metot: GB hastalarından alınan tümör dokuları 0,5-1 mm'lik parçalara ayrıldı ve 14 gün boyunca organoid ortamında, orbital çalkalama altında kültüre edildi. Organoid oluşumundan önce ve sonra GFAP, OLIG2, IDH-1, p53, ATRX ve Ki67 ekspresyonunu değerlendirmek için immünohistokimyasal analiz yapıldı.

Bulgular: Tümör örneklerinin tamamı 14 gün içinde küresel organoidler oluşturdu. Histolojik analiz, nükleer atipi ve pleomorfizm de dahil olmak üzere GB'ye özgü morfolojik özelliklerin korunduğunu gösterdi. GFAP, OLIG2 ve ATRX ekspresyon paternleri, ana tümörler ve organoidler arasında uyumluydu. IDH-1 ekspresyonu üç hastada tutarlıydı. Ki67 ve p53 ekspresyon seviyeleri, organoidler ve karşılık gelen tümörler arasında benzer dağılım gösterdi.

Sonuç: Hasta kaynaklı GB organoidleri, temel histopatolojik özellikleri korur ve GB modellemesi için bir platform olarak hizmet edebilir.

Anahtar Kelimeler: Glioblastoma, Organoid kültürü, Tümör heterojenitesi

Corresponding Author / Sorumlu Yazar:
Berrin Tunca
Bursa Uludag University Faculty of Medicine Medical Biology Department, Gorukle, Bursa, Türkiye.
E-Mail: btunca@uludag.edu.tr

Article Info / Yayın Bilgisi:
Received / Gönderi Tarihi: 14/01/2026
Accepted / Kabul Tarihi: 05/03/2026
Published / Online Yayın Tarihi: 21/06/2026

Cited / Atıf: Melis Ercelik, et al. A Patient-Derived Glioblastoma Organoid Model that Preserves Intra- and Intertumoral Heterogeneity. Online Türk Sağlık Bilimleri Dergisi 2026;11(2):196-204. doi: 10.26453/otjhs.1862625

INTRODUCTION

Glioblastoma (GB) represents the most prevalent and lethal malignant brain tumor in the adult population.[1] Despite standard treatment approaches for GB, including surgical resection followed by chemoradiotherapy, the 5-year survival rate remains dismally low at only 3–7%.[2] Although novel therapeutic strategies are being explored for these patients, intertumoral and intratumoral molecular heterogeneity significantly limits the effectiveness of such treatments.[3] Despite the use of various research models to elucidate the biological mechanisms of GB and to develop novel therapeutic strategies, these models exhibit significant limitations. Traditional preclinical models include cultured or patient-derived in vitro 2-dimensional (2D) tumor cell models and in vivo xenograft or genetic mouse models.[4] 2D cell culture models, including spheroids, have limited utility in GB research due to their inability to recapitulate cell-cell interactions and the influence of the tumor microenvironment, as well as their tendency to acquire additional mutations during clonal expansion and amplification processes.[5] In vivo models can mimic multicellular architecture and inter-tissue interactions but fail to fully recapitulate human cancer–specific genetic features and intratumoral heterogeneity.[2] Therefore, there is an urgent need for disease models that structurally and functionally mimic human tissues and organs while incorporating a greater number of tumor microenvironment components, in order to elucidate the underlying pathogenesis of GB and evaluate novel drug candidates.[2]

Organoids are 3-dimensional (3D) structures that can mimic human diseases, and are typically derived from self-organized stem cells or resected diseased tissues.[6-8] Compared to conventional in vitro models, tumor organoids more accurately recapitulate the cellular architecture and physiological characteristics of the human body due to their enhanced ability to resemble the tumor microenvironment.[9] Furthermore, organoids can be extensively expanded in culture while preserving the heterogeneity of the primary tumors and maintaining genomic stability.[10, 11] Owing to these features, organoid-based malignancy models are emerging as valuable and indispensable tools in cancer research.[7,8]

In this study, we aimed to generate patient-derived GB organoids directly from fresh tumor tissues using a defined culture medium without single-cell dissociation, with the goal of establishing a living biobank. Furthermore, we sought to evaluate whether these organoids preserve key histological features and reflect intertumoral and intratumoral heterogeneity of the original tumors. Ultimately, this model was developed to explore its potential as a platform for investigating GB biology and assessing personalized therapeutic strategies.

MATERIALS AND METHODS

Ethics Committee Approval: This prospective study was approved by the Ethics Committee of Bursa Uludag University (ethics number: 2021-9/14) and conducted in accordance with the Declaration of Helsinki.

Patients: This prospective study includes four patients diagnosed with primary GB, who were followed at the Department of Neurosurgery, Bursa Uludag University Hospital, between 2022 and 2024. Tumor tissue samples for organoid production were collected during microsurgical resection, before the initiation of radiotherapy or chemotherapy, using an operating microscope (Zeiss OPMI Pentero, Carl Zeiss Inc., Oberkochen, Germany). Inclusion criteria consisted of patients who underwent surgical resection for suspected high-grade brain tumors, validated as GB by neuropathologists according to WHO criteria. Conversely, exclusion criteria included patients who underwent only stereotactic biopsy, had a known family history of glioma, presented with a coexisting malignancy, or died within 30 days post-surgery due to non-tumor related causes.

Collection, Dissociation and Preparation of Tumor Samples: During the surgery, the resected GB tumor tissue was placed in sterile phosphate-buffered saline. To avoid compromising the quality of organoid production, the tumor tissue was processed within a maximum of 30 minutes following surgical resection. For organoid culture establishment, the tissue was transferred to Hibernate™- A medium (BrainBits) maintained at 4°C. The tumor tissue was then transferred into a sterile cell culture dish containing H+GPSA medium for dissection under a stereomicroscope (Zeiss) in a laminar flow biosafety cabinet. H+GPSA medium consisted of Hibernate™-A Medium, 1X GlutaMAX™ supplement (Thermo Fisher Scientific), 1X penicillin-streptomycin (Thermo Fisher Scientific) and 1X amphotericin B (Thermo Fisher Scientific). The tumor tissue was cut into pieces approximately 0.5 to 1 mm in diameter using a scalpel, and cell debris was washed with H+GPSA medium. The tumor tissue was incubated at room temperature for 10 minutes with gentle rotation in 1X red blood cell lysis buffer (Thermo Fisher Scientific) to lyse most of the contaminating red blood cells. The tumor tissue was then removed from the red blood cell lysis buffer and washed with H+GPSA medium.

Organoid Generation from Patient-Derived GB Tissue: For the establishment of a GB organoid culture, tumor pieces were added to 4 mL of organoid medium (OM) containing 2.5 µg/ml human insulin (Sigma-Aldrich) per well in ultra-low attachment 6-well culture plates (Corning), and incubated in a humidified sterile incubator at 37°C with 5% CO_2 on an orbital shaker rotating at 120 rpm. OM consisted of 50% DMEM/F-12 (Thermo Fisher Scientific), 50% Neurobasal™ (Thermo Fisher Scientific), 1X GlutaMAX™ (Thermo Fisher Scientific), 1X MEM Non-Essential Amino Acids Solution (Thermo Fisher Scientific), 1X N2 supplement (Thermo Fisher Scientific), 1X B27 supplement without vitamin A (Thermo Fisher Scientific), and 1X 2-mercaptoethanol (Thermo Fisher Scientific). During the first week of organoid production, the medium became turbid as the tumor pieces typically shed cellular and blood debris. Therefore, 75% of the medium was replaced every 48 hours, and the plates were tilted at a 45° angle while the fresh medium was carefully added to the tissues. Tumor pieces generally formed spherical organoids within 1-2 weeks, depending on the tissue quality and patient-specific tumor growth characteristics.[12] Following 14 days of incubation, the organoids typically exhibited sizes varying from 0.5 mm to 1.5 mm. Since the microdissection process was performed manually, their sizes showed a natural variability compared to single-cell derived models. To prevent necrotic cells in larger organoids in the future, organoids exceeding 1 mm in diameter were routinely cut into smaller pieces of approximately 0.5 mm.

Tissue Processing and Immunohistochemistry Analysis: Parental tumor samples and organoid fragments were fixed in 4% paraformaldehyde for 24 h and embedded in paraffin (FFPE). 3 µm serial tissue sections were processed for hematoxylin and eosin (H&E) staining and immunohistochemistry (IHC) for GFAP (BioGenex, AM020-5M Lot: AM0200617), OLIG2 (Atlas, HPA003254), IDH-1 (Biocare, ACI3253), p53 (Dako, IR606 Lot: 41654182), ATRX (Medaysis, MCD987), and Ki67 (Dako, IR609 Lot:41718523). Sections were manually processed with primary antibodies for 30 min. Then, they were washed with PBS and incubated with a Dako single bond HRP kit containing a secondary antibody and avidin-biotin system for 20 min. Following this step, the slides were rinsed with PBS and developed using 3,3'-diaminobenzidine as the chromogenic substrate for peroxidase activity, resulting in brown-colored staining of positive cells. Subsequently, the sections were washed again with PBS, counterstained with hematoxylin, dehydrated through graded alcohols, cleared in xylene, and mounted with Entellan®. Slides were washed again with PBS, counterstained with hematoxylin, dehydrated, and coverslipped using Entellan®. Prepared slides were examined with an ECLIPSE Ci Series (Nikon, NY, USA) light microscope. Immunolabeling was evaluated by two independent pathologists without being affected by other analyses.

Establishment of GB Organoid Biobank: GB organoids were cut into approximately 100 µm diameter pieces, and washed with OM to remove cell debris. Organoid pieces were incubated with OM supplemented with 10 µM Y-27632 (StemCell Technologies) for 1 h and then resuspended in a freezing medium consisting of OM supplemented with 10 µM Y-27632 and 10% DMSO (Sigma-Aldrich). Approximately 20 organoid pieces were placed in cryotubes and kept at room temperature for 15 min before being slowly cooled to −80°C using a CoolCell® freezing container (Thermo Fisher Scientific) placed in a −80°C freezer. Frozen GB organoids were stored at −152°C for long-term storage.

IHC Scoring: Data were analyzed using descriptive statistics to evaluate the concordance between parental tumors and organoids. For IHC characterization, a semi-quantitative scoring (SQS) system was integrated with the Labeling Index (LI). GFAP, OLIG2, IDH-1, and ATRX expression were categorized as positive (+) or negative (−) based on the presence of staining. Specifically, p53 and Ki67 were quantified as the percentage of positive nuclei (LI%) and categorized into a four-tier scale: 0 (negative), 1+ (weak, <10%), 2+ (moderate, 10-30%), and 3+ (strong, >30%)[13]. This scoring was independently validated by neuropathologists to ensure objective characterization.

RESULTS

Tumor tissues were obtained from four patients (3 males and 1 female) with a mean age of 71.7 ± 3 years (range: 68–76). In one patient, the lesion was located in the parietal lobe, while in the remaining three it was primarily in the temporal lobe—extending into the temporo-basal region in two patients and the occipital region in one. All patients had normal preoperative neurological examinations. Macroscopic total resection was achieved during a single surgical session in all patients except one (Patient #4), who required two consecutive sessions. Postoperative magnetic resonance imaging (MRI) was performed within the first 24 hours after surgery (Figure 1A-D). Total resection was confirmed in all cases, except for Patient #1, who had a minimal residual tumor in the left temporal region. No postoperative complications were observed. Histopathological analysis confirmed GB in all patients. All received postoperative radiotherapy and temozolomide (TMZ) treatment. At the end of the mean follow-up

period of 6.2 ± 4.3 months (range: 2–12 months), all patients had died except for Patient #3, who remains under follow-up.

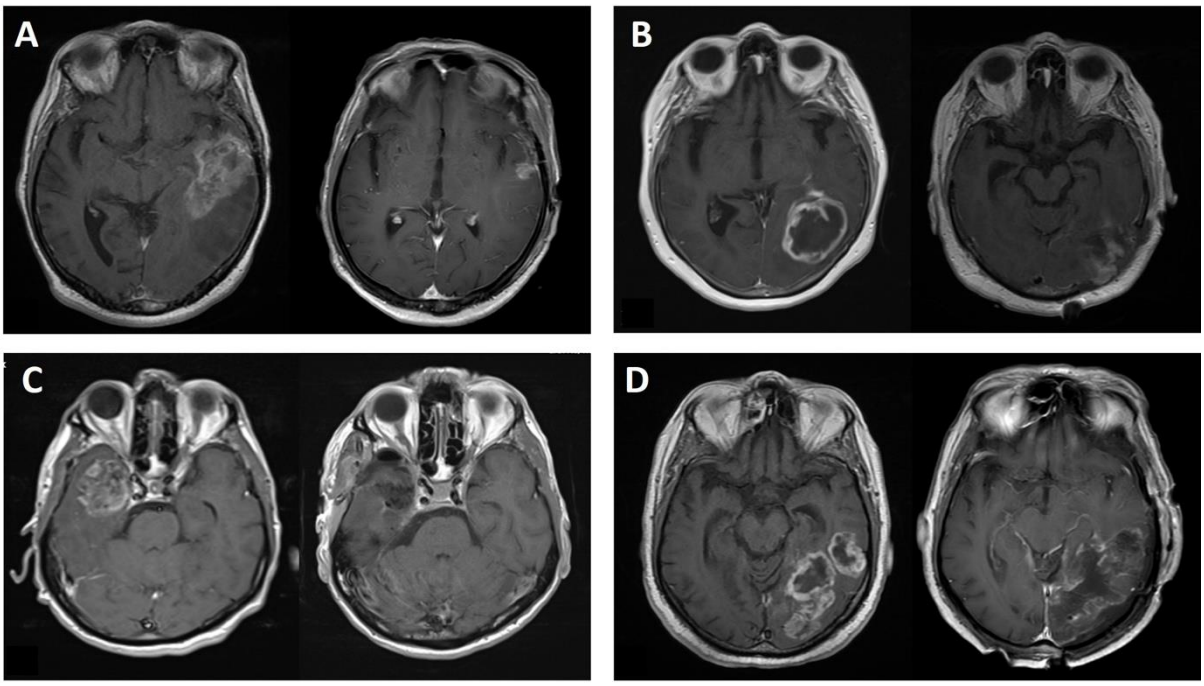

Figure 1. Preoperative and postoperative MRI scans of four patients diagnosed with GB. (A–D) correspond to Patients #1 through #4, respectively. In each subpanel, the left image represents the preoperative MRI, while the right image shows the postoperative MRI obtained within 24 hours following surgery. Macroscopic total resection was achieved in all cases except Patient #1 (a), who exhibited a minimal residual tumor in the left temporal region.

Fresh GB tissue was obtained intraoperatively from GB patients by experienced neurosurgeons during surgical resection. To ensure successful organoid formation, special care was taken to collect tumor tissue from the tumor margins, specifically from regions with minimal necrosis and limited surrounding normal brain tissue. The resected tumor tissue was dissected into small pieces approximately 0.5 to 1 mm in diameter using a scalpel, and cellular debris was removed by washing with H+GPSA medium (Figure 2A).

These pieces, cleared of red blood cells and residual tissue debris, were then transferred to ultra-low attachment 6-well culture plates containing OM and placed in an orbital shaker inside an incubator to support organoid formation and enhance nutrient and oxygen diffusion (Figure 2A). The criteria for successful generation of patient-derived organoids included the survival of microdissected tumor pieces for at least two weeks, acquisition of a spherical morphology, and sustained growth in culture. Under these conditions, tumor pieces formed spherical organoid structures within 14 days (Figure 2B). To prevent the development of necrotic cell death in the central regions of the organoids, they were subsequently passaged by sectioning into smaller pieces of approximately 0.5 mm in diameter.

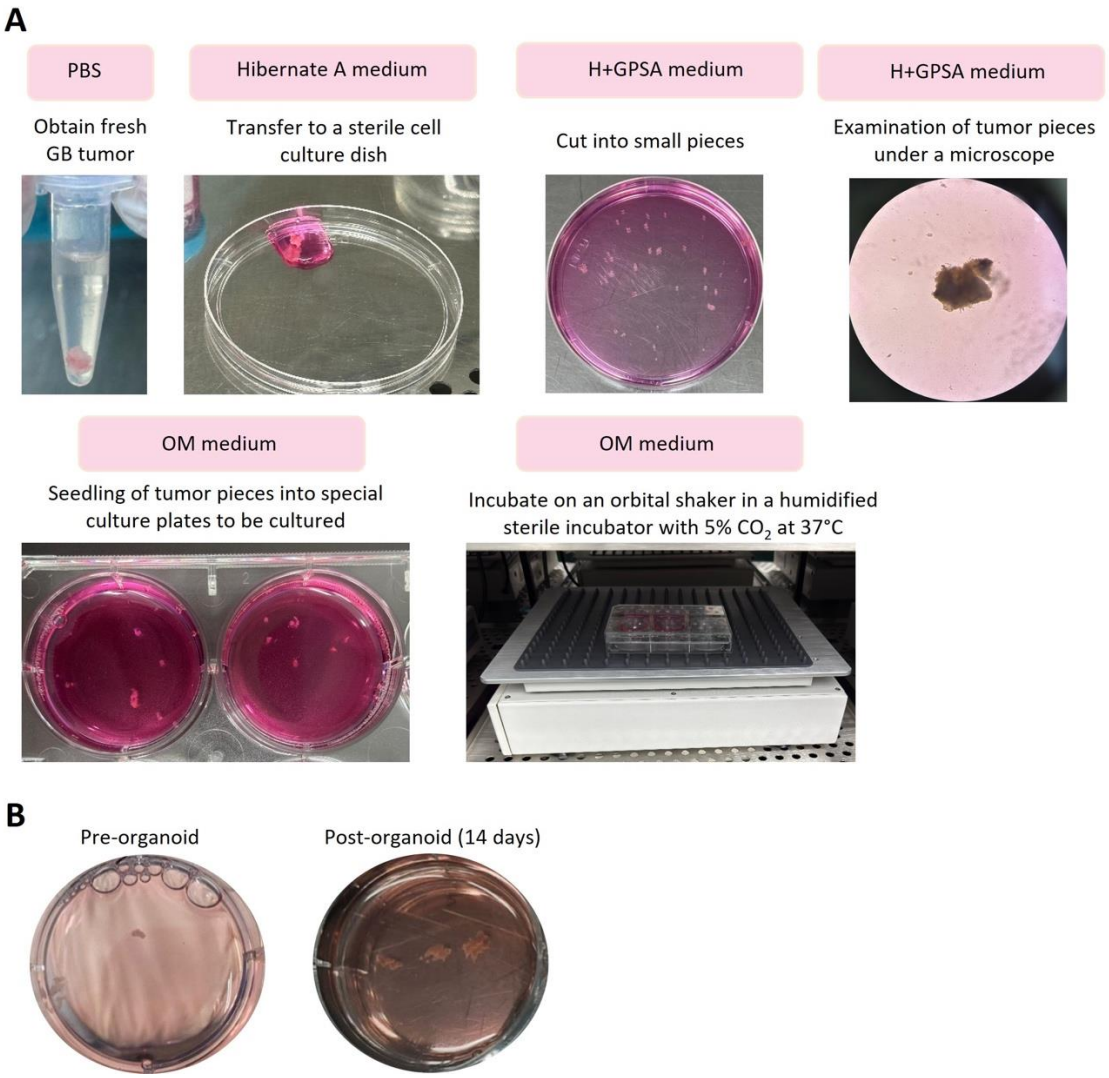

Figure 2. (A) A schematic of the procedure for organoid generation from tumors of GB patients. (B) Image of morphologically grown organoids.

Histological analyses were performed to evaluate whether the patient-derived organoids resembled their parental tumors. Examination using H&E staining revealed that the organoids preserved GB-specific morphological features, which was independently confirmed by a neuropathologist (Figure 3). The organoids exhibited cellular and nuclear atypia of the patient tumors, including abundant mitotic figures, marked pleomorphism, and nuclear irregularities. Furthermore, they displayed various morphological features of the parental tumors, such as gemistocytic cells, multinucleated giant cells, and cells with prominent cytoplasmic vacuolization. All organoids were diagnosed as GB, as were patient tumors. To better characterize the cellular identities, the glial marker GFAP, the glioma stem cell marker OLIG2, and molecular markers such as IDH-1, ATRX, and p53 to assess mutational status, and Ki67 immunostaining to evaluate the proliferation index were used. The expression levels of these markers were evaluated using a SQS system to ensure diagnostic objectivity.

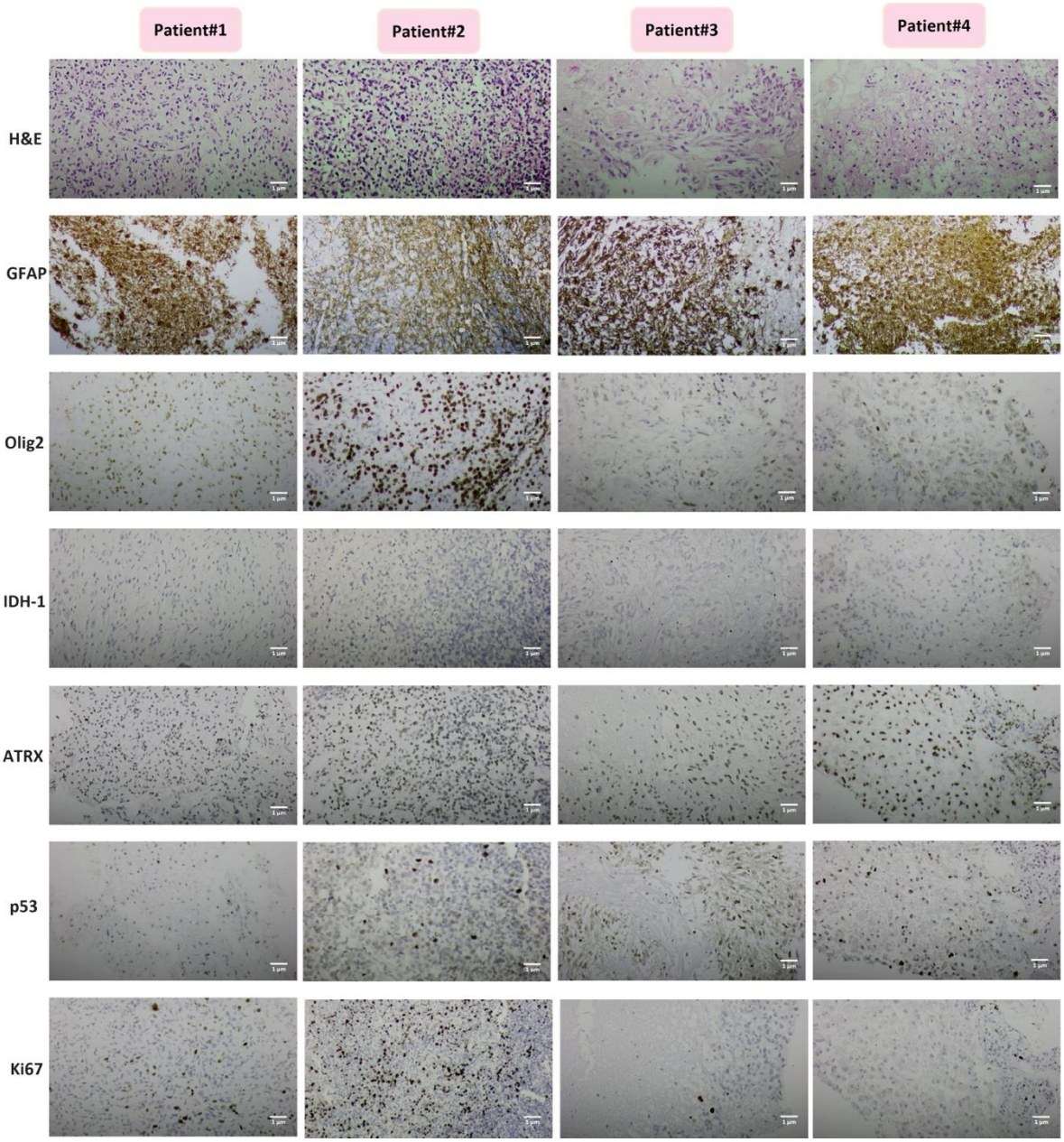

Figure 3. Images of IHC staining for different markers showing maintenance of parental tumor cell populations in organoid samples for four patients. Scale bar: 1 μm.

In all four patient samples, parental tumors and organoid tumors were completely concordant concerning GFAP, OLIG2 and ATRX (Table 1). In Patient #1, Patient #2 and Patient #4 samples, parental tumors and organoid tumors were identical in terms of IDH-1, while IDH-1 positivity was observed in the organoid tumor derived from Patient #3. p53 and Ki67 markers showed that organoids had relatively similar cell densities to their corresponding parental tumors, and all patients were p53 positive. For these markers, the labeling index (percentage of positive nuclei) was categorized into a four-tier intensity scale (0 to 3+) for comparative analysis. These findings indicate that the organoids exhibited histopathological features consistent with those of the parental tumors and highlight the importance of individual tumor heterogeneity in organoid-based modelling.

Table 1. Marker quantifications from immunohistochemical staining of parental and organoid tumors.

	Parental tumors						Organoids					
Cases	**GFAP[1]**	**Olig2[1]**	**IDH-1[1]**	**ATRX[1]**	**p53 (LI%)[2]**	**Ki67 (LI%)[2]**	**GFAP[1]**	**Olig2[1]**	**IDH-1[1]**	**ATRX[1]**	**p53 (LI%)[2]**	**Ki67 (LI%)[2]**
					20%	35%					Low positive	10%
Patient #1	+	+	-	-	(2+)	(3+)	+	+	-	-	(1+)	(1+)
					30%	35%					5%	40%
Patient #2	+	+	-	-	(3+)	(3+)	+	+	-	-	(1+)	(3+)
					20%	13%					30%	45%
Patient #3	+	+	NS	-	(2+)	(2+)	+	+	-	-	(3+)	(3+)
					10-15%	27%					10%	1-3%
Patient #4	+	+	-	-	(2+)	(2+)	+	+	-	-	(1+)	(1+)

Abbreviations: LI, Labeling Index; NS, Nonspecific; (+), Positive; (-), Negative: [1] Binary scoring (Presence/Absence): [2] SQS (Semi-quantitative Score): Based on LI%: <10% (1+), 10-30% (2+), >30% (3+).

DISCUSSION AND CONCLUSION

Here, we present a model in which patient-derived GB organoids are generated without single-cell dissociation, in the absence of serum and extracellular matrix, allowing them to expand while preserving natural cell–cell interactions and maintaining features similar to their parental tumors.

The identification of genotype-specific molecular profiles underlies personalized treatment strategies in many cancers, including GB.[14,15] Although genotype-based therapies are available for breast cancer, lung cancer, and leukemia, extending personalized treatment to most patients remains challenging.[15] Currently, only 7% of cancer patients benefit from genome-guided therapies,[16] largely because predicted clinical responses often fail to reflect actual outcomes. GB, the most aggressive primary brain tumor, was among the first cancers analyzed by The Cancer Genome Atlas Project.[17,18] Genomic studies have shown that GB comprises heterogeneous molecular subtypes defined by RNA expression, DNA methylation, and multi-omics profiling.[14,19,20] Single-cell RNA sequencing has further demonstrated marked intratumoral heterogeneity, highlighting cellular plasticity and diverse phenotypes.[21] Despite this complexity, treatment remains largely limited to surgery, radiotherapy, and TMZ-based chemotherapy.

A major limitation of GB treatment is the lack of suitable models for effective drug screening. Conventional in vitro cultures fail to fully replicate the parental tumor's genetic architecture, as additional mutations acquired during expansion lead to loss of intratumoral heterogeneity. Furthermore, commercial matrices such as Matrigel and the use of serum or growth factors may alter tumor cell phenotypes.[22] Organoid cultures better preserve tumor heterogeneity and structural features.[23] Recently, patient-derived organoids have gained attention for drug screening applications. Hubert et al. (2016)[24] optimized in vitro conditions to generate GB organoids from patient-derived cells, xenografts, and genetically engineered mouse gliomas. Jacob et al. (2020)[12] later developed a faster protocol and showed that GB organoids retained intra- and intertumoral heterogeneity through transcriptomic and exome analyses, including single-cell sequencing.

In this study, we successfully generated patient-derived tumor organoids using a mechanical dissection approach that preserved the native architecture of the tumor microenvironment. By avoiding enzymatic dissociation and excluding exogenous growth factors, we preserved the intrinsic phenotypic and molecular characteristics of the original tumor cells. We demonstrated the robustness and reproducibility of our organoid culture system, with organoid fragments sustainably surviving and retaining the capacity to proliferate for two weeks, with the acquisition of a spherical morphology. IHC profiling revealed a high degree of molecular fidelity between parental tumors and corresponding organoids, particularly in the expression of glial markers such as GFAP, OLIG2 and ATRX. Single-cell RNA sequencing studies have identified distinct expression patterns within organoids, suggesting that prolonged culture periods may lead to the emergence of unique molecular characteristics not present in the original tumors. These findings underscore the dynamic nature of organoid cultures and point to potential limitations in accurately recapitulating the original tumor under extended culture conditions spanning several months.[25] In this study, although there was a similarity in the expression of IDH-1 markers in organoid sections relative to the parental tumor, one patient had an IDH-1 mutation after organoid transplantation. Analysis of p53 and Ki67 markers showed that organoids had relatively similar cell densities to their corresponding parental tumors. This demonstrated that the organoid platform can reliably recapitulate key lineage features of glial tumors in vitro.

Organoid biobanks have been established for various cancer types, including pancreatic, liver, prostate, and breast cancers.[26] In GB, the pioneering study by Jacob et al. laid the groundwork for patient-derived organoid models.[12]

In the present study, we generated a GB organoid biobank from four patients, demonstrating strong histopathological similarity to the parental tumors. This biobank represents a reliable in vitro model and a valuable platform for future biological research and preclinical testing, supporting the development of personalized therapeutic strategies in GB.

In our developed organoid model, we focused on the characterization of early-passage organoids within a culture period of 2 to 4 weeks. This strategic timing was chosen to prioritize the preservation of the natural heterogeneity of the primary tumor and to minimize clonal drift. This is because long-term in vitro cultivation in organoid models can lead to the emergence of dominant clones and a subsequent shift from the original molecular profile. However, it is important to acknowledge that even in short-term cultures, the possibility of clone selection or culture-induced variations cannot be entirely ruled out, as certain cell populations may adapt more readily to in vitro conditions. Furthermore, while our model effectively recapitulates the tumor architecture, the inherent absence of a functional vascular network and an intact immune microenvironment remains a common limitation of current in vitro organoid systems. While our biobanking protocol ensures the recovery and use of these organoids for later applications, maintaining them at early passages ensures that experimental results continue to represent the patient's primary disease state. Therefore, while the organoid model supports long-term viability, we advocate for the use of early-passage cultures to maintain maximum translational relevance. However, since the entire tumor tissue from the patient could not be isolated for organoid generation, minor variations may occur due to intratumoral heterogeneity. While this represents a limitation of the study, it also highlights the need for longitudinal monitoring and more comprehensive analyses to fully understand tumor biology. As established in this study, a standardized cryopreservation protocol for the biobank has been developed. In future research, quantitative analysis of post-thaw viability and cell health across patient samples will be conducted to more clearly demonstrate the long-term success of this system, which will enable the development of patient-specific treatment strategies and the use of more reliable models in GB research.

In conclusion, our findings support the utility of patient-derived organoid models as reliable yet dynamic platforms for studying glioma biology. Additionally, the preservation of key glial markers from the parental tumors in the organoids underscores their potential applications in translational research, such as drug screening and personalized therapeutic strategies.

***Ethics Committee Approval*:** Ethical approval for this study was granted by the Bursa Uludag University Ethics Committee (Dated 20.06.2021, decision no: 2021-9/14). All patients were fully informed about the study objectives, confidentiality measures, and their right to withdraw at any time, and written informed consent was obtained prior to participation. The study was conducted in full accordance with the Declaration of Helsinki.

Conflict of Interest: No conflict of interest was declared by the authors.

Author Contributions: Concept – ME, CT, BT, AB; Supervision – BT, AB; Materials – ME, CT, PE, AB**;** Data Collection and/or Processing – PE, AB, HK, MOT; Analysis and/or Interpretation – ME, CT, PE, AB; Writing – ME, PE, BT.

Peer-review: Externally peer-reviewed.

Financial Support: This research was funded by Bursa Uludag University Scientific Research Projects Coordination Unit (TAY-2022-584).

Acknowledgements: We greatly acknowledge to the Scientific and Technological Research Council of Turkey (TÜBİTAK) for supporting author Cagla Tekin within the scope of the 2211-A doctoral program scholarship. We thanks to Prof. Dr. Şükrü Yıldırım from Istanbul Maltepe University, Prof. Dr. Şahsine Tolunay and Assoc. Prof. Dr. Mine Ozsen from Bursa Uludag University for their support in IHC analysis. We would also like to thanks to Prof. Dr. Bahattin Hakyemez for special assistance with MRI imaging.

REFERENCES

1. Ostrom QT, Cioffi G, Gittleman H, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012-2016. Neuro Oncol. 2019;21:v1-v100. doi:10.1093/neuonc/noz150
2. Xu C, Yuan X, Hou P, et al. Development of glioblastoma organoids and their applications in personalized therapy. Cancer biology & medicine. 2021;20(5), 353–368. doi:10.20892/j.issn.2095-3941.2023.0061
3. Neftel C, Laffy J, Filbin MG, et al. An integrative model of cellular states, plasticity, and genetics for glioblastoma. Cell. 2019;178:835-849. doi:10.1016/j.cell.2019.06.024

4. Haddad AF, Young JS, Amara D, et al. Mouse models of glioblastoma for the evaluation of novel therapeutic strategies. Neuro-Oncol Adv. 2021;3:vdab100. doi:10.1093/noajnl/vdab100
5. Zhang C, Jin M, Zhao J, Chen J, Jin W. Organoid models of glioblastoma: advances, applications and challenges. American journal of cancer research. 2020;10(8):2242-2257.
6. Zhang DY, Song H, Ming G. Modeling neurological disorders using brain organoids. Semin Cell Dev Biol. 2021;111:4-14. doi:10.1016/j.semcdb.2020.05.026
7. Veninga V, Voest EE. Tumor organoids: opportunities and challenges to guide precision medicine. Cancer Cell. 2021;39:1190-201. doi:10.1016/j.ccell.2021.07.020
8. Xu H, Jiao D, Liu A, Wu K. Tumor organoids: applications in cancer modeling and potentials in precision medicine. J Hematol Oncol. 2022;15:1-20. doi:10.1186/s13045-022-01278-4
9. Jin MZ, Han RR, Qiu GZ, Ju XC, Lou G, Jin WL. Organoids: an intermediate modeling platform in precision oncology. Cancer Lett. 2018;414:174-180. doi:10.1016/j.canlet.2017.11.021
10. Behjati S, Huch M, van Boxtel R, et al. Genome sequencing of normal cells reveals developmental lineages and mutational processes. Nature. 2014;513:422-425. doi:10.1038/nature13448
11. Duarte AA, Gogola E, Sachs N, et al. BRCA-deficient mouse mammary tumor organoids to study cancer-drug resistance. Nat Methods. 2018;15:134–140. doi:10.1038/nmeth.4535
12. Jacob F, Salinas RD, Zhang DY, et al. A patient-derived glioblastoma organoid model and biobank recapitulates inter- and intra-tumoral heterogeneity. Cell. 2020;180:188-204.e22. doi:10.1016/j.cell.2019.11.036
13. Qin LX, Tang ZY, Ma ZC, et al. P53 immunohistochemical scoring: an independent prognostic marker for patients after hepatocellular carcinoma resection. World J Gastroenterol. 2002;8(3):459-463. doi:10.3748/wjg.v8.i3.459
14. Wang C, Sun M, Shao C, et al. A multidimensional atlas of human glioblastoma-like organoids reveals highly coordinated molecular networks and effective drugs. npj Precis. Onc. 2024;8:19. doi:10.1038/s41698-024-00500-5
15. Moscow JA, Fojo T, Schilsky RL. The evidence framework for precision cancer medicine. Nat. Rev. Clin. Oncol. 2018;15, 183–192. doi:10.1038/nrclinonc.2017.186
16. Marquart J, Chen EY, Prasad V. Estimation of the percentage of US patients with cancer who benefit from genome-driven oncology. JAMA Oncol. 2018;4:1093-1098. doi:10.1001/jamaoncol.2018.1660
17. Brennan CW, Verhaak RG, McKenna A, et al. TCGA Research Network: The somatic genomic landscape of glioblastoma. Cell. 2013;24;157(3):753. doi:10.1016/j.cell.2013.09.034
18. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. Nature. 2008;455(7216):1061–1068. doi:10.1038/nature07385
19. Louis DN, Perry A, Wesseling P,et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. Neuro Oncol. 2021;23(8):1231-1251. doi:10.1093/neuonc/noab106
20. Wang LB, Karpova A, Gritsenko MA, et al. Clinical Proteomic Tumor Analysis Consortium: Proteogenomic and metabolomic characterization of human glioblastoma. Cancer Cell. 2021;39(4):509-528.e20. doi:10.1016/j.ccell.2021.01.006
21. Venteicher AS, Tirosh I, Hebert C, et al. Decoupling genetics, lineages, and microenvironment in IDH-mutant gliomas by single-cell RNA-seq. Science. 2017;355(6332):eaai8478. doi:10.1126/science.aai8478
22. Wang X, Sun Y, Zhang DY, Ming GL, Song H. Glioblastoma modeling with 3D organoids: progress and challenges. Oxf Open Neurosci. 2023;2:kvad008. doi:10.1093/oons/kvad008.
23. Shamir ER, Ewald AJ. Three-dimensional organotypic culture: experimental models of mammalian biology and disease. Nat Rev Mol Cell Biol. 2014;15(10):647-664. doi:10.1038/nrm3873
24. Hubert CG, Rivera M, Spangler LC, et al. A three-dimensional organoid culture system derived from human glioblastomas recapitulates the hypoxic gradients and cancer stem cell heterogeneity of tumors found in vivo. Cancer Res. 2016;76:2465-77. doi:10.1158/0008-5472.CAN-15-2402
25. Taurin S, Alzahrani R, Aloraibi S, Ashi L, Alharmi R, Hassani N. Patient-derived tumor organoids: A preclinical platform for personalized cancer therapy. Transl Oncol. 2025;51:102226. doi:10.1016/j.tranon.2024.102226
26. Bleijs M, van de Wetering M, Clevers H, Drost J. Xenograft and organoid model systems in cancer research. EMBO J. 2019;38(15):e101654. doi: 10.15252/embj.2019101654