### Abstract

Glialbloma (GBM) has a median survival of less than 2 years due to intratumoral heterogeneity, diffuse infiltrations of adjacent brain tissue, and a lack of effective therapies. Development of more efficacious therapies will require better GBM models for the testing and identification of novel agents. Towards this end, we have successfully developed a GBM 3D tissue model that can provide in vitro, patient-specific compound screening. Stable populations of glioma stem cells (GSC) from 24 of 41 patient samples have been successfully established and cultured long-term with minimal changes. To confirm stemness of the GSC population, we have successfully established a limiting-dilution series within SCID/Bliss mice and characterized the resultant tumors. 4 of these lines have been used to establish patient-derived xenograft (PDx) models in mice. The original, primary patient tissue established GSC populations, and the resultant PDX tissues have been characterized by flow cytometry, IHC, RNA expression, NGS, and MGMT methylation status. With the goal of better modeling the patient tumor tissue in vitro, our GSC populations have also been used to establish complex microtumors within the KIYATEC 3DKUBE® perfusion system, consisting of monoculture GSCs, GSCs co-cultured with human brain endothelial cells (HBECs), and GBSCs co-cultured with HBECs and CD34+ peripheral blood mononuclear cells. Our monoculture microtumors consisting of only GSCs show a maintenance of GSC markers Nestin and Sox2 by both IHC and qRT-PCR. Interestingly, when these cells are produced to PDX, they up-regulate GFAP as a marker of differentiation that is not observed in the mouse or human monoculture microtumors. We have shown these 3D models to be viable for more than 1 month in perfusion and to be effective models for drug compound screening by dosing the microtumors on a weekly basis with temozolomide (TMZ). We have correlated TMZ response to MGMT methylation as reported both clinically and measured in vitro. Finally, in vitro drug response has been compared to both matched PDX in vivo drug response, and the patient’s clinical response to TMZ and MGMT methylation. Our data supports that this complex, 3D, patient-derived GBM model can be used to effectively screen, identify and characterize novel treatments of GBM.

### Methods

**Clinical Drug Response**

- **Clinical Drug Response**
  - **Tissue Samples**
  - **Patient-Derived Xenograft (PDX)**
  - **Ex vivo Drug Response**
  - **3D Drug Response Assay**
  - **Drug Response**

**Sensitivity to TMZ Modulated by Microenvironment Complexity**

- **BNA17**
  - **Microenvironment**
  - **Drug Response**
  - **Perfusion Microtumor Drug Response**

**Characterization of Cultured GSC Neurospheres**

- **H&E**
- **Ki67**
- **Nestin**
- **SOX2**
- **GFAP**

**Correlative Drug Response in PDX Models**

- **BNA17**
  - **Stage IV GBM**
  - **Stage IV Glioblastoma**
  - **BNA19**
  - **BNA31**

### Conclusions

- **Glioma stem cells that maintain stemness during long-term can be consistently isolated and cultured from GBM patient samples**
- **Patient-derived GSCs can be cultured in 3DKUBE® system alone or as a complex microtumor (with or without drug treatment) for more than 1 month**
- **3DKUBE® TMZ drug responsiveness matches clinical and PDX outcomes, suggesting that this complex culture system could be used to screen and personalize GBM patient treatments**

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