Profiling Patient-Specific Glioblastoma Drug Response In Vitro Using Complex 3D Microtumors

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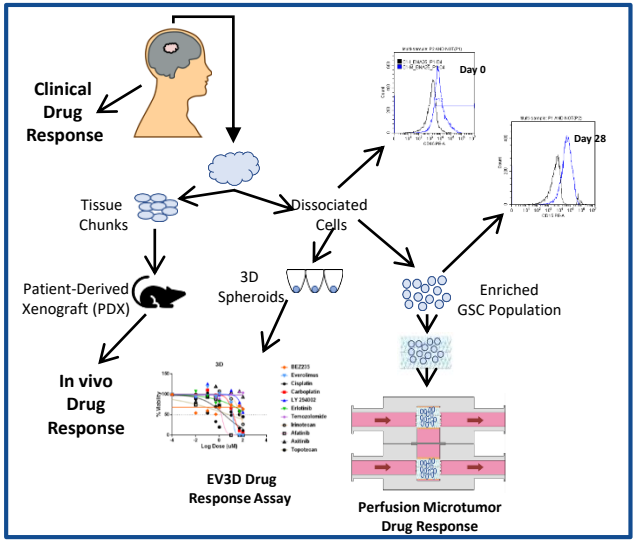
Abstract

Glioblastoma (GBM) has a median survival of less than 2 years due to intra-tumoral 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/Bg 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 GSCs co-cultured with HBECs and CD14+ peripheral blood mononuclear cells. Our monoculture microtumors consisting of only GSCs show a maintenance of GSC markers Nestin and Sox2 by both IHC and mRNA. Interestingly, when these cells are used to produce PDX, they up-regulate GFAP as a marker of differentiation that is not observed in the neurosphere or monoculture microtumor cultures. 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



Sensitivity to TMZ Modulated by Microenvironment Complexity

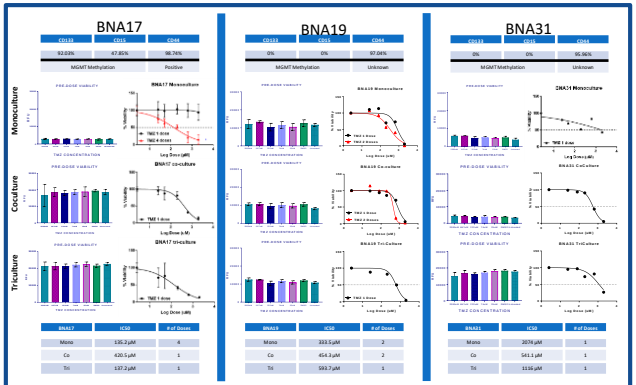


Figure 1. Microtumor dosing with Temozolomide (TMZ). Three different types of microtumors were created: GSC only (monoculture), GSC + HBEC (co-culture), and GSC + HBEC + CD14+ PBMC (triculture). GSC populations were characterized by flow cytometry for the presence of known stem cell markers CD133, CD15, and CD44. Methylation status of MGMT was reported from clinical pathology of the primary tumors. Viability of the microtumors was assessed with PrestoBlue prior to drug addition and throughout experiments. Microtumors were treated with TMZ on day 1 and perfused for 4 days, then changed to fresh media for 3 days. Every 7th day the microtumors were collected for viability testing. The microtumors were exposed to TMZ under perfusion until the viability of the highest-dose cohorts was too low to continue. N ≥ 4, lines represent non-linear regression, and error bars represent standard deviation.

Characterization of Cultured GSC Neurospheres

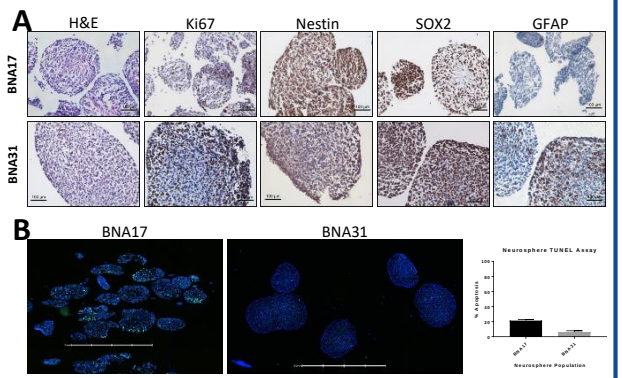


Figure 3. Enriched GSC population characterization from neurospheres. GSCs propagate as neurospheres (NS). NS were fixed and embedded in paraffin, then cut into 5µm sections. Representative images are used for all panels. (A) NS sections were stained with antibodies against proliferation marker Ki67, neural stem cell marker Nestin, stem cell marker SOX2, and neural differentiation marker GFAP. H&E staining was used to delineate the structure of the NS. (B) The amount and localization (if any) of apoptotic cells was determined with a fluorimetric TUNEL assay. BNA17 NS used in the assay were between 300-600 µm in size; BNA31 NS were >1 mm in size. Images were acquired and analyzed using Nexcelom Celigo. BNA17 was 21.2% positive for apoptotic cells and BNA31 was 6.2% positive. TUNEL assay n = 3.

Sensitivity to Axitinib Modulated by Microenvironment Complexity

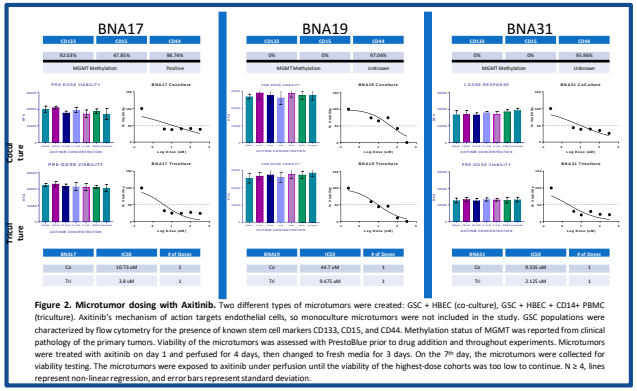


Figure 2. Microtumor dosing with Axitinib. Two different types of microtumors were created: GSC + HBEC (co-culture), GSC + HBEC + CD14+ PBMC (triculture). Axitinib's mechanism of action targets endothelial cells, so monoculture microtumors were not included in the study. GSC populations were characterized by flow cytometry for the presence of known stem cell markers CD133, CD15, and CD44. Methylation status of MGMT was reported from clinical pathology of the primary tumors. Viability of the microtumors was assessed with PrestoBlue prior to drug addition and throughout experiments. Microtumors were treated with axitinib on day 1 and perfused for 4 days, then changed to fresh media for 3 days. On the 7th day, the microtumors were collected for viability testing. The microtumors were exposed to axitinib under perfusion until the viability of the highest-dose cohorts was too low to continue. N ≥ 4, lines represent non-linear regression, and error bars represent standard deviation.

Correlative Drug Response in PDX Models

A

Patient	Tumor Type	Clinical MGMT Methylation	Treatment	Progression Free Survival	Ex vivo MGMT Methylation	Ex vivo TMZ response
BNA17	Stage IV GBM	Methylated	TMZ	>12 months	Methylated	137 µM
BNA19	Stage IV Gliosarcoma	Unknown	None	2 months	Unmethylated	594 µM
BNA31	Stage IV GBM	Unknown	TMZ	>16 months	Unmethylated	1116 µM

B

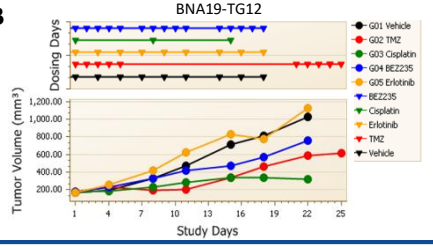


Figure 4: Correlative Response in the Clinic and in PDX. (A) Summary of clinical information, including response to TMZ. (B) PDX response to a number of agents, including TMZ. The top graph indicates the dosing schedule for each drug The bottom graph shows PDX tumor growth in mice treated according to the dosing schedule. When it became obvious that a response was not going to be detected in the TMZ-dosed cohort, StemMed increased the dosing schedule. General toxicity was observed in the cisplatin group.

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

This work is supported by NCI SBIR Contract #: HHSN261201400019C

With Special Thanks to the Biorepository

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