Evaluating Invasion of Glioblastoma Cells in Rat Cortical Spheroids

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Introduction

- Glioblastoma (GBM) is highly aggressive and malignant primary brain tumor.
- At most 15 months of median survival rate even if patients undergo resection surgery, chemotherapy or radiation therapy.
- Suitable in-vitro models are necessary for translation of GBM research to clinical application.
- Traditional extracellular matrix (ECM) based GBM invasion assay[1,2] fails to mimic the complex invasion behaviors of GBM in the patients.
- Human induced pluripotent stem cell (iPSC) derived organoids possess differentiated neurons only in the superficial layer, with the center filled with progenitor cells[3].

Objectives

- We use primary rat cells to create robust and fully differentiated cortical spheroids and use them to evaluate GBM invasiveness.
- Using patient derived GBM cells, we developed two assays to evaluate the infiltrative behavior of GBM cells in rat cortical spheroids that is also observed in human specimens[3]. We added dissociated GBM cells or GBM spheroids to cortical spheroids in assays 1 and 2, respectively.

Methods

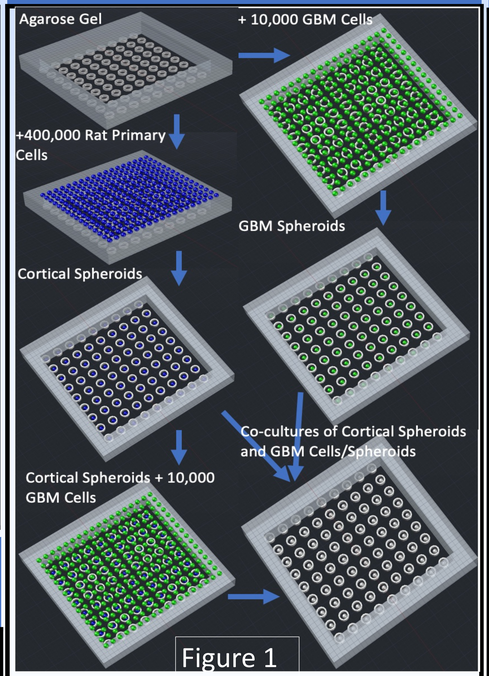

Figure 1

- Neurons were transfected with jRGECO1a, which is a genetically encoded calcium indicator, and GBM cells were tagged with enhanced green fluorescent protein (eGFP) using pAAV virus (Addgene).
- Co-cultures were fixed on DIV 16, stained using anti-bodies to NeuN and GFP.

Results

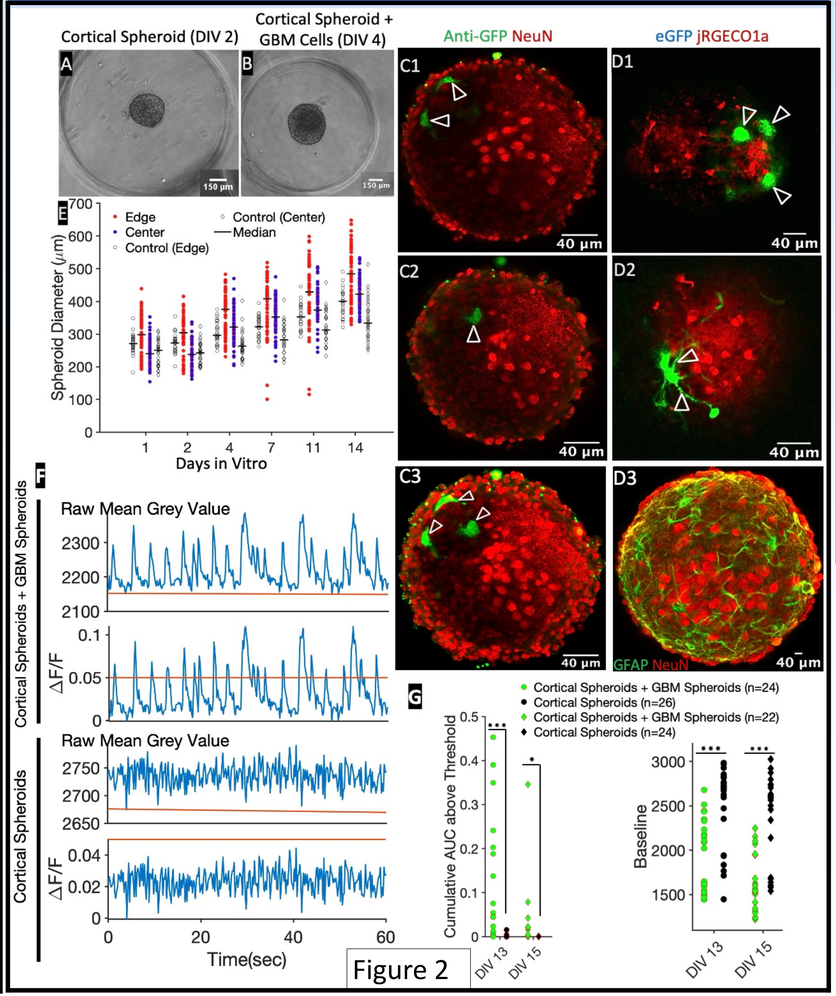

Figure 2

- Phase contrast imaging showed that GBM cells merged with cortical spheroids within two days (Fig. 2B).
- We report that spheroid diameter has increased on DIV 4 (Fig. 2B, E) because GBM cells were added to cortical spheroids after DIV 2.
- We noticed that median diameter of the spheroids at the edge were larger than the spheroids at the center of the agarose gels (Fig. 2E).
- Confocal imaging of both live (Fig. 2D1, D2) and fixed (Fig. 2C1, C2) samples showed that GBM cells were inside the cortical spheroid and near the neurons, but not mixed with them (Fig. 2C2, C3).
- Live confocal imaging also depicts the signature invasive protrusion of GBM cells (Fig. 2D2).
- Calcium imaging shows that co-cultures of cortical and GBM spheroids showed higher activity compared to cortical spheroids (Fig. 2F, G).

Conclusions

- Our work illustrates the highly invasive behavior of GBM cells.
- Finding these invasive characteristics in our in-vitro model, we can conclude that our assays can demonstrate reliable modeling of GBM invasion which researchers could use for future experiments.
- Compared to using iPSC derived organoids which sometimes takes 2 months of experimental timeline[3], our assay is significantly faster.

References:
1. Diao W. et al. Sci. Rep. 2019; 9.
2. Vinci M. et al. J. Vis. Exp. 2015; 52686
3. Goranci-Buzhala et al., 2020, Cell Reports (31)