Contrasting effects of 2D vs. 3D hydrogel microenvironments on the behavior of glioblastoma multiformes

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Statement of Purpose: Every year, ~ 22,500 individuals in the United States are diagnosed with brain cancer (annual incidence rate ~ 5 in 100,000 cases)[1], of which 70% are contributed by malignant gliomas. Glioblastoma Multiforme (GBM), the specific tumor investigated in this work, is a primary tumor of astrocytes (i.e., glial cells in the central nervous system). A characteristic feature of this primary brain tumor is its high invasion potential. Despite recent advancements in treatment techniques (e.g., chemotherapy), median survival rate for GBM patients remains dismally low (~12-15 months) [1]. This is, in part, a consequence of our poor understanding of the molecular pathogenesis of GBMs. To refine existing techniques and expand present therapeutic options, there is a need for new models to explain complex GBM migration behavior. Most existing models (e.g., scratch assay [4]) of tumor cell migration utilize 2D rigid substrates (e.g., plastic) that do not replicate the complex *in vivo* environment. In this work, we utilized Matrigel, an extract of Engelbreth-Holm-Swarm mouse sarcoma (rich in extracellular matrix components) as a model hydrogel system to investigate migration of GBM brain tumors. Compared to plastic, matrigel provides a less rigid mechanical microenvironment. Further, these tumors are believed to spread along basement membrane structures (e.g., glia limitans) [2] and matrigel is an excellent *in vitro* mimic for these structures. In addition, given that cell behavior is drastically altered in 3D environments [3]; we compared and contrasted GBM behavior in 2D vs. 3D matrigel microenvironments.

Methods: *OSU2 cell culture*: GBM cells were procured from primary brain tumor of a single human patient. Cells were sub-cultured for experimental use and developed into a robust OSU2 cell line. Briefly, patient derived tumors were washed thoroughly with cell culture media containing 200 unit penicillin, 200 µg streptomycin, & 0.5 µg/ml amphotericin B, digested by treatment with 200U/ml type 1A collagenase (4 h), centrifuged at 250g, 5 min and re-suspended in cell culture media containing 10% fetal bovine serum, 100unit penicillin, 100µg streptomycin, 0.25 µg/ml amphotericin B. The cell solution was then transferred into a petri-dish and incubated at 37°C, 5% CO_2. *2D vs 3D OSU2 culture using BD Matrigel*: For 3D cell culture in Matrigel, pre-stained OSU2 cells (~ 4000 cells/well) were mixed with matrigel (ice bath) at 50, 60, 70 and 80 % (v/v) matrigel concentrations. Solutions were allowed to gel at 37°C, 0.5h and then ~50µl media was added on top of the gels. For 2D culture, matrigel solutions at similar concentrations were allowed to gel at 37°C, 0.5h and pre-stained OSU2 cells (~ 4000 cells/well) were placed on top of hydrogels. *Histology*: Patient tumor samples containing tumor cells were stained using standard hemotoxylin and eosin (H & E) staining. Some samples were also stained with Nissl stain. *Microscopy*: OSU2 cells in matrigel, for both 2D and 3D culture were observed using confocal microscopy for 2 days.

Results: There were striking differences in OSU2 cell morphology when cultured on top versus inside the hydrogel. Counterintuitively, OSU2 cells on top of the hydrogel showed a distinct rounded morphology with very few processes, whereas cells encapsulated in the hydrogel spread out and extended long processes (for all range of matrigel concentrations). OSU2 cell behavior in 3D was similar to *in vivo* behavior as observed using histology (Figure 1, 2). In addition, video microscopy showed that OSU cells cultured in 3D were more likely to extend processes than cells cultured in 2D.

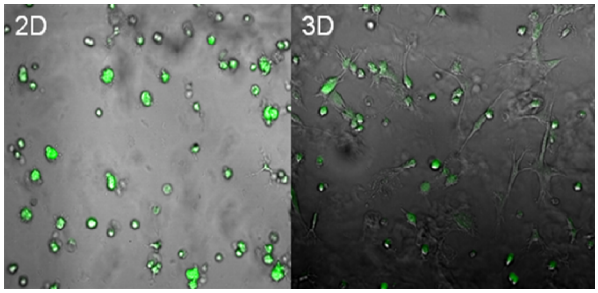

Figure 1. OSU2 cell morphology. (2D vs 3D culture in 70% v/v matrigel microenvironment (day 2)).

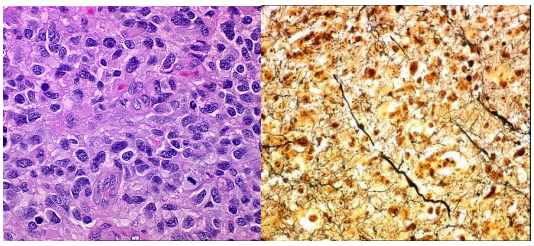

Figure 2: (left) GBM stained with H&E and (right) patient tumor cell morphology (Nissl stain) as visualized using histology.

Conclusions and Future Work: Clear differences in 2D vs. 3D environments reiterate the importance of 3D *in vitro* models. Although culturing cells on top of hydrogels is still considered 3D by many researchers, cell function is drastically altered in this environment and may not be representative of the *in vivo* situation. Future studies will further explore these differences using actin & glial fibrillary acidic protein (GFAP) staining. In addition, cell spreading will be quantified. Further, matrigel stiffness will be measured to directly correlate substrate modulus with cell behavior. Our *ultimate* goal is to develop an *in vitro* brain mimic that would permit testing of different migration hypotheses and recapitulate *in vivo* phenomena, reducing the need for animal studies.

References: [1] P.Y. Wen et al., *N Engl J Med*. 2008. **359** (5); 492-507. [2] A.P. Amar et al., *J Neurooncol*. 1994. **20** (1); 1-15. [3] E. Cukierman et al., *Science*. 2001. **294** (5547); 1708-1712. [4] A. Valster et al., *Methods*. 2005. **37** (2); 208-215.