In Vitro Model Of Glioblastoma To Study Subpopulation Induced Extracellular Matrix Changes

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Introduction

Glioblastoma (GBM) is a lethal, incurable Grade IV glioma. It comprises 48% of primary brain tumors and has a median patient lifespan of 14.5 months.[1] GBM is extremely heterogeneous and is defined by multiple genetically and phenotypically distinct subpopulations. The different subpopulations are often categorized as mesenchymal, proneural, classical, or neural. The relative distribution of each subpopulation changes over time and each subpopulation responds differently to treatment. Clinically, the classical subpopulation has been associated with tumor cell proliferation while the mesenchymal subpopulation has been associated with worse outcomes.

GBM cells secrete signaling molecules, cytokines, which leads to increased inflammation and impaired vessel function. These factors also increase tumor cell proliferation and promote remodeling of the extracellular matrix (ECM). Often, this modification is observed as an increase in the brain's local elastic moduli. A deeper understanding of each GBM subpopulation's role in this remodeling process will be useful to physicians, allowing them to predict changes to brain composition based on the makeup of each patients' unique tumor. In this study, 3D tumor organoids in which GBM cells are suspended in extracellular matrix (ECM) mimetic-hydrogels were used to study each subpopulation.

Project Goals and Objectives

- Develop 3D hydrogel systems capable of supporting different subpopulations
- Utilize the 3D hydrogel system to study GBM proliferation and ECM remodeling

Methods

- **Hydrogel Composition:** The 3D hydrogel used in this study consists of thiol modified hyaluronic acid (HA), thiol modified gelatin, and a poly(ethylene glycol) diacrylate (PEGDA) crosslinker. The hydrogels are crosslinked with UV light for 2 seconds.
- **GBM Cell Line Characterization:** Four distinct GBM cell lines (U87, U87 EGFR VIII, A172, U373) were chosen to represent genetically and phenotypically distinct populations. Organoids for each cell line were created by suspending 50,000 cells in a 10 µL hydrogel droplet and crosslinking with UV light. Viability was qualitatively assessed through a Live/Dead assay (n = 4). Images were acquired with the Nikon A1R live cell confocal microscope (OSU CMIF).
- **Drug Study:** Proliferation in response to common chemotherapeutic temozolomide (TMZ) was assessed using the CellTiter-Glo 3D Luminescent Cell Viability Assay (n = 4). Drug concentrations of 10 µM, 100 µM, and 1000 µM were tested along with a blank and 1% DMSO control. Assays were performed at multiple time points (days 1,4, and 7) to characterize changes over time. Drug concentrations were replenished on Day 4.
- **MMP Characterization:** Matrix metalloproteinase (MM activity was assessed using fluorescent peptide sensors from Leight Lab (n = 8).[2] These peptide sensors were incorporated into the hydrogels at a concentration of . Fluorescence was measured at 494 nm excitation and 521 nm emission. Protein expression of specific MMPs (MMP-2, MMP-9, MMP-13) was quantified through ELISAs (n = 3).

Results and Discussion

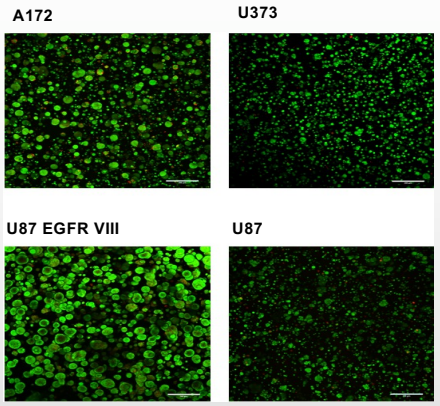


Figure 1: Day 7 Live/Dead Maximum Intensity Projection images. Green = Live, Red = Dead. Scale = 200 µm

- 3D hydrogel system displays high viability of different populations
- System can demonstrate differences in morphology

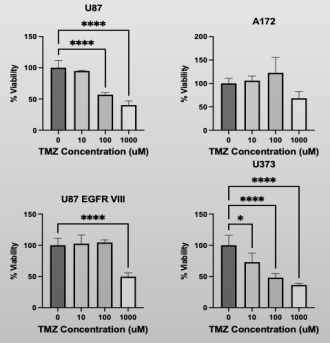
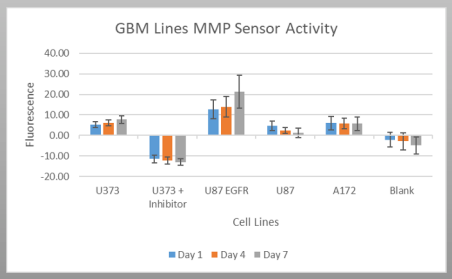


Figure 2: Day 7 ATP assay of GBM cell lines under various drug concentrations. Readings are standardized to the 1% DMSO control

- Hydrogel system can recapitulate heterogeneous drug response
- As evident by the decrease in viability in response to increasing TMZ concentrations



- Sensors are able to measure MMP activity (verified by MMP2 ELISA)
- Need to optimize procedure in order to avoid "fluorophore expiration"

Figure 3: Different GBM lines pan-MMP activity tracked through Leight Lab Sensor. Note: Readings were standardized to Day 0 and the inhibitor used was GM6001

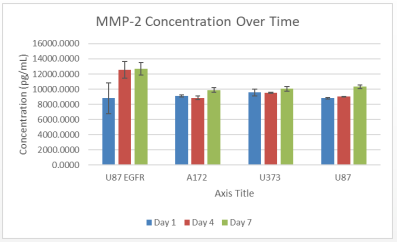


Figure 4: MMP-2 Concentration measured via ELISA

- Hydrogel system allows us to measure MMP-2 concentration
- MMP-2 is a matrix metalloproteinase which are defined by remodeling the ECM, showing this system's ability to measure ECM remodeling

- System is able to show difference in certain MMP concentrations (MMP-2 scale is 10 times greater than MMP-13 scale)
- Allows us to get a more MMP specific view than the pan-MMP activity from the fluorescent sensors

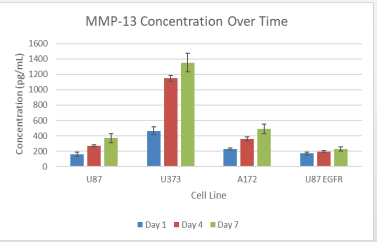


Figure 5: MMP-13 Concentration measured via ELISA

Conclusions and Future Directions

- 3D Hydrogel system supports different populations and allows us to study matrix remodeling in their respective systems
- Further characterization techniques will be employed to understand ECM rigidity and collagen production in these different cell line systems
- Patient derived tumor cells will be sorted into the four different subpopulations and will be characterized using the developed techniques

Acknowledgements

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References

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[2] Leight, J. L., Alge, D. L., Maier, A. J. & Anseth, K. S. Direct measurement of matrix metalloproteinase activity in 3D cellular microenvironments using a fluorogenic peptide substrate. Biomaterials 34, 7344- 7352, doi:10.1016/j.biomaterials.2013.06.023 (2013).