Brain Cancer Cell-Derived Matrix Alters Neural Astrocyte Migration and Metabolic Activity

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Statement of Purpose: The extracellular matrix (ECM) plays an instructive role during both physiology and disease. For example, the ECM secreted by tumor cells is compositionally distinct from the native tissue directly supports cancer cell invasion and disease progression[1]. In glioblastoma, the most aggressive primary brain tumor, cancer-derived ECM molecules recruit neural and immune cells within the adjacent tumor micro-environment and promote immuno-suppressive, pro-tumor phenotypes. Better characterization of cancer ECM could therefore lead to new therapies for preventing tumor microenvironment education or potentially promoting immunosuppression or regeneration after tissue injury. Cancer ECM is often studied a single molecule at a time or within the complex, uncontrollable environment of small animal tissues. An alternative approach is adding macromolecular crowding agents into traditional cell culture to better mimic a crowded, tissue-like environment and enhance ECM deposition. We report macromolecular crowding (MMC) as an effective strategy to generate glioma-derived matrices for downstream analyses and evaluating effects on migration and metabolism of cells in the brain tumor microenvironment, namely astrocytes.

Methods: Mouse glioblastoma cell line GL261 was plated at 17,700 cells/cm^2 in a 12-well plate, cultured to confluency, then for 1 week in DMEM+10% FBS with or without the MMC agents: carrageenan, Ficoll 70/400, or high molecular weight hyaluronic acid. MMC agents were used at 0.075 mg/mL, 37.5/25 mg/mL, and 0.5 mg/mL, respectively. We measured deposition of total collagen and sulfated glycosaminoglycans using quantitative assays and other matrix components semi-quantitatively by immunostaining. To isolate the matrix, we decellularized the wells by adding 10 µm raptinal, a rapid-acting apoptosis agent[4], for 2 hours at 37°C. The wells were washed three times with 1X PBS for 10 mins each, followed by a 2-day wash in 1X PBS. Human cortical astrocytes (Sciencell) were grown to confluency on either Ficoll-crowded GL261 matrix or collagen I-coated controls in Astrocyte Medium. A 10 µL pipet tip was used to generate a scratch 'wound'. The wound area was imaged every 4 hours and quantified. Samples then received fresh medium with 1X alamarBlue HS, were incubated for 4 hours at 37°C and analyzed on a BioTek Cytation 3 plate reader. Statistics were performed using Graphpad Prism version 9.2.

Results: We evaluated ECM deposition in cultures of GL261 glioma cells with and without macromolecular crowding (cartoon shown in **Fig. 1A**). Based on immunofluorescence staining of untreated controls (**Fig. 1B**) and MMC samples, only Ficoll (**Fig. 1C**) increased collagen type I. Total collagen content, as measure by quantitative assay, was significantly increased in the presence of both carrageenan and Ficoll (*p<0.05; **Fig. 1D**). We find these two crowding agents also increased accumulation of fibronectin, based on semi-quantitative immunostaining (not shown). All three MMC agents increase glycosaminoglycan content (not shown). We chose to focus on Ficoll crowding for future studies since this agent consistently performed best. Cell cultures crowded with Ficoll for 1 week were decellularized by treating with

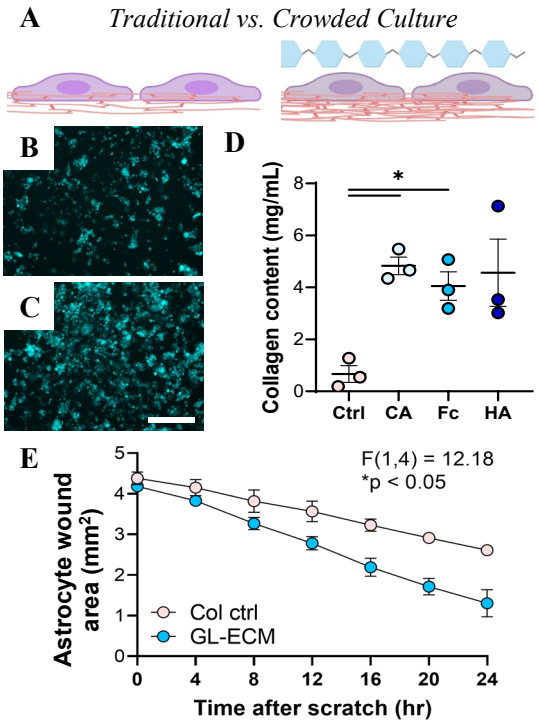

***Figure 1**: A) Cartoon of traditional vs. crowded culture, from Biorender.com. B-C) Collagen I immunostaining after 1 wk of no treatment (B) or Ficoll crowding (C). D) Quantification of total collagen content. E) Scratch wound area over 24 hrs in confluent astrocyte monolayers. *p<0.05.*

the small molecule raptinal, a rapid-acting apoptosis drug[4]. The decellularized wells were then seeded with human cortical astrocytes towards evaluating the neural cell response to the cancer-derived matrix. Wells coated with rat tail collagen I were used as controls, as this substrate is used for routine cell propagation. Astrocytes migrated significantly faster (*p<0.05) on GL261-produced ECM (GL-ECM) versus on collagen (**Fig. 1E**), as evidenced by a faster rate of 'wound' area closure. In support of this result, astrocytes on GL-ECM were also more metabolically active and had larger, less organized cellular focal adhesions (data not shown).

Conclusions: Macromolecular crowding was found to be an effective technique to increase accumulation of glioma-derived ECM. The resulting matrix can then be used to study effects of cancer matrix on neural cells in the tumor micro-environment. Here, glioma-derived matrices increased astrocyte metabolism and migration related to closure of a scratch wound. These results may have implications for understanding the role of astrocytes in tumor progression and suggests a role for using cancer-derived matrix to promote normal wound healing. Further studies could explore using MMC to study the role of glioma ECM in biasing neural cells toward tumor-promoting, immunosuppressive phenotypes.

References:

[1](Marinkovic et al., JBMR Part A. 2021; 109:1803-1811.)
[2](Gaspar et al., Acta Biomaterialia. 2019; 88: 197-210.)
[3](Shendi et al., Acta Biomaterialia, 2019; 100: 292-305.)
[4](Palchaudhuri et al., Cell Rep. 2015; 13:2027-2036.)