Studying Matrix-Derived Metastatic Cues in a Biomimetic Hydrogel System

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Statement of Purpose: Key aspects of the tumor environment that promote metastasis have not been fully identified. A cancer model cell line from a subcutaneous metastasis of mouse lung adenocarcinoma cells (344.SQ) forms lumenized epithelial structures when cultured in Matrigel-based matrices and can exhibit an epithelial-to-mesenchymal transition (EMT) under appropriate signaling conditions[1]. Using a tunable PEG-based hydrogel system, we explored key factors of the tumor environment that influence EMT including 344.SQ "scribble" cells with a knockdown of a polarity gene (scrib), substrate mechanical properties, proteolytic degradation, and adhesive peptides.

Methods: Peptide Conjugation: Bioactive peptides were reacted with monoacrylate-PEG succinimidyl carboxymethyl (PEG-SCM) for incorporation into hydrogels, including: PEG-RGDS for cell adhesion, PEG-IKVAV and PEG-LQVQLSIR as laminin peptides, and PEG-PQ-PEG to form the hydrogel backbone with a protease-sensitive sequence (GGGPQGIWGQGK) incorporated for cell-mediated degradation of hydrogels.
Hydrogel Formation: Hydrogels were prepared by photocrosslinking PEG-PQ-PEG using 1 mM eosin Y in the precursor solution and exposing to white light for 30 seconds. 3 mM PEG-RGDS was added unless otherwise noted, and 344.SQ wild type or scribble knockdown cells were encapsualted at 3,000 cells/µl. Substrate stiffness was altered by varying PEG-PQ-PEG concentration (5%, 7.5%, 10%). In some studies, PEG-IKVAV or PEG-LQVQLSIR was added at 3 mM.
Imaging: Lumen formation by encapsulated cells was imaged with a Zeiss Axiovert 135 inverted fluorescent microscope. Cells were fixed and stained for polarity markers with primary antibodies directed against ZO-1 for tight junctions (apical surface), α6-integrin (basolateral), and TOPRO-3 (nucleus).

Results: The use of a cell-adhesive and proteolytically degradable PEG-based matrix allowed formation of lumenized spherical structures from wild type cells with similar morphology observed in Matrigel[1] (Fig. 1). Scribble cells with a knockdown of a polarity gene did not form lumenized structures. Altering substrate mechanical properties by varying polymer concentration led to differences in structure morphology with larger structures forming in softer hydrogels (Fig. 2).
The addition of immobilized PEG-IKVAV and PEG-LQVQLSR to cultures led to no clear alteration in structure morphology at the concentration tested with all cultures forming typical lumenized spheres (Fig. 3).
Lumenized structures formed by 344.SQ wildtype cells were stained for polarity markers and were found to exhibit apical-basolateral polarity both with laminin peptides and with RGDS (Fig. 4).

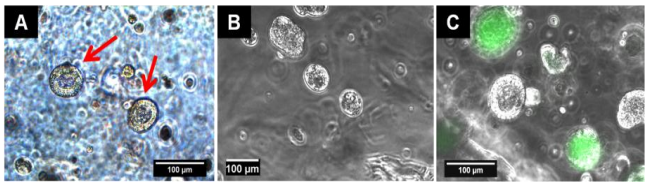

Fig 1: **344.SQ in PEG-based Matrix**. (A) Wild type cells form lumenized spherical structures (arrows). (B) Structures with scribble cells do not lumenized and (C) co-cultures show lumenized wildtype cells adjacent to non-lumenized, GFP-labeled scribble structures.

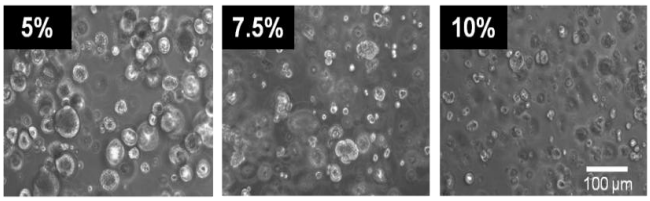

Fig 2: Altered Mechanical Properties Affected Morphology
Substrate stiffness altered structure morphology as larger structures formed in softer hydrogels (5%) compared to stiffer hydrogels (10%).

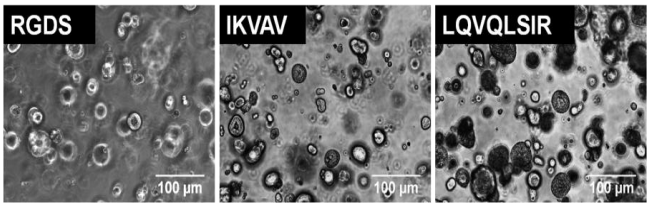

Fig 3: Cellular Morphology Unaltered by Addition of Laminin Peptides
Lumenized epithelial structures formed in samples with laminin peptides covalently incorporated with no observed change relative to RGD-only hydrogels at the concentration tested.

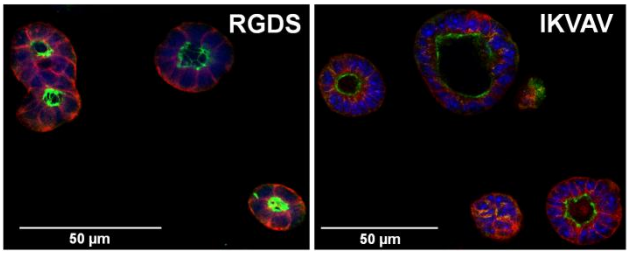

Fig 4: Lumenized Structures Exhibited Polarity
Lumenized epithelial structures exhibited apical-basolateral polarity with ZO-1 marking apical tight junctions (green) and α6-integrin marking the basolateral surface (red). TOPRO-3 (blue) nuclear counterstain.

Conclusions: 344.SQ lung cancer cells form lumenized epithelial structures with organized polarity when encapsulated in PEG-based matrices. The addition of matrix-tethered peptides derived from laminin, an ECM protein thought to be important to native tumor environment signaling, did not profoundly alter morphology, but altering hydrogel mechanical properties did induce morphologic differences in structure size.

References:

1. Gibbons, D.L. 2009. *Genes and Dev*. 23: 2140-2151.