**Assessing Influences of Vascular Cells on the Behavior of Lung Adenocarcinoma in a Three-dimensional Biomimetic Tumor Angiogenesis Model**

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**Statement of Purpose:** Tumor angiogenesis is the process of blood vessel recruitment to a tumor site in order to facilitate tumor growth. This blood vessel network also acts as a route by which cancer cells can metastasize to distant organs and is the primary route by which drugs are delivered for therapy [2]. Its importance in cancer has thus led to the need for advanced approaches to study the process that allow for control over cell-cell and cell-matrix interactions. Meeting this need would enable researchers to elucidate key mechanisms and identify potential targets for therapies. Since tissue engineering approaches offer solutions to meet these needs, they were employed to create a 3D *in vitro* model. The model is composed of tumor and vessel cells that are encapsulated in bioactive poly(ethylene glycol) (PEG) hydrogels, which have tunable bioactivity and mechanical properties. This model was used to study vascular cell soluble and cell-cell influences on the behavior of a mouse model of metastatic lung adenocarcinoma (344SQ).

**Methods:** Bioactive PEG hydrogels were constructed by incorporating peptides, RGDS to allow for cell adhesion to the matrix, and GGGPQGIWGQGK (PQ) to allow for cell-mediated matrix degradation, into the matrix. Briefly, peptides were reacted with a heterobifunctional PEG derivative, PEG-succinimidyl valerate, in an acqueous buffer to yield PEG-RGDS and PEG-PQ-PEG. A dual hydrogel set-up was used for the tumor angiogenesis model, whereby 2 pre-polymer solutions were prepared: a cancer component and a vessel component (Fig. 1A).

Cancer component: 344SQ encapsulated at 2 $x10^6$ cells/mL. 344SQ have been shown previously to form epithelial lumenized spheres in PEG hydrogels and respond to treatment with transforming growth factor beta 1 (TGF-β1) by undergoing an epithelial-to-mesenchymal transition (EMT) and losing polarity and lumenization [1].

Vessel component: human umbilical vein endothelial cells (HUVEC) and human vascular pericytes (HVP) encapsulated at 30 $x10^6$ cells/mL in a 4:1 ratio, respectively. Cells were incorporated into the pre-polymer solutions composed of a photoinitiator, Eosin Y, 3% PEG-PQ-PEG, and 3.5 mM PEG-RGDS. The cancer solution was exposed to white light for crosslinking for 10 seconds followed by addition of the vessel component and a second exposure for 30 seconds. Cells were cultured in hydrogels for 7-10 days, then fixed, stained and imaged on a Zeiss LSM 510 inverted confocal microscope. ELISAs were performed to assess angiogenic factor secretion from 344SQ cells (VEGF and PDGF-BB). Secretion of TGF-β1, a potent stimulator of EMT, by HUVEC and HVP was also assessed via ELISA.

**Results:** Interactions of vessel cells with 344SQ in the tumor angiogenesis model led to changes in cancer cell morphology not seen previously with this cell type when

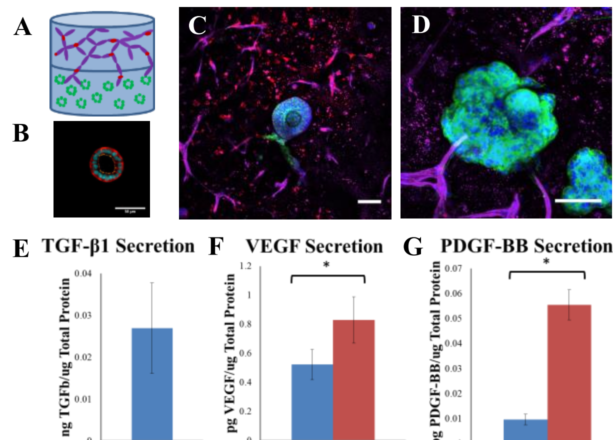


Figure 1. (A) Schematic of tumor angiogenesis model (344SQ = green, HUVEC = purple, HVP = red), (B) 344SQ sphere in a bioactive PEG hydrogel with polarity markers, ZO-1 (yellow) and β-catenin (red) (sb = 50 µm)[1], (C and D) images of vessel-cancer interactions in tumor angiogenesis model with DAPI (blue), phalloidin (green), human nuclear antibody (red), and PECAM (purple) (sb = 100 µm), (E) TGF-β1 secretion from HUVEC and HVP, (F) VEGF secretion from 344SQ (blue = untreated, red = TGF-β1 treated, $*p < 0.05$) and (G) PDGF-BB secretion from 344SQ (blue = untreated, red = TGF-β1 treated, $*p < 0.05$).

encapsulated alone (Fig. 1B). 344SQ morphology changes largely varied when interacting with vessel cells and observations have included cancer cells forming projections from their original spheres and increasing in size (Fig. 1C and 1D). Because 344SQ epithelial morphology has been previously perturbed with TGF-β1 treatment, TGF-β1 secretion from HUVEC and HVP hydrogels was assessed via ELISA [1]. Results show an average TGF-β1 concentration of 26.9 pg/µg total protein, which is more than 5 times that used in previous studies to elicit an EMT response from 344SQ (Fig. 1E) [1]. Angiogenic factor secretion from 344SQ was also assessed. Results showed that 344SQ secrete VEGF and PDGF-BB and there is a 12-fold increase in VEGF secretion and a 5-fold increase in PDGF-BB secretion when treated with TGF-β1 (Fig. 1F and 1G). Thus both vessel cells and cancer cells are involved in paracrine signaling that could account for morphological changes observed in the model.

**Conclusions:** This research shows the use of a 3D *in vitro* tumor angiogenesis assay to begin to elucidate paracrine signaling and direct cell-cell contact interactions between vessel cells and cancer cells in a PEG hydrogel system that allows for a high amount of control over cell-cell and cell-matrix interactions.

**References:** 1. Gill, BJ. Cancer Res. 2012; 72: 6013-6023. 2. Verbridge, SS. Tissue Eng. 2010; 16: 2147-2152.

**Abstract #348**
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