A 3D Hydrogel Model of Tumor Angiogenesis to Study the Role of Vascular Cells in Tumor Progression

Laila C. Roudsari[1], Amber S. Witt[1], Jonathan M. Kurie[2], Jennifer L. West[1]
[1]Duke University, Durham, NC, [2]The University of Texas MD Anderson Cancer Center, Houston, TX

Statement of Purpose: Cancer cells require vasculature to sustain growth and the process by which blood vessels are recruited to a tumor site is known as tumor angiogenesis. Tumor vasculature is also the route cancer cells use to metastasize to distant organs, which is the leading cause of death from cancer. The importance of this process has led to the development of anti-angiogenic therapies. However, minimal clinical success has exposed the limitations of current methods to study tumor angiogenesis, making clear the need for approaches that better mimic the in vivo microenvironment. Tissue engineering gives us a unique toolset for simulating tissue structure and function, so we have employed these strategies to develop a 3D biomimetic vascularized tumor model. Our model is a dual layer poly(ethylene glycol) (PEG) hydrogel system comprised of a cancer cell layer containing a mouse model of metastatic lung adenocarcinoma known as 344SQ, and a vascular cell layer containing endothelial cells and pericytes. The use of PEG hydrogels gives us freedom to customize the bioactivity of the system and control over cellular organization into distinct hydrogel layers. This system has allowed us to explore how vascular cells can directly induce changes in cancer cells, beyond delivering nutrients and removing waste as observed in vivo. Our goal is to elucidate the role of vascular cells in tumor progression.

Methods: Transwell invasion assay: Human umbilical vein endothelial cells (HUVEC) were seeded at a concentration of $1.5x10^5$ cells/well in a 24-well plate. 344SQ ($5x10^4$ cells) were seeded on a transwell membrane in the well plate and allowed to invade for 16 hours. Cells that remained on the top of the membrane were removed and invaded cells were fixed, stained, and counted. Control wells contained HUVEC media without cells. Materials: Bioactivity was incorporated into PEG hydrogels by conjugation of a heterobifunctional PEG derivative (acrylate-PEG-succinimidyl valerate) to 2 peptide sequences: RGDS, to allow for cell adhesion to the matrix, and GGGPQGIWGQGK (PQ), to allow cell degradation. Conjugation was performed in an aqueous buffer to yield PEG-RGDS and PEG-PQ-PEG. Cells were encapsulated in PEG hydrogels with a composition of 4% w/v PEG-PQ-PEG and 3.5 mM PEG-RGDS, using Eosin Y as the photoinitiator and white light for crosslinking. Cells: The vascular cell component was comprised of HUVEC and human vascular pericytes (HVP) at a concentration of $30x10^6$ cells/mL and a ratio of 4:1, respectively. The cancer cell component, 344SQ, were incorporated at a concentration of $1.5x10^6$ cells/mL. Model: For the tumor model, 344SQ were suspended in the prepolymer solution, added to a PDMS well, and partially polymerized for 15 seconds. This was followed by suspension of the vascular cells in prepolymer solution, which was added to a second PDMS well that was stacked onto the original well. The entire construct was polymerized for 30 seconds, followed by removal of the PDMS wells (Fig. 1B). To assess changes in cancer cell morphology correlated to distance from the interface, the cancer hydrogel was doped with fluorescently tagged PEG-RGDS. The hydrogels were cultured for 7-14 days, followed by immunostaining, imaging, and analysis.

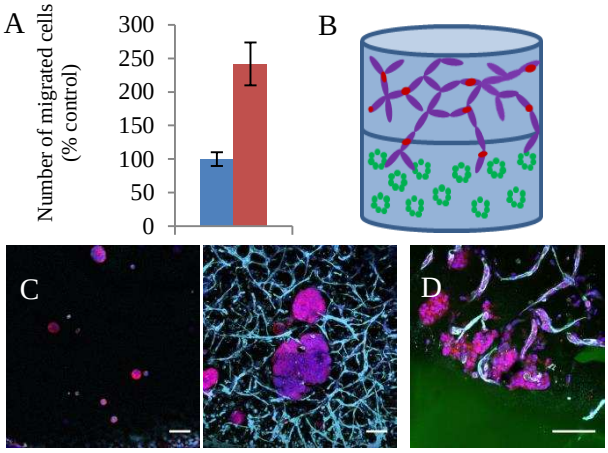

Figure 1. (A) 344SQ invasion in co-culture with HUVEC (blue = HUVEC media, red = seeded HUVEC), (B) tumor angiogenesis model schematic (HUVEC = purple, HVP = red, 344SQ = blue), (C) images of 344SQ spheres in the bulk cancer gel (left) and at the interface (right) (phalloidin = red, PECAM and human nuclear antibody = cyan, DAPI = blue), and (D) a cross sectional image of the interface (PEG-RGDS in cancer gel = green) (scale bars = 100 μm).

Results: 344SQ were more invasive when co-cultured with HUVEC (Fig. 1A). This is evidence that the presence of endothelial cells can alter the invasive nature of lung adenocarcinoma cells. When encapsulated alone in PEG hydrogels, 344SQ form spheres with epithelial polarity that mimic the lung acini while HUVEC and HVP form tubule networks that mimic native microvascular networks. In the tumor angiogenesis model, tubule networks were observed at day 12 in the vascular hydrogel layer (Fig. 1C). Also, 344SQ far from the interface formed sphere structures similar to those found when encapsulated alone (Fig. 1C). However, at the interface between the 2 layers, an overall increase in sphere area of the cancer cells was observed, as well as instances of invasive cancer cells not exhibiting epithelial morphology (Fig. 1C). It is evident that the proximity of cancer cells to vascular cells has an impact on cancer cell behavior. Direct interaction between vascular cells and cancer cell aggregates at the interface was also observed when looking at a cross section of the hydrogel (Fig. 1D).

Conclusions: This research aimed to explore the role of vascular cells in lung cancer progression. To do so, a 3D vascularized tumor model was developed to better understand how these cells behave together in an environment where soluble cell signaling as well as direct cell-cell interactions can be evaluated.