A Biomimetic Hydrogel Matrix Facilitates Evaluation of Cancer-associated Fibroblast Contributions to Tumor Angiogenesis

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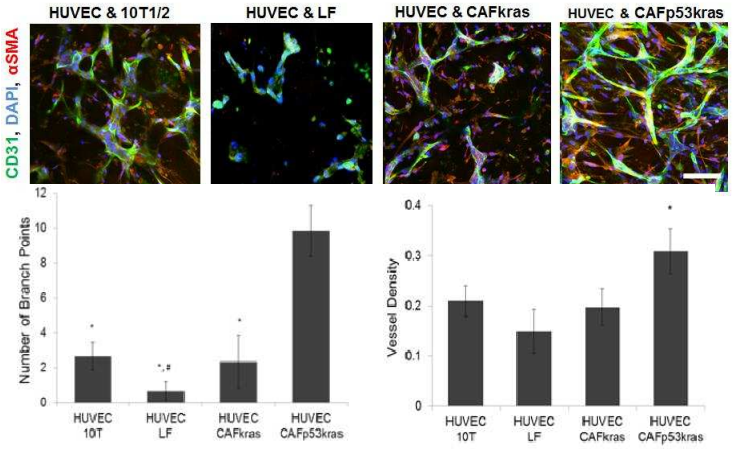
Statement of Purpose: The formation of new blood vessels around a tumor is facilitated by the complex interplay between cells in the tumor stroma and the surrounding microenvironment. Understanding this interplay and its dynamic interactions is crucial in identifying promising targets for cancer therapy. Current methods in cancer research involve 2D monolayer cultures on polystyrene plates. However, cell-cell and cell-ECM interactions that are important in vascularization cannot be recapitulated in 2D. To obtain more biologically relevant information, it is essential to mimic the tumor microenvironment in a 3D culture system. Matrigel has been extensively utilized as a 3D culture system; however, Matrigel allows little experimental control over matrix bioactivity and makes comparing studies difficult due to its batch to batch variability and growth factor contamination. In this work, we have demonstrated the use of poly(ethylene glycol) diacrylate (PEGDA) hydrogels to recapitulate and study the tumor vascularization in 3D. Unlike Matrigel, poly(ethylene glycol) (PEG) acts as a blank slate that can be modified to recapitulate the functions and properties of the ECM. We utilized degradable PEG hydrogels to evaluate the effects of cancer associated fibroblasts (CAFs) on tumor vascularization. CAFs comprise a majority of the cells in the tumor stroma and are rich source of secreted factors. The inherent characteristics of CAFs suggest that they may influence other cells in the vicinity such as endothelial cells (ECs) to promote and direct formation of new vessel structures. To investigate this theory, CAFs were isolated from non-metastatic prone tumor stroma of lungs of Kras mice (labeled here as CAFkras) and from metastatic prone mice expressing both the Kras and Tp53 mutations (labeled as CAFp53kras) and co-cultured with HUVECs in our PEGDA hydrogel system. In comparison to normal lung fibroblasts (LF), CAFs accelerated the formation of new vessels. Examination of metastatic prone CAFs, non-metastatic prone CAFs, and normal lung fibroblasts co-cultured with ECs revealed clear differences in vessel assembly, morphology, and density. We show that CAFs express αSMA and closely associate with vessel cells in a fashion similar to pericytes to stabilize new vessels. These findings suggest that CAFs play a crucial role in enhancing tumor vascularization and subsequently direct the malignant conversion of benign tumors to metastatic cancer.

Methods: To synthesize degradable hydrogels sensitive to cell-secreted matrix metalloproteases, specific sequence GGGPQGIWGQGK (abbreviated PQ) was reacted with heterobifunctional acryloyl-PEG succinimidyl valerate (PEG-SVA) [1]. The PQ peptide sequence is MMP2 and MMP9 sensitive. Cell-adhesive peptide RGDS was incorporated into PEG materials by attaching the free amine of the peptide to a bifunctional PEG molecule, leaving the other end to crosslink into the hydrogel. HUVECs were encapsulated in degradable hydrogels along with either 10T1/2 cells, CAFp53kras, CAFkras, or LF at a ratio of 4:1 at 30,000 cells/uL. Hydrogels were formed from prepolymer solution composed of 10% (w/v) PEG-PQ-PEG and 3.5 µmol/mL in sterile HEPES buffered saline (HBS) containing 1.5% (v/v) triethanolamine, 1 mM Eosin Y, and 3.95 µL/mL N-vinyl-2-pyrrolidone (NVP). The test groups for this study were the following: (1) HUVECs with CAFp53kras, (2) HUVECs CAFkras, and (3) for the control HUVECs with LF and (4) HUVECs with 10T1/2. Previous work has shown that HUVECs and pericyte-precursor 10T1/2 cells encapsulated in degradable hydrogels undergo tubule network formation; therefore, HUVECs encapsulated with 10T1/2 cells was utilized as a positive control group [1].The polymer solution with cells was photopolymerized via white light. Hydrogels were immersed in EGM-2 media and cultured up to 6 days. Vessel formation of the encapsulated cells was assessed by immunostaining ECs with CD31 and the fibroblasts or pericytes with α-smooth muscle actin (αSMA).

Results: HUVECs encapsulated with CAFs, LF, or 10T1/2 cells in degradable hydrogels formed tubule networks as shown in Figure 1. Immunohistochemistry was performed to confirm the expression endothelial and smooth muscle cell markers in tubules formed by co-cultures of HUVEC and CAF, LF, 10T1/2 cells in hydrogels. Visible differences in tubule formation and morphology were observed between the different co-culture groups. The degree of network formation varied with the metastatic potential of the CAFs. For example, hydrogels with CAFs from lung tumors in Tp53 and kras mutant mice induced vessel formation with a particularly heightened response than CAFs from mice that express K-ras allele. In comparison with all other co-culture groups, CAFp53kras cells in association with ECs arranged in tubule networks with significantly greater vessel density and branching.

Figure 1



Conclusions: In this work with PEGDA hydrogels, we show that CAFs induce vessel formation and assembly in the tumor microenvironment. CAFs also stabilize nascent vessels by closely associating with ECs like mural cells. This study offers insight on how the complex crosstalk established between ECs, CAFs and their surrounding ECM affects cancer progression and tumor vasculogenesis. Controlling this complex crosstalk can provide means for developing new therapies to treat cancer.

References: [1] Moon JJ, Saik JE, Poche RA, Leslie-Barbick JE, Lee SH, Smith AA, Dickinson ME, West JL. Biomaterials. 2010. 31: 3840-3847.