An artificial desmoplasia-mimetic microenvironment for studying EMT in pancreatic ductal adenocarcinoma

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Statement of Purpose: The development of pancreatic ductal adenocarcinoma (PDAC), a form of highly metastatic and lethal pancreatic cancer, is heavily influenced by soluble factors, cell-matrix, and cell-cell interactions presenting from the local tumor microenvironment, or desmoplasia.[1] Using conventional two-dimensional tissue culture techniques and animal models, significant progresses have been made toward understanding PDAC.[2] However, PDAC is still difficult to diagnosis and its survival rate is extremely low, in large part due to the limited understanding of the complex interplay between PDAC cells and pancreatic desmoplasia. The inherent limitations associated with current tumor cell culture techniques prompted us to design well-defined and highly tunable biomimetic matrices for studying PDAC cell fate. We have reported bio-orthogonal thiol-ene hydrogel platforms for controlling the growth, morphogenesis, and drug resistance in PDAC cells culture in 3D.[3,4] Built upon our prior work, current project focuses on fabricating synthetic desmoplasia-mimetic microenvironment for examining the synergistic influence of immobilized integrin-binding ligand and soluble factors on epithelial-mesenchymal transition (EMT) in PDAC cells.

Methods: COLO-357, a TGFβ-responsive PDAC cell line, were encapsulated in radical-mediated orthogonal thiol-ene hydrogels cross-linked by multi-arm PEG-tetra-norbornene (PEG4NB) and peptide crosslinker KCGPLGLYAGCK (MT1-MMP-sensitive sequence). Gels were also functionalized with ECM-mimetic peptides (e.g., collagen I-derived CGFOGER (5mM), O: hydroxyproline) to evaluate the effect of integrin binding on PDAC cell growth and morphogenesis under the influence of soluble TGFβ (0.5nM) and EGF (1nM). Cell survival and proliferation were quantified by total DNA content and AlamarBlue reagent while cell morphology was observed using Live/Dead staining, immuno-fluorescent staining, and confocal imaging. The expression levels of epithelial and mesenchymal markers were detected using western blot and real time PCR.

Results: More than 90% of the encapsulated COLO-357 cells remained alive following the gelation process and the cells proliferated to form clusters with different morphology, depending on the growth factor supplements (Fig. 1). Specifically, cell proliferation was suppressed in the presence of TGFβ1 and EGF without collagen-1 mimetic peptide, CGFOGER. On the other hand, cells encapsulated in CGFOGER-functionalized gels exhibited extensive protrusions and adopted irregular shapes when TGFβ1 and EGF were added to the culture media. Intensive MT1-MMP expression was detected in the leading edges of the cells encapsulated in CGFOGER-immobilized gels and with the treatment of EGF/TGFβ1. On the mRNA level, CGFOGER peptide alone was sufficient to induce a 2-fold increase in vimentin (a mesenchymal marker) expression and a 14% decrease in E-cadherin (an epithelial marker) expression (Fig. 1B). We also found that MT1-MMP mRNA expression was independent of CGFOGER peptide but was significantly up-regulated (2.4-fold) by soluble TGFβ1 and EGF (Fig. 1C). On the protein level, the presence of EGF/TGFβ1 down-regulated the expression of epithelial markers (E-cadherin and β-catenin) while up-regulated mesenchymal marker (vimentin), indicating enhanced EMT.

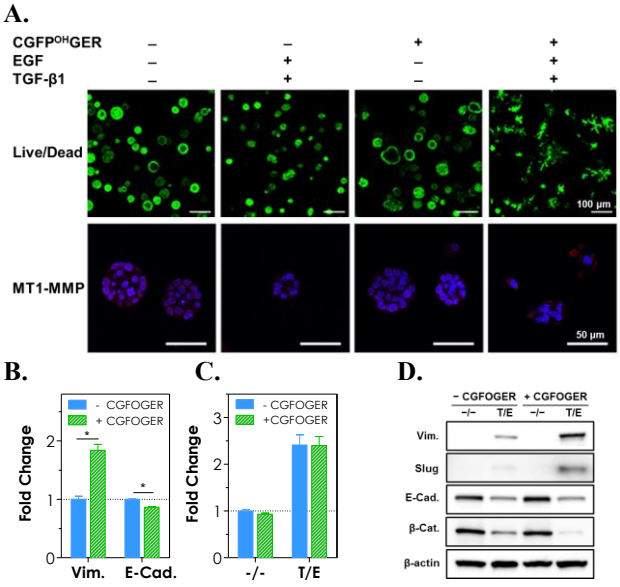

Figure 1. (A) Live/Dead (top) and immunofluorescence (bottom) staining images (red, MT1-MMP; blue, nuclei) of COLO-357 cells encapsulated (10-day) in MT1-MMP-sensitive hydrogels with or without 5mM CGFOGER and TGFβ1 (T: 0.5nM) and EGF (E: 1nM). (B) Vimentin and E-Cadherin mRNA expression. (C) MT1-MMP mRNA expression (n = 3, mean ± SEM, * $p < 0.05$). (D) Western blot of EMT markers.

Conclusions: We have developed a desmoplasia-mimetic hydrogel system suitable for PDAC research. The use of MT1-MMP-sensitive linker permits cell-mediated matrix cleavage while the use of CGFOGER provides an integrin binding motif for cell-attachment. Through this modular cross-linking approach, we revealed the differential influence of integrin ligand binding and soluble factor signaling on tumor cell EMT processes, a critical step leading to tumor metastasis. This material platform may serve as a promising platform for studying PDAC *in vitro* or for screening tumor-targeting drugs in a pathophysiologically relevant microenvironment.

References: [1] Krantz et al. *Mol Cancer Res*. 2011;9: 1294. [2] Dangi-Garimella et al. *Cancer Res*. 2011;71: 1019. [3] Raza et al. *Biomaterials*. 2013;34: 5118. [4] Ki et al. *Biomacromolecules*. 2013;14: 3017.