Synergy of matrix stiffness and EGFR inhibition in apoptosis of pancreatic tumor cells in 3D

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Statement of Purpose: Pancreatic cancer is one of most lethal cancers. The diagnosis of pancreatic cancer is difficult in the early stages and the options of clinical treatments are very limited. The diagnosis and treatments of pancreatic cancer can benefit from a biomaterial platform that facilitates the understanding of tumor cell progression and response due to the presence of external stimuli. While many pioneering studies have elucidated the crucial influence of mechanotransduction in cancer cell progression,[1,2] the effect of matrix stiffness on pancreatic cancer cell fate remains elusive. Furthermore, studies have indicated the importance of epidermal growth factor receptor (EGFR) signaling in pancreatic cancer cell development and several EGFR inhibitors have been identified for suppressing metastasis of pancreatic cancer cells.[3] Most of these studies, however, are conducted in 2D cell culture platforms that do not resemble a tumor microenvironment. Therefore, the objective of this study is to study the synergistic influences of matrix stiffness and EGFR inhibition on pancreatic ductal adenocarcinoma cells (PANC-1) apoptosis in a highly tunable 3D synthetic tumor microenvironment.

Methods: PANC-1 cells were photoencapsulated (365 nm, 5 mW/cm^2) in poly(ethylene glycol)-octa-norbornene (PEG8NB) hydrogels formed by a step-growth photo-click reaction.[4] Dithiothreitol (DTT) or matrix metalloproteinase-sensitive peptide (KCGPQGIWGQCK) was used as gel crosslinker and lithium arylphosphanate (LAP, 1 mM) was used as the photoinitiator. EGFR peptide inhibitor NYQQNC (0-4 mM) was incorporated in the gels *in situ* via the same thiol-ene reaction. Matrix stiffness was modulated by controlling the content of macromer in the precursor solution. Encapsulated PANC-1 cells were cultured in 10% FBS supplemented DMEM for 10 days. Cell viability was evaluated with cellular metabolic activity (Alamarblue reagent), DNA content, live/dead staining, and apoptosis assays. The expressions of EGFR and Akt in the encapsulated cells were assessed by Western blotting.

Results: Synthetic peptide, NYQQN, has been shown to inhibit soluble EGFR activation.[5] However, it is not clear whether this peptide has inhibitory effect for pancreatic cancer cells. In an attempt to evaluate the efficacy of this peptide drug in inhibiting pancreatic cell proliferation, we first conducted a 2D study on tissue culture plates and found that soluble NYQQN peptide induced cell death only at a concentration of 4 mM or higher. We further evaluated the viability of PANC-1 cells in thiol-ene hydrogels. The encapsulated PANC-1 cells exhibited high viability (85-90%) post-encapsulation and proliferated to form cell clusters. When EGFR inhibitor NYQQN was immobilized within the hydrogel network, its effect on cell proliferation was largely affected by the gel compositions. For example, when a non-cleavable linker DTT was used, the encapsulated cells became irresponsive to the peptide drug. More interestingly, the responsiveness of PANC-1 cells to this peptide drug was largely dependent on the stiffness of the hydrogel matrix (Figure 2A). Specifically, the concentration of peptide drug needed to induce cell death decreased as we increased gel stiffness (G' was ~2, 7, and 12 kPa for 4, 6, and 8 wt% PEG8NB gels, respectively) and 2 mM of NYQQN was sufficient in inducing cell death (in contrast to 4 mM in 2D culture). After 10-day of 3D culture, PANC-1 cells formed clusters in the softer matrix (4 wt%) even in the persistent presence of peptide drug (Figure 2B, top image) while cells encapsulated in stiffer gels did not survive (Figure 2B, bottom image).

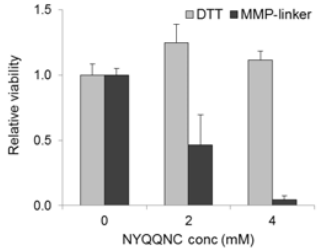

Figure 1. Influence of gel crosslinker on the effect of NYQQN-induced PACN-1 cell death. (8wt% PEG8NB, day-10 post-encapsulation).

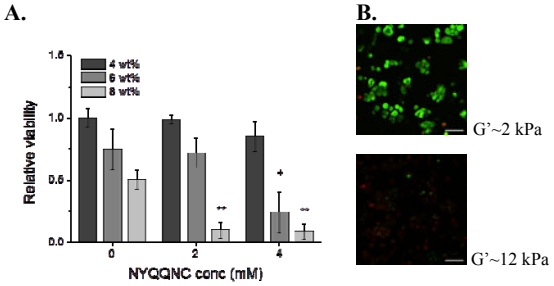

Figure 2. (A) PANC-1 cell viability in PEG8NB gels crosslinked by MMP-sensitive linker in the presence of immobilized NYQQN peptide (day-1 post-encapsulation). (B) Live/dead staining (confocal Z-stack images: Scale: 100 μm) of PANC-1 cells encapsulated in gels with different stiffness and immobilized with 4 mM NYQQN (10 days 3D culture).

Conclusions: This study highlights the importance of using a 3D matrix to study the effect of therapeutic drugs on pancreatic cancer cell survival. An EGFR peptide inhibitor, NYQQN, was found to be effective only in cell-responsive and stiffer matrices. Furthermore, the effective peptide dosage on inducing cell death was lower for cells encapsulated in a stiffer matrix than that used in a conventional 2D culture (i.e., 2 mM in 3D vs. 4 mM in 2D). In summary, this gel platform provides a highly tunable synthetic tumor microenvironment with precise controls over matrix properties for *in vitro* cancer cell research.

References: [1] Nemir S et al. *Biotechnol Bioeng.* 2010;105:636-44. [2] Leight JL. *Mol Biol Cell.* 2012;23:781-91. [3] Mendelsohn J, Baselga J. *Oncogene.* 2000;19:6550-65. [4] Fairbanks BD et al. *Adv Mater.* 2009;21:5005-10. [5] Abe M. *Biopolymer.* 2007;89:40-51.