Modeling Cancer Metastasis Using a Bioorthogonally Integrated Hydrogel Platform

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Statement of purpose

Prostate cancer is the second most common cause of cancer related deaths in men (1). Cancer growth and metastasis requires intricate and dynamic interactions between cancer cells and the tumor microenvironment. Thus, physiologically relevant three-dimensional prostate tumor models presenting essential cell signaling cues have great potential for mechanistic investigation of tumorigenesis and for testing of anticancer therapies (2,3). Previously, we showed the formation of multicellular tumoroids from LNCaP cells entrapped as single cells in a hyaluronic acid (HA)-based hydrogel with a multivalent presentation of cell adhesive peptides (4). The presence of RGD signal promoted the development of larger tumoroids, however individual cells remained immobilized as spheroids without any sign of dissemination or invasion into the surrounding matrix. With an overarching goal of modeling cancer metastasis, we engineered a HA-based protease degradable hydrogel platform, with cell adhesive properties tunable in a spatial and temporal fashion employing the rapid, bioorthogonal and extremely efficient cycloaddition reaction between tetrazines (Tz) and trans-cyclooctene (TCO) derivatives (5). Hydrogel exhibited biochemical and biomechanical signals that promoted tumor formation, induced phenotypic changes, and stimulated cancer cell migration/dissemination in 3D.

Materials and methods

Synthesis of hydrogel building blocks: The carboxylate groups on HA were modified with methylphenyl tetrazine hydrazide to yield HA-Tz (6). The matrix metalloprotease (MMP) cleavable peptide was modified with norbornene N-hydroxy succinimide ester to yield the SMR-bisNb crosslinker. Fibronectin derived peptide was treated with TCO 4-nitrophenyl carbonate to obtain RGDSP-TCO (7).

3D culture: DU145 cells were suspended in HA-Tz and SMR-bisNb solution to form a homogenous gel. On day 7, RGDSP-TCO was added to the media to initiate interfacial ligation. Cultures were maintained in EMEM media for 21 days.

Immunostaining and confocal imaging: Spheroid morphology were visualized after 7 and 21 days in culture via laser scanning confocal microscope. Hydrogel constructs were permeabilized using 0.2% Triton X-100 in PBS and blocked in 3%BSA. Primary antibody against target protein was added, followed by Alexa Fluor 488 secondary antibody. Constructs were stained for F-actin using Alexa Fluor 568-phalloidin and nuclei stained with 4',6-diamidino-2-phenylindole (DAPI) for 2 hours at room temperature. Samples were washed thoroughly with PBS and images were captured using confocal microscope.

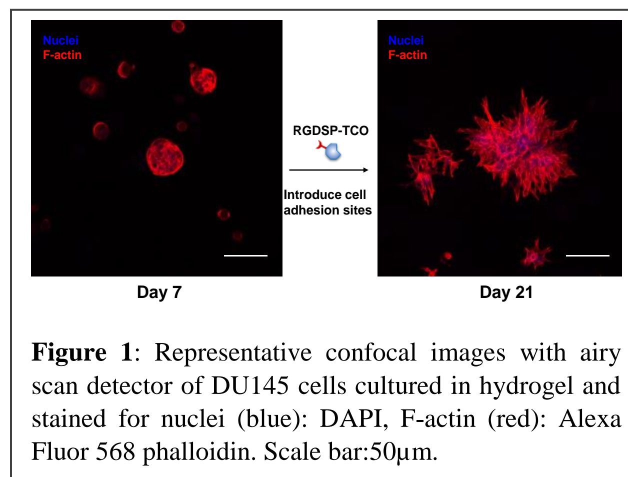

Figure 1: Representative confocal images with airy scan detector of DU145 cells cultured in hydrogel and stained for nuclei (blue): DAPI, F-actin (red): Alexa Fluor 568 phalloidin. Scale bar:50µm.

Results

When encapsulated in the hydrogel, DU145 prostate cancer cells spontaneously formed multicellular spheroids after 7 days of culture. Conjugation with RGDSP-TCO cell adhesive peptide promoted proliferation and formation of cellular protrusions by day 21 (Figure 1). Characterization of 3D cellular constructs by immunocytochemistry and qPCR analyses revealed that RGDSP signal promoted epithelial to mesenchymal transition. After RGDSP conjugation, spheroids exhibited protrusive invadopodia like structures, indicating ECM degradation and invasion.

Conclusions

A bioorthogonal 3D cell culture platform was developed employing tetrazine ligation. Cells maintained in this matrix adapted mesenchymal phenotype and migratory behaviors reminiscent of cancer metastasis. This platform is tunable and experimentally tractable and can be used to identify potential molecular targets and accelerate advances in cancer therapeutics.

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