An Engineered Inert Matrix for In-Vitro Maintenance of Cancer Stem Cells

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Statement of Purpose: Cancer stem cells (CSCs) are responsible for cancer initiation and metastasis as well as drug resistance. Self-renewal and differentiation of CSCs is regulated by their microenvironment. Among different factors in the microenvironment, matrix stiffness or elastic modulus plays an important role in regulating stem cells function. However, it is difficult to study the effect of individual biophysical and biochemical factors presented in the microenvironment by in vitro 3D cell culture system with naturally derived matrices, because these matrices contain many ligand-receptor interaction molecules. The purpose of this study was to use a PEG based 3D cell culture matrix with a wide range of mechanical and physical properties and biocompatibility for the investigation of the effect of gel modulus and cell surface binding peptides on CSCs growth and differentiation.

Methods: To investigate the effect of gel modulus on breast cancer CSC, 4T1 (mouse breast cancer cell), MCF7 and MDA-MB231 (human breast cancer cells), or MCF10a (human mammary epithelial cell) were mixed with a precursor solution containing different concentrations of PEGDA macromer in the initiator solution. After photopolymerization, the hydrogel had a modulus range from 2 to 70kPa. Cells encapsulated in the hydrogel were cultured in the DMEM-F12 medium. Cell morphology and gene expression were examined at different time points by fluorescent microscopy and real time PCR, respectively. To study the effect of cell binding peptide, an acrylamide-terminated CD44-binding peptide (RLVSYNGIIFFLK) was synthesized in the solid phase by Fmoc chemistry. The peptide was conjugated to the PEGDA hydrogel (2%), and cells were encapsulated and cultured in the peptide containing hydrogel.

Results: Tumorsphere formation in the PEGDA hydrogel depended strongly on the gel modulus. As the matrix modulus was increased from 2 kPa to 5, 26, 47 and 68 kPa, the average tumorsphere size and cell number density changed dramatically in 4T1, MDA-MB-231 and MCF7 breast cancer cells but not in MCF10a normal human breast epithelial cells (Figure 1). Breast cancer cells in the gel with modulus of 5 kPa formed the largest spheres, highest cell number density and highest expression levels of breast CSCs markers (CD44 and ABCG2) after 9 days. The effect of cell type on tumorsphere formation was investigated in the gel with 5kPa modulus. After 9 days of incubation, the expression of breast CSC markers CD44 and ABCG2, the density and size of 4T1 and MDA-MB-231 tumorspheres were higher than that of MCF7. Nearly 60% of MDA-MB-231 and 40% of the 4T1 spheres were larger than 70 μm while most of the MCF7 spheres were between 40 and 60μm. MCF10a remained as single cells with no significant change of CSC markers.

The effect of CD44 binding peptide on tumorsphere formation was investigated in the PEGDA gel with 5kPa modulus after 9 days of incubation. Conjugation of CD44 binding peptide to the PEGDA hydrogel abolished tumorsphere formation in 4T1, MDA-MB-231, and MCF7 tumor cells.

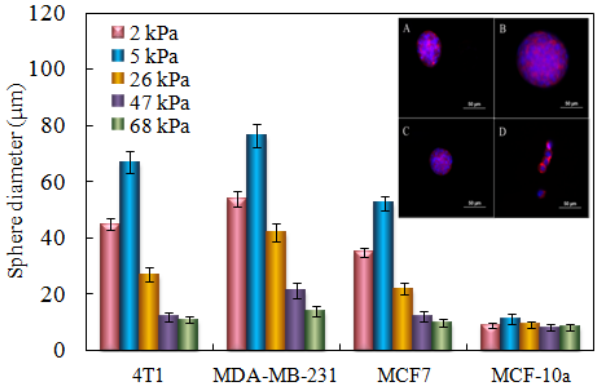

Figure 1. Tumorsphere diameter after 9 days; Evolution of breast cancer cells and sphere formation after 9 days in PEGA with different moduli; A: 4T1, B: MDA-MB-231, C: MCF7, D: MCF10; in the inset the nuclei and cytoskeleton are stained with DAPI (blue) and phalloidin (red).

Conclusions: The formation and maintenance of breast cancer stem cells is modulated by the elastic modulus of the matrix. This engineered inert 3D matrix provides a novel tool to control and investigate the effect of factors in the microenvironment on the maintenance of CSCs in vitro. Studying the effect of matrix stiffness with different breast cancer cell lines revealed that matrix stiffness can dramatically affect the cell growth. Furthermore, the growth of CSCs was not only dependent on the type of cancer cell but also on their malignancy. Cells with a high malignancy such as 4T1 and MDA-MB-231 formed more spheres than MCF7 or normal MCF-10a cells when encapsulated in the gel, suggesting that spheres originated from the CSC subpopulation of cancer cell lines and the PEGDA gel with a certain modulus promote CSCs proliferation. The result of conjugation of a CD44 binding to the gel in this study indicates that this 3D cell culture system can be used as a model to investigate individual tumor microenvironmental factors on the maintenance and differentiation of CSC.

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