Multiplex Biomaterial Matrix Cues Modulate Cancerous Progression of Adult Stem Cells

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Statement of Purpose: Microenvironmental cues determine the fate of stem cells[1]. These cues include chemical and mechanical factors as well as competitive cell-cell versus cell- matrix adhesion events. Adult stem cells can be transformed to be malignant through epigenetic alternations and/or lineage transitions ("cancer stem cells"). Our laboratory has shown that synthetic polymer substrates impact cytoskeletal remodeling, proliferation, spreading and apoptosis in benign osteosarcoma cells through outside-in signaling, but the effects of polymer matrix-derived cues on cancerous transformation of adult stem cells have not been evaluated[2]. Potential outcomes will be advantageous to elucidate a matrix-dependent mechanism involving metastasis and transformation of adult stem cells. Here we employed a library of poly(ε-caprolactone) (PCL)-based combinatorial polymers as culture substrates for human mesenchymal stem cells (hMSCs). Each polymer contained a different molar ratio of three polymeric subunits: (1) polyethylene glycol (PEG; Mw =5,000), (2) a hydrophobic PCL, and (3) a negatively charged carboxylated PCL (cPCL). Cancerous transformation was induced via prolonged treatment with a metal carcinogen (i.e., nickel sulfate); these groups were compared to untreated controls for each polymer type. The expression of oncogenes, stemness markers, cancer stem cell markers, proliferation, apoptosis at the gene and protein levels, as well as intra- and extra-cellular ROS production, indicates that chemical and mechanical properties of the synthetic polymeric matrix impact cancerous progression of hMSCs via an anti-oxidative mechanism.

Methods: Polymer substrates were prepared by spin coating 1% w/v solutions onto round cover glass slips. hMSCs were seeded on the substrates in 24-well plates and were exposed to prolonged, periodic treatment with nickel sulfate to induce cancerous transformation. End point measurements include quantitative RT-PCR, colorimetric assays, fluorescence microscopy, flow cytometry, and Western blots.

Results: End point analyses indicate that the hydrophobic PCL, negatively-charged cPCL, and hydrophilic PEG all regulate stemness, oncogenic, and tumor suppression activities of hMSCs differently in response to carcinogen treatment. In general, extensively more colony formation was observed under carcinogen treatment compared to the untreated condition. However, spontaneous colony formation was also observed on PEG- and/or cPCL-containing polymers in untreated conditions, indicating non-malignant colony formation (Figure 1A). This result indicates a possible spontaneous change in the anchorage-dependent phenotype in response to the chemical, mechanical, and morphological properties of PEG- and cPCL-containing polymers. The incorporation of a negative charge into the surface chemistry reduced the pro-oxidative effects of PEG in intracellular ROS levels, indicating an anti-oxidative activity. Analysis of RT-PCR data revealed changes in the expression of self-preserving defense mechanisms relative to cancer related proteins (Figure 1B). Interestingly, the presence of PEG and/or CPCL dramatically increased P 53 expression compared to glass and PCL. A relative increase in C-Myc on cPCL-containing polymers indicates increased proliferation with a simultaneous decrease in tumor suppressor activity. These data indicate that PEG and the negative charge play a significant role in changes in cancerous progression of adult stem cells on the matrix surface. A series of possible material properties, including surface and bulk modulus, redox potential, and phase separation, are under scrutiny.

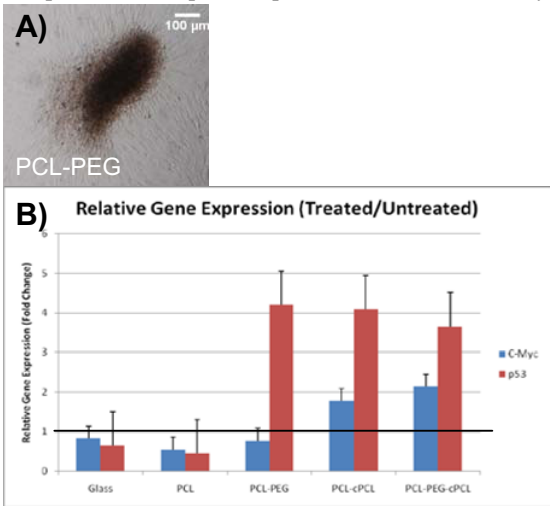

Figure 1: (A) Colony formation of hMSCs on PCL-PEG, **(B)** Relative gene expression from RT-PCR for hMSCs on different polymer types. For each group, values represent treated conditions normalized to untreated conditions.

Conclusions: The data suggest that synthetic polymeric components influence cancerous transformation of hMSCs differently in response to carcinogen treatment. Incorporation of a negatively charged subunit and/or PEG into the polymer backbone results in a dramatic increase in expression of the P53 tumor suppressor while cPCL alone increases cMyc expression. Ongoing work includes further confirmation of our results as well as investigations of the fundamental mechanisms involved in these processes. Further studies are underway to elucidate the molecular components and signaling events responsible for inductive or inhibitive effects of synthetic polymers on stem cell transformation. This work directly contributes to the development of the next generation of biomaterials to control stem cell fate and promote healthy tissue regeneration while preventing cancer progression.

References:

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