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RNA-seq reveals diverse effects of substrate stiffness on mesenchymal stem cells

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Abstract

Substrate stiffness has been recognized as an important regulator of cell fate and function, but an understanding of the full extent of processes affected by stiffness is lacking as its transcriptome-wide effects have not been mapped. This limited understanding has restricted the contexts in which engineers can employ stiffness as an engineering design parameter. To address these limitations, we performed RNA-seq on mesenchymal stem cells (MSCs) cultured in alginate hydrogels over a range of moduli to broadly map the transcriptome-wide changes associated with stiffness sensing. We found a large number of stiffness-sensitive genes, and that many genes respond to stiffness in nonlinear ways. Informed by these differential expression results, we explored a hypothesis related to current MSC clinical activity, and found that stiffness can regulate the expression of MSC immunomodulatory markers in response to cytokine stimulation. Overall, these results reveal previously unknown features of MSC stiffness response and demonstrate the value of coupling –omics approaches with biophysical experiments.

For almost two decades, engineers and biologists have studied the effects of adhesion substrate properties on cell phenotype[1, 2]. Over that time, approaches to control and study these features have evolved, revealing myriad ways in which cells sense and respond to their culture substrates[3]. For instance, Tekin et al found significant transcriptome-wide changes associated with 2D versus 3D tissue culture[4]. Within 3D cell culture, material characteristics such as adhesion ligand composition and density[5-7], time-dependent material properties[5, 8], nanoscale topography[9], and degradability[10],