Rational Approaches to Direct human Embryonic Stem Cell Differentiation

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***Short Abstract* — In order to study the effect of the mechanical microenvironment on the differentiation of hESCs we constructed 3D synthetic scaffolds with variable mechanical properties. After evaluating gene expression patterns of cells growing in the scaffolds we constructed protein interaction networks that describe pathways predominant to each microenvironment. In order to predict the differentiation state of the cells we inferred the function from the constructed networks by assigning Gene Ontology attributes. We finally utilized the networks as a guide for perturbing the cellular state with small interfering RNAs and identified critical genes that when knocked down can control the hESC differentiation.**

I. The Mechanical Microenvironment Controls hESC Differentiation

STEM cell behavior is correlated with cues that lie in their extracellular microenvironment [1]. These cues operate on different spatial and temporal scales to pattern specific cellular responses that drive tissue morphogenesis and differentiation [2]. To characterize the effect of the mechanical microenvironment on the differentiation of human embryonic stem cells, the cells were grown on scaffolds of matrigel coated binary poly-L-lactide acid (PLLA) and co-polylactic/glycolic acid (PLGA) blends at varying ratios and of elastic moduli (EM) [3].

In an effort to draw correlative associations between scaffold stiffness and hESC differentiation, the expression of 96 genes unique to each of the three germ layers; ectoderm, mesoderm and endoderm, was quantified after 14 days in culture. To evaluate the gene expression patterns, we performed hierarchical clustering analysis of the expressed genes. We identified clusters of genes that are uniquely upregulated in hESC grown on low elasticity modulus, intermediate EM or high EM scaffolds. To identify the significance of these sets of genes first we expanded the gene data sets by constructing extended protein-protein interaction networks. We then assigned Gene Ontology attributes. This analysis revealed that genes upregulated in hESCs grown on low EM scaffold correlated with ectoderm development, genes upregulated in hESCs grown on intermediate EM scaffold with endoderm morphogenesis and genes upregulated in hESCs grown on the high EM scaffold with mesoderm morphogenesis [3].

II. Using siRNA to Perturb hESC Differentiation State

One of the most significant nodes in the extended network of the genes that eventually give rise to mesoderm is KDR/VEGFR2. We hypothesized that the differentiation state of the hESCs could be perturbed by knocking down KDR using small interfering RNA and examined the effect of KDR knockdown on the expression of genes representative of the three germ layers [4]. All examined mesodermal representative markers were significantly upregulated, the expression of ectodermal markers was slightly upregulated and all examined endodermal markers were downregulated.

In order to understand the causative role of the KDR knockdown on the observed gene expression pattern, we constructed a protein-protein interaction network that connected the undifferentiated cell state and a differentiated state that leads to the expression of the mesoderm specific genotype. We created two separate protein-protein interaction networks, one that includes KDR (initial network) and another where KDR is virtually knocked out. We then identified a "reduced" network of the proteins that are uniquely expressed during the virtual KDR knockout. Analyzing the connectivity of the reduced network led to identification of a predominant gene, androgen receptor (AR). In the subset of the extracellular proteins at the reduced network we can identify that integrins signal downstream towards AR. We hypothesized that the androgen receptor affects the expression of the mesoderm, endoderm and ectoderm germ layer genes. To test this hypothesis we knocked down AR. As predicted, AR knockdown led to a decrease in the expression of endoderm representative genes and upregulation of the mesoderm and ectoderm genes, similar to the effect of the KDR knockdown.

References

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