Translating Extracellular Matrix to Intracellular Signaling to Proliferation

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Statement of Purpose: The chemical composition of a biomaterial certainly affects cell behavior, but it is not clear how the cell determines that it is on a particular substrate. If we understand exactly how the cell makes this determination, we may be able to trick cells into behaving as we want them to by designing materials with desirable surface chemistries.

Here we vary the extracellular matrix (ECM) proteins adsorbed to a cell culture plate with which human umbilical vein endothelial cells (HUVECs) are brought into contact. These cells are then assayed at various times to determine the activation of four important signaling pathways by quantification of a member of that pathway: extracellular-signal-regulated kinase (ERK), c-Jun kinase (JNK), IκB kinase (IKK), and Akt. We then quantify the rate of cell proliferation on the same substrates. A systems biology tool, Partial Least Squares Regression Analysis (PLS), is used as an aide for data interpretation.

Methods: The activities of four signaling kinases (JNK, IKK, Akt, and ERK1/2) are measured for HUVECs in contact with materials to which one of nine ECMs (Gelatin (Gel), Collagen I (ColI), Collagen IV (ColIV), Laminin (Lama), Matrigel (Matr), Fibronectin (Fn), Vitronectin (Vn), Chondroitin Sulfate (CS), Heparan Sulfate (HS)) or no coating (PS) is adsorbed. HUVECs (Cascade Biologics) are brought into contact with the surfaces, frozen at the desired time point, lysed, and the lysate is immunoprecipitated (IP) with an antibody for a kinase of interest. The IP surface is exposed to substrate for the kinase in the presence of P^{32}-labeled and unlabeled ATP, filtered through a vacuum filter plate, then the filters are analyzed by liquid scintillation. We also quantify cell proliferation on the same ten substrates using the Cell Titer 96 (Promega) assay by plating 3000 cells/well, culturing for 72 hrs, then assaying for cell number. ERK inhibition experiments add 10mM U0126 (Sigma) which inhibits MEK which acts to activate ERK.

Results: Cell number after 72hr incubation is input into a matrix as a column matrix with ten rows, one for each substrate composition (values shown in Figure 1).

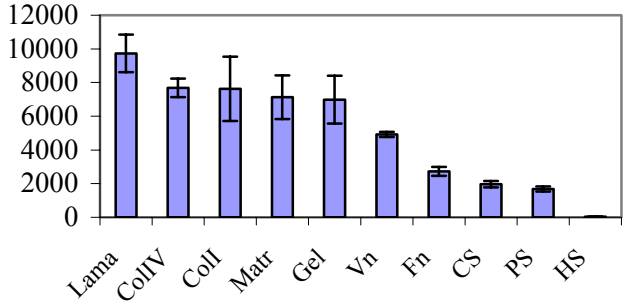

Figure 1. Cell number vs. ECM composition of substrate

Kinase assay data for 12 time points (0, 5, 10, 15, 30, 45, 60, 90, 120 minutes, 24, 48, and 72 hours), maximum, mean, and area-under-the-curve (AUC) for each kinase and for each substrate were inserted into a matrix with the kinase time-points as columns (60) and the substrate composition as rows (10). These two matrices are analyzed by PLS to yield scores and loadings vectors.

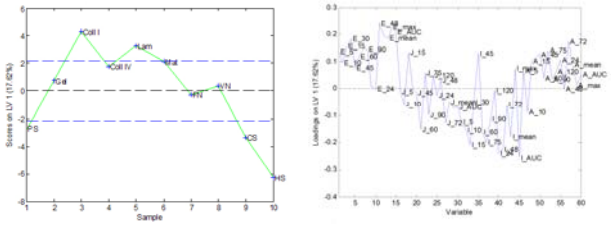

Figure 2. Scores (Left) and Loadings (Right) for PLS on kinase assay data and proliferation for various substrate compositions. Blue dashed line indicates 50% confidence interval. ERK, JNK, IKK and Akt time-points are variables 1-15, 16-30, 31-45, and 46-60 respectively.

The scores show that all of the "high" proliferators (Lama, ColIV, ColI, Matr, Gel, Vn) have positive scores, and all of the "medium" (Fn, CS, PS) and "low" (HS) proliferators have negative scores. Loadings show that scores correlate positively with ERK activity and negatively with IKK activity and do not correlate particularly positively or negatively with IKK or Akt.

This suggests the hypotheses that inhibiting ERK might decrease proliferation and that inhibiting IKK might increase proliferation. We repeated the proliferation experiment in the presence of an ERK inhibitor (results shown in Figure 3).

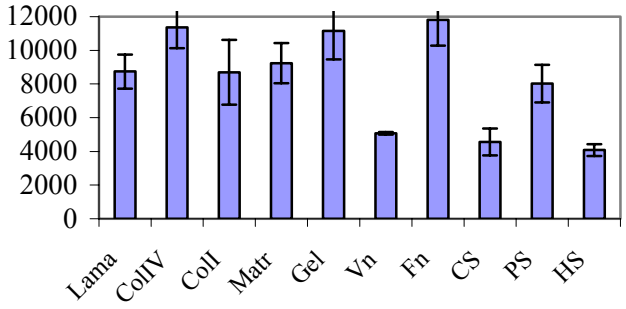

Figure 3. Cell number with ERK inhibition.

Conclusions: ERK and IKK are likely important in mediating the effect of ECM substrate on cell proliferation. The specific hypothesis that ERK activity increases proliferation was tested by inhibiting ERK; however, a few substrates exhibited increased proliferation (Fn, PS, HS) and none exhibited decreased proliferation. Nevertheless, inhibition of ERK proved to be important in regulation of cell proliferation via ECM.

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