Intracellular Signaling: the key to understanding why cells behave differently on different materials

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Statement of Purpose: Rational design of biomaterials requires understanding how cellular microenvironments elicit particular cell behaviors. Although the mechanisms of intracellular signal transduction are relatively well understood, how the cells can distinguish among thousands of microenvironments is not. This study proposes that a systems level view of intracellular signaling is necessary to interpret differences in cell interactions with different substrates. Here we study how different substrates (9 different extracellular matrix (ECM) components and un-coated polystyrene) elicit different patterns of intracellular signaling. We then correlate an important cell behavior, cell adhesion, and a molecular mediator of that behavior, β_1-integrin subunit expression, with those intracellular signals to generate a hypothesis: that increased ERK and JNK activity cause increased cell adhesion. Testing this hypothesis by inhibiting these kinases provides specific knowledge regarding what pattern of signaling is elicited by particular materials and what pattern of signaling is required to achieve desired cell behaviors.

Methods: Human umbilical vein endothelial cells (HUVECs) were exposed to 10 substrates: collagen I (C1), gelatin (G), collagen IV (C4), matrigel (M), laminin (L), fibronectin (Fn), vitronectin (Vn), chondroitin sulfate (CS), heparin sulfate (HS), and uncoated tissue culture polystyrene (PS). The activities of 4 intracellular signaling kinases (extracellular signal-related kinase (ERK), c-Jun N-terminal kinase (JNK), protein kinase B (Akt), and IκB kinase (IKK)) were quantified for cells on each substrate as a function of time. The matrix of data, including calculated mean, maximum, and integrated concentration with respect to time for each kinase, was analyzed using Principal Component Analysis (Matlab PLS Toolbox) to correlate signaling activity with variation in substrate.

Cell adhesion of HUVECs on the same 10 substrates was measured by incubating HUVECs with the surface for 1 hr, inverting the plates, washing once with PBS, fixing, staining with toluidene blue, incubating with 1% SDS, and then measuring absorbance of the supernatant at 670nm. β_1-integrin subunit expression was measured by fluorescence-activated cell sorting (FACS). Partial least squares (PLS) regression analysis was used (Matlab PLS Toolbox) to correlate signaling data with these cell behaviors. Inhibition assays were performed repeating the cell adhesion and FACS for β_1-integrin in the presence of inhibitors for the four kinases: ERK (10 μM U0126), JNK (5 μM SP600125), IKK (10 μM PS1145 dihydrochloride), Akt (20 μM LY294002).

Results: PCA indicates that mean activities of ERK, JNK, Akt, and IKK correlate well with the first three principal components (PCs). These were defined as "high" (H) and "low" (L) based on statistical differences shown by Fischer's least significant difference method (p<0.4, high p-value is justified because many samples are required to study system behavior). "Medium" (M) activities were sometimes statistically different as noted in Table 1. The mean activities of these 4 kinases distinguish almost all of these substrates – 39 of 45 pair-wise comparisons.

Table 1. Pattern of activity for each substrate

	PS	G	C1	C4	L	M	Fn	Vn	CS	HS
ERK	L	H	H	M*	M*	M*	H	L	L	L
JNK	M†	H	H	H	M	H	L	L	L	L
Akt	H	H	M	M	M	L	L	H	H	L
IKK	M‡	M‡	M‡	L	H#	M‡	M‡	M‡	H	H

* C1 (p<0.25), † Vn (p<0.4), ‡ HS and CS (p<0.4), # CS (p<0.4)

Cell adhesion and β_1-integrin subunit expression were then quantified. Cell adhesion is ranked low to high – "Low": CS, HS, PS, L; "Medium": Vn, Fn, M, C4; "High": G, C1. β_1-integrin subunit expression is ranked low to high – "Low": HS, PS, CS, L; "Medium": Vn, Fn, M; "High": C4, G, C1. "Low" and "High" are different (p<0.1). PLS correlates both adhesion and β_1-integrin expression primarily with ERK and secondarily with JNK activities. This hypothesis, that ERK and JNK cause increased adhesion, was tested by inhibiting the 4 kinases.

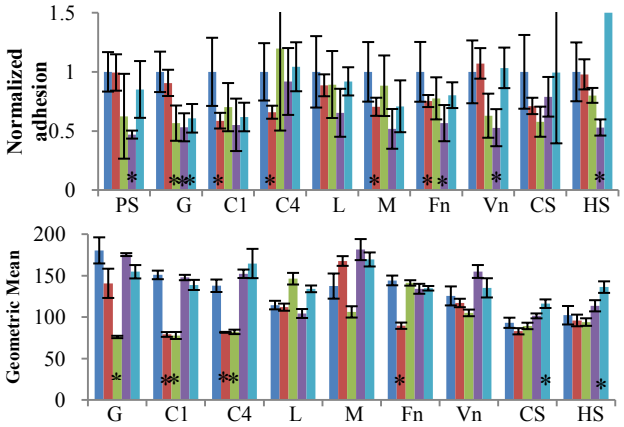

Figure 1. Adhesion assay (top) and FACS for β_1-integrin (bottom) with no inhibition (dark blue), or inhibitors for ERK (red), JNK (green), IKK (purple), Akt (light blue).
* Different from no inhibition control (p<0.05).

Conclusions: ERK inhibition decreases adhesion of C1, C4, M, and Fn which are four of the five substrates with the greatest ERK activity. The fifth, G, is the only substrate to show different adhesion (less) upon JNK inhibition. Likewise, ERK inhibition decreases β_1-integrin expression on C1, C4, and Fn. JNK inhibition does likewise on G, C1, and C4. IKK and Akt decrease adhesion of some samples and slightly increase β_1-integrin expression on CS and HS and adhesion on HS.

We have shown that each substrate elicits a different pattern of intracellular signaling which in turn cause differences in cell behavior. Specifically we have shown that activation of ERK (e.g., by C1) causes increased β_1-integrin expression and stronger cell adhesion.