Migration of endothelial cells on fibronectin gradients subject to soluble migration promoters and inhibitors.

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Introduction. The modulation of cell motility using soluble factors has been widely characterized in the fields of immunology, development and cancer research. This study takes advantage of the versatility of our free cell assay to conduct the first investigations into the coordinated effects of haptotactic gradients and soluble factors. Acidic fibroblast growth factor (aFGF), a known migration promoter, and the migration inhibiting phosphatidylinositol 3-kinase inhibitor, LY294002, were used to modulate human microvascular endothelial cells (hMEC) migration in this study.

Methods. Subconfluent hMEC were cultured on FN gradients produced by chemical coupling of FN to cross-diffused alkane thiols as described in previously (Smith 2004). Migration of hMEC was recorded by time-lapsed video microscopy using the "Free Cell Migration Assay" as described previously (Smith 2006). Images were collected up to 24 hours for conditions without and with either aFGF or LY294002.

Results. Cells migrating on the most stimulatory FN haptotactic gradients (1.23ng Fn/mm^3) were treated with uniform dosing of the soluble factors. 10^{-8}M acidic fibroblast growth factor stimulation was observed to increase S_d from 4.3 ± 1.6 to 6.8±1.2μm/hour with a concurrent decrease of S_r from 8.8 ± 1.0 to 6.3 ± 0.9μm/hour. The migration inhibitor LY294002 was titrated into the migration media for the pro-migratory aFGF + gradient condition and shown to eliminate the increase in S_d at low dosing and lead to cell death as concentration increased. 5mM LY294002 decreased S_d from 4.3 ± 1.6 to 0.6 ± 1.1μm/hour with only 18% mortality (Figure 1).

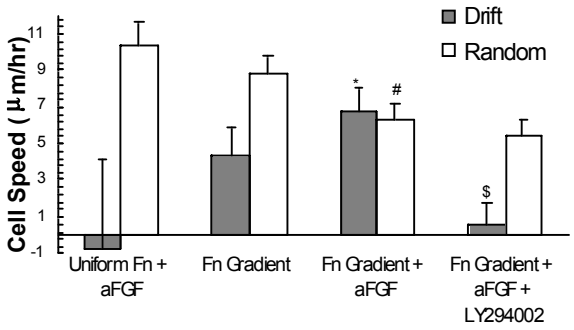

Figure 1. aFGF treatment increases S_d on haptotactic fibronectin gradients and LY294002 specifically inhibits S_d in optimal migration conditions. Error bars represent standard error of the mean. * S_d on gradient with aFGF is greater than both no gradient and no aFGF conditions ($P<.05$). # S_r on gradient with aFGF is less than no gradient condition ($P<.05$). $ S_d on gradient with aFGF and 5mM LY294002 is lower than both gradient and gradient + aFGF conditions ($P<.05$).

Conclusions. These experiments demonstrate the utility of the newly developed Free Cell Migration Assay for characterization of chemotactic and haptotactic mediators of endothelial cell migration.

Citations.

Smith JT, Tomfohr JK, Wells MC, Beebe TP, Jr., Kepler TB, Reichert WM. (2004) Langmuir 20(19):8279-86.

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