Predicting how cells spread and migrate
Focal adhesion size does matter

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**Introduction**

Efficient cell migration is central to the normal development of tissues and organs and is involved in a wide range of human diseases, including cancer metastasis, immune responses, and cardiovascular disorders. Mesenchymal migration is modulated by focal-adhesion proteins, which organize into large integrin-rich protein complexes at the basal surface of adherent cells. Whether the extent of clustering of focal-adhesion proteins is actually required for effective migration is unclear. We recently demonstrated that the depletion of major focal-adhesion proteins, as well as modulation of matrix compliance, actin assembly, mitochondrial activity, and DNA recombination, all converged into highly predictable, inter-related, biphasic changes in focal adhesion size and cell migration. Herein, we further discuss the role of focal adhesions in controlling cell spreading and test their potential role in cell migration.

**Morphology and dynamics of focal adhesions**

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**Recapitulation of Biphasic Relationship Between Focal Adhesion Size and Cell Migration Speed**

Fast-moving fish keratocytes, human leukocytes, and Dictyostelium discoideum cells display small focal adhesions at their basal surface, while slow-moving fibroblasts and endothelial cells display large focal adhesions.12-14 Therefore, a superficial comparison among migratory cells suggests that cells that feature small focal adhesions migrate more rapidly than cells that feature large focal adhesions. This disparate data suggests that the extent of clustering of focal-adhesion proteins into basal adhesion plaques would inversely correlate with cell migration. However, a rigorous assessment of the role of focal-adhesion clustering in the migration of isotypic cells has been lacking.

To assess the potential interplay between focal adhesion formation and cell migration, we measured the speed and persistence of migration of control...
manipulated the expression and activity of proteins that were (spatially and functionally) progressively further away from focal adhesion complexes. For instance, disassembly of actin filaments to block actomyosin-mediating force relay\(^\text{25}\) and depletion of the F-actin-crosslinking protein \(\alpha\)-actinin, which is functionally associated with force transduction between adhesion site and cytoskeleton\(^\text{26,27}\), induce changes in cell speed that are robustly predicted by corresponding changes in focal adhesion size. Deactivation of mitochondria and DNA recombination, which cell migration speed were biphasically related (Fig. 1D), i.e., as focal adhesion size increased, cell moved more rapidly; past a maximum threshold speed, cell migration decreased for increasing focal adhesion size. Importantly, neither the shape of focal adhesions, nor their number or the relative cell surface occupied by focal adhesions, nor the molecular composition of focal adhesions seems to predict cell migration.\(^8\)

To test the predictive power of this biphasic relation between focal adhesion size and cell migration speed, we manipulated the expression and activity of proteins that were (spatially and functionally) progressively further away from focal adhesion complexes. For instance, disassembly of actin filaments to block actomyosin-mediating force relay\(^\text{25}\) and depletion of the F-actin-crosslinking protein \(\alpha\)-actinin, which is functionally associated with force transduction between adhesion site and cytoskeleton\(^\text{26,27}\), induce changes in cell speed that are robustly predicted by corresponding changes in focal adhesion size. Deactivation of mitochondria and DNA recombination, which

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**Figure 1.** Focal adhesion size is a unique predictor of cell migration speed. (A–C) Effect of changes in substrate compliance—rigid glass (black), stiff (gray), and soft (white) polyacrylamide gels coated with collagen I, and depletion of focal adhesion proteins (FAK, paxillin, talin, and zyxin) on focal adhesion size (A), cell size (B), and cell migration speed (C). At least 30 cells per condition were analyzed to assess focal-adhesion and cell morphology and >50 cells per condition were tracked to assess cell motility. Error bars represent SEM. Multiple comparison to the control (i.e., WT cells on stiff substrates) was performed by 1-way analysis of variance (ANOVA) using the Dunnett post test. Significant statistical difference are shown as follows, ***\(P<0.001\), **\(P<0.005\), *\(P<0.01\). (D–F) Assessment of regression among focal adhesion size, cell size, and cell speed. Mean size of focal adhesion is biphasically and linearly correlated with cell speed (D) and cell size (E), respectively, while cell size is weakly correlated with cell speed either biphasically \((r^2 = 0.51)\) or linearly \((r^2 = 0.32)\). Gaussian (nonlinear) and linear models were tested to the data set ranged between 0 and 1 after normalization as \((x - x_\text{min})/(x_\text{max} - x_\text{min})\). Error bars represent SEM. Note that cell size is not statistically related to cell speed. (G) Schematic of prediction of cell speed by focal adhesion size. Cell speed is predicted by the mean size of focal adhesion not through regulation of cell size. Panels (A, C, and D) were reprinted from ref. 8.
had not been previously reported to play a role in cell migration or in the formation of focal adhesions,28-30 modulated focal-adhesion formation, and cell migration in ways quantitatively predicted by the pre-established biphasic relation. Finally, the biphasic relationship established with MEFs was further validated with HT-1080 cells, a highly tumorigenic human fibrosarcoma cell line. Together these results establish that focal-adhesion size uniquely and robustly predicts cell migration across cell types and extracellular conditions.8

The Interplay Between Cell Migration and Spreading

The adhesion between an adherent cell and its underlying substrate regulates cell migration speed biphasically.35 Cell-matrix adhesion strength may depend on the contact area between the cell and its adhesive substrate (i.e., cell spreading size),32 cell mechanics and contractility,33 the level of expression and activation of focal adhesion kinase (FAK) for integrin-stimulated cell migration, we have addressed a long-standing question in cell biology: whether morphology of focal adhesions is functionally related to cell migration. The power of such analysis is increased substantially by using a combination of genetic and mechanical perturbations as well as blind tests. Results from this analysis show that: (1) the mean size of focal adhesions—not their shape or their number per cell—predicts cell migration across cell types and (2) the mean size of focal adhesions predicts cell spreading, while cell spreading does not predict cell migration.

These results may have important implications in biomedical research: defects in organ and tissue development or disease resulting from the onset of or defects in cell migration may occur through misregulated changes in focal adhesion size. This provides for a conceptually new pharmacological target of disease: not a specific molecular target, but a morphological descriptor of an organ—focal adhesion size.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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