CELL MIGRATION AND POLARITY ON MICROFABRICATED GRADIENTS OF EXTRACELLULAR MATRIX PROTEINS

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1. INTRODUCTION

During embryonic development, cells migrate to form spatially segregated, specialized tissues. This targeted cell migration is required for proper tissue formation. In particular, substrate gradients are thought to direct cell migration in the epithelium of the small intestine.[1] Stem cells proliferate and undergo differentiation while migrating from the base of the crypt of Lieberkühn to the base of the villus.[2] The differentiated cells then continue to migrate upward to the tip of the villus where they are finally exfoliated into the intestinal lumen. Extracellular matrix (ECM) proteins beneath the epithelium reportedly influence multiple cellular functions such as proliferation, differentiation, migration, and tissue-specific gene expression.[1] The most significant finding yet to support a possible relationship between ECM protein expression and intestinal cell functions is the spatial gradient of laminin isoforms along the crypt – villus axis (Figure 1A). The expression of laminin-1 gradually increases from the crypt – villus junction to the villus tip. Conversely, the laminin-2 expression decreases with the distance from the base of the villus. The reciprocal expression of these laminin isoforms also complements the expression of their cell-membrane counterparts, the integrins. Hence, the relationship between the spatial patterns of laminin and integrins suggest that the migration of undifferentiated cells from the crypt may be directed to the villus by their interactions with the ECM proteins. Here we explore the effects of the surface density and concentration profiles of extracellular matrix proteins on the migration of rat intestinal IEC-6 cells.

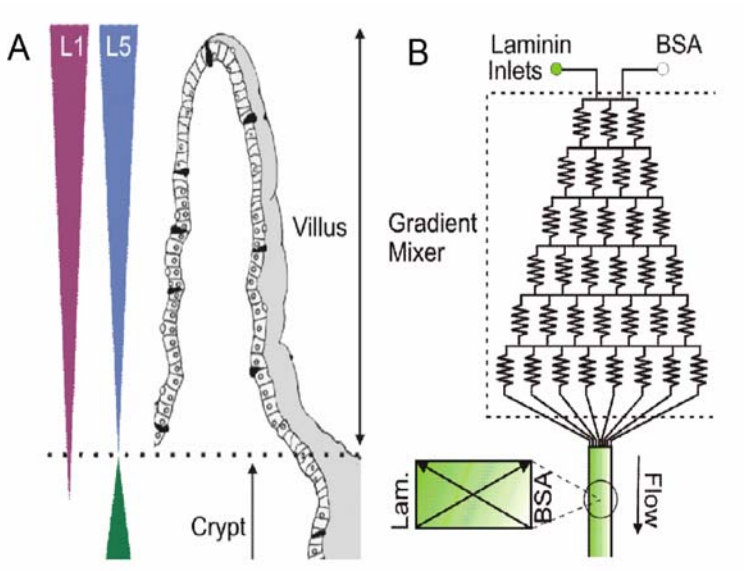

Figure 1. (A) *In Vivo* expression profile of laminin isoforms along the crypt – villus axis of the small intestine: laminin-1 (L1), laminin-5 (L5), and laminin-2 (L2). (B) Microfluidic network design used to recreate ECM protein gradients.

2. EXPERIMENTS

Microfluidic devices were used to create linear, immobilized gradients of laminin (Figure 1B). The bulk concentrations of proteins in the feed streams in the mixing device determined the gradient profile and the local concentration of laminin in the device. Two sets

of gradients were used to explore cell migration directedness: (i) gradients with similar change in local concentration, i.e. the same gradient steepness, and (ii) different gradients with similar local concentrations. IEC-6 migration was monitored on these substrates using time-lapse microscopy. Cells were also stained for actin and vinculln localization.

3. RESULTS

IEC-6 cells migrated up the gradients, independent of the steepness of the gradients used in this study. At the same local laminin concentration, the migration rate was independent of the gradient steepness. However, cell directedness decreases as the local concentration (laminin surface density) increased. Actin and vinculin staining revealed that cell morphology was polarized at the lower laminin surface densities of the gradient. When looking at the laminin gradient generated using a solution concentration of 25µg/mL, vinculin was punctuated at the lamellipodium and at the rear of the cell. Actin formed long stress fibers along the migration axis. In contrast, the rear end of the cell broadened and rounded at high local laminin density. The cells formed greater numbers of focal adhesion at both the front and rear of the cell, so that the difference between the front and rear is less apparent.

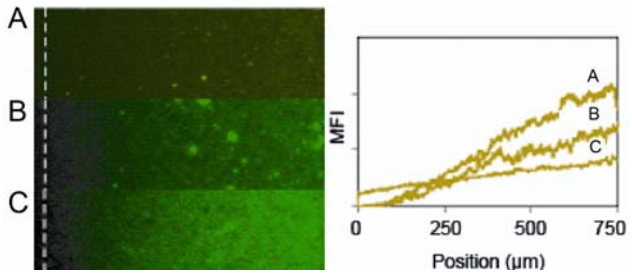

Figure 2. Fluorescence micrographs of linear gradients of laminin covalently bound to the alkanethiol monolayer. The gradient slope was adjusted by altering the initial laminin concentration: (A) 5 (10), (B) 10 (15), and (C) 25 µg/mL (34 pg/dm^2•µm).

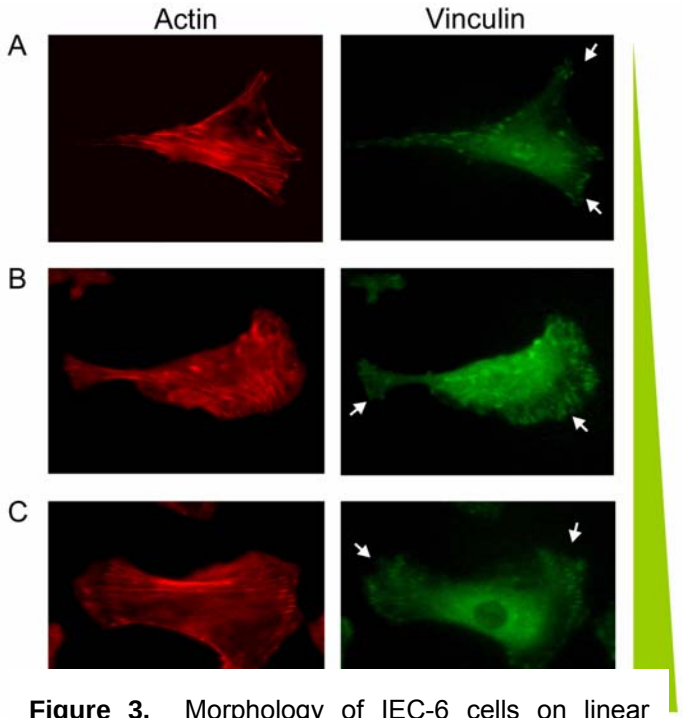

Figure 3. Morphology of IEC-6 cells on linear gradients of laminin at 34 pg/dm^2 •µm). Cells on the gradients were stained with anti-vinculin antibody (green) and phalloidin (red). The fluorescent image of cells was taken within ±75 µm of threee positions on the 750-µm wide gradients (A) 100, (B) 375 and (C) 650 µm from the channel side where laminin concentration is the lowest.

4. REFERENCES

1. Beaulieu, J.-F., *Recent Work with Migration/Patterns of Expression: Cell-Matrix Interactions in Human Intestinal Cell Differentiation.* The Guts as a Model in Cell and Molecular Biology, ed. D.W. F. Halter, and N.A. Wright. 1997, Hingham, MA: Kluwer Academic Publishers. 165-179.
2. Beaulieu, J.-F., *Integrins and Human Intestinal Cell Functions.* Frontiers in Bioscience, 1999. **4**: p. d310-321.