Differential Response of Epithelial Cells to Surfaces Containing Both Cell-Cell and Cell-Matrix Ligands

Tsai J, Patel L, and Kam LC
Department of Biomedical Engineering, Columbia University, New York, NY

Introduction

Proper tissue integrity and function depends on each cell balancing cell-cell and cell-matrix interactions; alterations of this balance have large implications in tissue function, and lead to a variety of pathological conditions, such as tumor metastasis. Alternation of epithelial (E)-cadherin function, for example, has high correlation with invasive potential. This report compares the integration of cell-cell and cell-matrix interactions by normal and metastatic epithelial cells. Specifically, MCF-7 and MDCK cells expressed different morphologies and concentrations of basolateral E-cadherin on surfaces coated with fibronectin, E-cadherin and a micropatterned mix of these proteins.

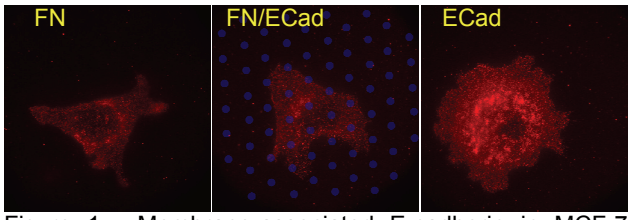

Figure 1. Membrane-associated E-cadherin in MCF-7 cells seeded on fibronectin (FN), fibronectin patterned E-cadherin (FN/Ecad), and E-cadherin (Ecad) modified surfaces. Blue dots indicate FN on the FN/Ecad surface. The field of view of each image is 80 μm.

Materials and Methods

Surface Preparation: Glass coverslips containing only fibronectin (FN, 25 μg/ml) or Ecadherin (ECad, 30μg/ml) alone were coated for 1 hour at 37C, then rinsed with phosphate buffered saline (PBS). Micropatterned FN/ECad surfaces were prepared by microcontact printing hexagonal arrays of 3-μm diameter dots of FN, spaced 10 μm apart, followed by coating with Ecad (30 μg/ml) for 1 hour at 37C. All substrates were blocked with 1 mg/ml bovine serum albumin for 30 minutes.

Cell Culture: MCF-7s and MDCKs were dissociated from tissue culture dishes with 0.05% trypsin with 0.5mM EDTA and re-suspended in either high glucose DMEM supplemented with 5% FBS, antibiotics, and 2mM L-glutamine (MCF-7) or low glucose DMEM supplemented with 5% FBS, antibiotics, 2mM L-glutamine, and 110 mg/mL sodium pyruvate (MDCK). Cells were seeded onto FN, Ecad, and FN/Ecad modified surfaces, allowed to adhere and spread for 3 hours, then fixed with 4% parafix and 0.05% triton X-100. MDCK cells expressing a DsRed-tagged E-Cadherin, were graciously provided by James Nelson (Stanford University).

Immunofluorescence staining: Immunostaining was performed using mouse anti-human-cytosolic-E-cadherin antibody (Invitrogen-Zymed) and followed by goat-anti-mouse-A568 (Invitrogen).

Results and Discussion

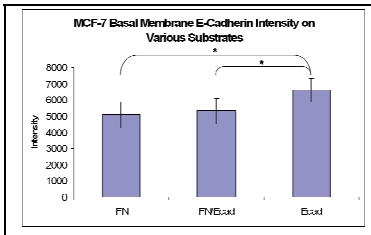

Figure 2. E-cadherin staining intensity, averaged over the area of the cell. *p < 0.01 by ANOVA, n=7 cells from a representative experiment.

On surfaces coated with FN alone, MCF-7 cells exhibited a motile morphology (Fig. 1). In contrast, MCF-7 cells on Ecad-only surfaces exhibited a symmetric, spread morphology, along with bright concentrations of this protein on the basolateral surface. Moreover, a higher, average level of E-cadherin was observed on the basolateral surface of cells on ECad than on FN (Fig. 2). On FN/Ecad surfaces, MCF-7 cells exhibited morphology similar to that on FN alone and a level of E-cadherin that was significantly less than on Ecad alone, suggesting that at this timepoint, cell-matrix interactions suppress cell-cell communication in these cells, even though FN only occupies a small fraction of the surface area.

In contrast, MDCK cells were able to recognize and respond to substrate-immobilized E-cadherin in the presence of FN dots (Fig. 3). This result suggests that early responses of normal epithelial cells to a complex environment may be determined more by cell-cell communication than by cell-substrate interactions.

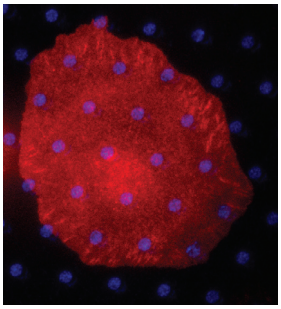

Figure 3: MDCK cells recognize and respond to substrate immobilized ECad in the presence of the cell-matrix protein FN (blue dots). This interaction is evidenced by the long, bright features of E-cadherin (red) evident in this representative image. FN dots (blue) are spaced 10 μm apart.

Conclusions

We present evidence that normal and tumorgenic epithelial cells respond differentially to an environment presenting both cell-cell and cell-matrix ligands. These data suggest a novel outside-in modulatory role of cell-matrix adhesion, most likely through integrin regulatory pathways, on E-cadherin expression and engagement by cells that have already started to differentiate to an invasive phenotype. These results may have long-term implications on the design of materials that take advantage of multiple signaling modalities to promote specific and desirable responses from cells.

Acknowledgements: This study was supported in part by the NIH (EY016586) and the Whitaker Foundation (SDA to BME at Columbia University).