Rigidity-Dependent Crosstalk between ECM and Cadherin Signaling

Jones Tsai and Lance C. Kam
Department of Biomedical Engineering, Columbia University, New York, NY

Statement of Purpose

Epithelial cells coordinate cadherin-based links with adjacent cells and integrin-mediated connections with an underlying ECM. An important example of crosstalk between these pathways is inhibition of cadherin function by integrin engagement (1-3). Recent experiments have separately established *in vitro* that integrin signaling is sensitive to the mechanical properties of the underlying substrate (4, 5), posing the possibility that integrin/cadherin crosstalk may be modulated by substrate rigidity. This study seeks to determine if integrin-cadherin crosstalk is indeed rigidity dependent by examining the ability of MCF-7 epithelial cells to concurrently engage fibronectin (FN) and E-cadherin (EcadFc) presented on substrates of varying rigidity. Substrates were patterned with 3-μm diameter FN dots, separated by 10 μm center-to-center, and surrounded by EcadFc in order to recapitulate the natural separation of these signals at basolateral surface of the cell (6, 7).

Materials and Methods

Substrate preparation. Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning) was prepared as millimeter-thick layers cast on glass. "Rigid" PDMS (E=5 MPa) and "soft" PDMS (E=60 kPa) was prepared by controlling the ratio of curing agent to polymer base and curing temperature. Stress-relaxation tests were conducted to verify the elastic moduli. Control surfaces were prepared by coating with either FN (human plasma, Sigma) at 100ug/ml or EcadFc (human E-cad/Fc fusion protein, R&D Systems) at 20ug/ml, both for 1 hr at 37°C. Patterned surfaces were prepared by microcontact printing using established methods (8), followed by coating with EcadFc. All surfaces were blocked in 100ug/mL BSA for 1 hr at 37°C.

Cell experiments. MCF-7 cells (ATCC) were cultured in DMEM supplemented with 10% FBS and penicillin / streptomycin. For experiments, cells were seeded at ~5,000 cells/cm^2. Five hours after seeding, cells were fixed and stained using antibodies for the cytosolic domain of E-cadherin (clone 4A2C7, Zymed) and paxillin (clone H-144, Santa Cruz Biotech).

Results

Inhibition of E-cadherin response by Fibronectin on glass substrates: On FN-coated controls glass surfaces, all cells formed clusters of paxillin, indicative of focal adhesions (FA), while cells on EcadFc-coated surfaces formed elongated cadherin structures (CA). On FN/EcadFc patterned glass, cells established FAs on the FN, but no CA structures on the EcadFc regions (Fig.).

Inhibition is dependent on substrate rigidity: To determine the effect of rigidity, these patterned FN/EcadFc experiments were carried out on elastomer substrates of controlled rigidity. MCF-7 cell response on the rigid PDMS substrates was similar to that on glass. In contrast, cells on patterned, soft PDMS substrates were able to concurrently form FAs and CAs (Fig. 1).

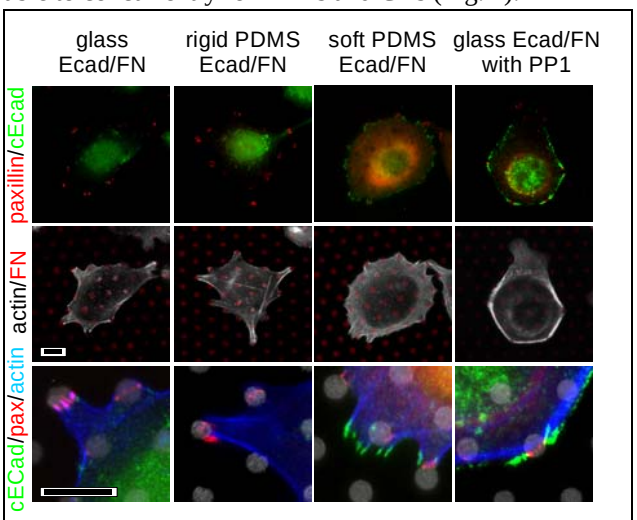

Figure 1: Integrin-cadherin crosstalk is dependent on rigidity and SFK activity. Scale bars = 10 μm.

ECM-cadherin Crosstalk as a Function of SFK: On patterned glass substrates, application of the SFK-inhibitor 10uM PP1 over the duration of cell interaction abrogated inhibition of CA formation and indeed promoted formation of dense CA structures (Fig. 1).

Discussion

Changes in tissue rigidity are associated with a range of physiological and pathological processes. In particular, inhibition of cadherin signaling as a result of increased integrin function is characteristic of epithelial-mesenchymal transformation, a step in the progression of cancer of epithelial tissues. While limited to interactions on the order of several hours, our demonstration that integrin-cadherin crosstalk is rigidity dependent sheds new light onto these processes. Our additional observation that this modulation occurs without large scale disruption of FA structures also suggests a new level of complexity in cell biomechanics.

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References

1)Chen *et al.* Curr. Op. Cell Bio. 18:572-8 2006. 2) Wang *et al.* PNAS 103:1774-9 2006. 3) de Rooij *et al.* JCB 171:153-164. 4) Choquet *et al.* Cell 88:39-48 1997. 5) Pelham *et al.* PNAS 94:13661-5 1997. 6) Perez *et al.* Langmuir 21:11963-8 2005. 7) Hunt *et al.* FEBS Letters 583:4539-4543. 8) Kam *et al.* JBMR 55;487-495 2001. 9) Tan *et al.* PNAS 100:1484-9 2003.