Actin and SRF transduce physical cues from the microenvironment to regulate keratinocyte terminal differentiation

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Statement of Purpose: The goal of this study was to develop micro-patterned substrates, which allow us to precisely and quantitatively control cell-ECM interactions of individual human keratinocytes, and to examine the role of these signals in terminal differentiation.

Methods: Micro-patterned substrates were generated by micro-contact printing and surface initiated polymerization of oligo(ethylene glycol methacrylate) (MW 300) brushes. The polymer brush served as a protein resistant background surrounding circular or elliptical gold islands with areas of 314-1963 μm^2 (20-50 µm diameter). The gold islands were coated with 1-20 µg/ml of type I collagen, laminin, or fibronectin and rinsed with PBS prior to cell seeding. Primary human keratinocytes were seeded onto the substrates at 25,000 per cm^2. Non-adherent cells were rinsed off after 1h and the adherent cells were cultured up to 24h in FAD medium alone or supplemented with the indicated cytoskeletal inhibitors. Expression of involucrin, transglutaminase, periplakin, and Ki67 was detected by immunofluorescence staining. The F-actin cytoskeleton was stained with TRITC-phalloidin and focal adhesions were visualized by vinculin immunostaining. Gene expression of individual keratinocytes was examined by single-cell PCR. Serum response factor (SRF) transcriptional activity was measured using an SRF luciferase reporter and the Dual-glow Luciferase kit (Promega) according to the manufacturer's instructions. All transfections (siRNA or cDNA) were performed using Lipofectamine 2000.

Results: The initial population of keratinocytes seeded onto the substrates was negative for involucrin and positive for Ki67 (Fig 1). After 24 hours, there was a significant increase in the number of involucrin positive cells on the smallest islands and an inverse correlation between the number of differentiated cells and adhesive area (Fig 1). The same relationship was also observed for the differentiation markers, transglutaminase 1 and periplakin (not shown). Limited adhesion also reduced Ki67 expression (Fig 1), BrDU incorporation, and expression of the stem cell genes, LRIG1 and DLL1 (not shown). The effect of island size on terminal differentiation did not depend on ECM type or density (not shown). However, there were significantly fewer involucrin positive cells on more elongated islands compared to circular islands of equal area (Fig 2). Keratinocytes on 20µm islands displayed a dense cortical shell of F-actin and reduced focal adhesions, whereas cells on 50µm islands had distinct F-actin filaments on the basal surface and robust focal adhesions (Fig 3). Treatment with Latrunculin A and Y27632 blocked shape-induced involucrin expression, and Jasplakinolide and Cytochalasin D induced involucrin on all substrates (Fig 3). The effects of Latrunculin and Cytochalasin correlated with SRF transcriptional activity, and knocking down SRF or its co-factor MAL inhibited differentiation (Fig 3). Overexpression of MAL stimulated differentiation on non-patterned substrates (Fig 3).

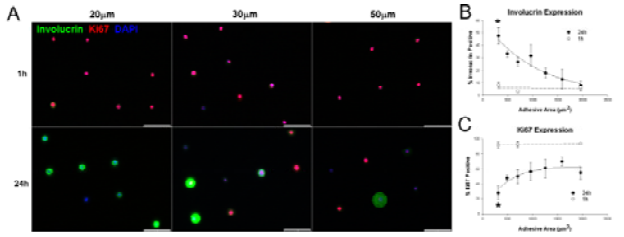

Figure 1: (A) Involucrin and Ki67 expression on micro-patterned substrates, 20-50µm diameter. (B-C) Quantification of involucrin and Ki67 positive cells. N=4 experiments, *P<0.05 relative to 1963µm^2.

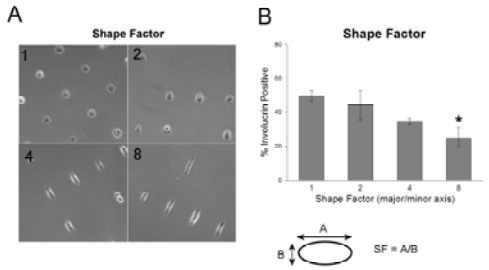

Figure 2: (A) Phase contrast images of keratinocytes on islands with shape factors 1-8. (B) Quantification of involucrin positive cells after 24h. N=4 experiments, *P<0.05 relative to SF1.

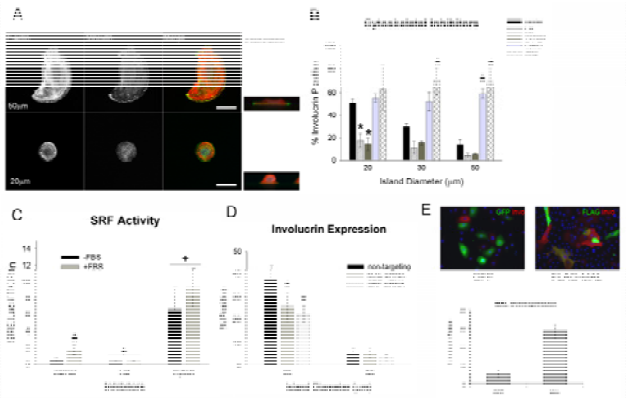

Figure 3: (A) F-actin and vinculin localization. (B) Involucrin expression on patterned substrates and treated with cytoskeletal inhibitors. *relative to carrier (C) Relative SRF reporter activity. *relative to –FBS, +relative to carrier (D) Involucrin expression on patterned substrates following MAL or SRF knockdown. *relative to non-targeting (E) Involucrin expression in keratinocytes over-expressing GFP or MAL on non-patterned surfaces. N=4 experiments, P<0.05 for all.

Conclusions: Together, these results indicate that the actin cytoskeleton mediates shape induced terminal differentiation in human keratinocytes via altered SRF transcriptional activity. Moreover, these findings demonstrate how biophysical cues from the surrounding microenvironment are transduced into transcription responses that regulate cell fate decisions.