Desmosomes pattern cell mechanics to govern epidermal tissue form and function

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Abstract

The epidermis exhibits polarization of structural and functional features across multiple cell layers, but how this polarity is created and preserved is poorly understood. Desmosomes (DSMs) are cell-cell adhesive organelles linking to intermediate filaments (IF) and are critical for signaling and tissue integrity. The DSM cadherin desmoglein 1 (Dsg1) is first expressed as cells commit to differentiate and stratify, with expression peaking in the granular layer (SG). We hypothesize the pattern of Dsg1 and associated IF is crucial for establishing proper epidermal polarity, especially mechanical polarity. To test this, we used laser ablation and whole mount imaging to assess layer-specific mechanical properties of 3D epidermal cultures. These data support an epidermal mechanical gradient: keratinocytes are under compressive forces in the basal layer and high tension in the suprabasal layers. We previously showed Dsg1 regulates basal cell forces to promote stratification. Here, a DSM/IF uncoupling mutant recapitulates Dsg1 loss, suggesting a requirement of IF attachment. High apical tension in simple epithelia helps generate barrier-forming tight junctions (TJs). TJs localize to the SG in epidermis, where tension is high and Dsg1 expression peaks. Thus, we assessed the ability of Dsg1 to polarize tension and TJs in the SG. Dsg1 loss disrupts the polarized SG distribution of the tension sensitive TJ component ZO1. Our data suggest Dsg1 modulates TJs through the mechanosensitive kinase ErbB2, which localizes to the TJ forming layer. Dsg1 loss disrupts this localization and decreases total and phosphorylated ErbB2. Uncoupling the DSM/IF linkage or inhibiting ErbB2 significantly decreases epidermal electrical barrier function. As mechanical forces regulate cell behavior, our data suggest a model in which patterning of DSM components regulates epidermal polarity by orchestrating the distribution of mechanical inputs to drive morphogenesis and development of a life-essential barrier.

Disrupting the desmosome/IF connection impairs stratification

Disrupting the desmosome/IF connection alters basal cell mechanics

Using laser ablation, we analyzed the mechanical properties of basal cells in early and mature raft cultures: Day 1 and Day 6 after lift to air/liquid interface, respectively. Cells expanded after ablation at early timepoints, indicating tension. Cells collapsed at late timepoints, suggesting cells were under compressive forces. At both timepoints, uncoupling the DSM/IF linkage with DPNTP reduced the amount of displacement observed. Expression of DPNTP had no effect on cell density (right).

Disrupting the desmosome/IF connection impairs epidermal barrier function

Epidermal raft cultures were grown on transwell inserts and transepidermal electrical resistance experiments were performed as the rafts developed. Confocal images show expression of GFP (control) and DPNTP-GFP expression in the granular layer, which contains barrier-forming tight junctions (shown with ZO1 staining). DPNTP-GFP expressing cells had reduced ZO1 staining and DPNTP-GFP expressing raft cultures showed reduced barrier function over the course of raft development.

Dsg1 modulates the protein levels of ErbB2

Previous work from our lab showed that Dsg1 can regulate the activity of members of the ErbB family of receptor tyrosine kinases. (A) Time course of ErbB2 and EGFR expression and activation in epidermal equivalent raft cultures. (B) ErbB2 immunostaining in normal human epidermis. It is localized where tight junctions form. (C) Depletion of Dsg1 results in a decrease in both total and active ErbB2 as well as a loss of cell-cell border localized ErbB2 in epidermal equivalent raft cultures.

Simple vs. stratified epithelia

The central question we want to address is how to go from a single layer simple epithelium to a multilayer stratified epithelium, like the epidermis. Similar to simple epithelia, the epidermis is polarized and components including tight junctions are localized to the most superficial living layer (the granular layer). While the classical cadherin family member E-cadherin is expressed in a mostly uniform pattern, components of the DSM are expressed in a differentiation-specific pattern. For example, Dsg1 is only expressed in stratified epithelia and begins to be expressed as cells transit from the basal layer and is concentrated in the upper layers of the epidermis.

Molecular components of epithelial desmosomes

(Left) Schematic representation of the major protein constituents of desmosomes: PM, plasma membrane; DP, desmoplakin; Dsg, desmoglein; Dsc, desmocollin; PKP, plakophilin; PG, plakoglobin. (Right) DPNTP is a dominant negative mutant of DP that lacks the ability to bind to IF and uncouples the IF cytoskeleton from desmosomes.

Is the desmosome/IF connection important for early stages of tissue development?

To examine the effect of disrupting the DSM/IF connection on early stage stratification, we performed sorting assays using a 2D normal human epidermal keratinocyte (NHEK) cell culture model. Genetically manipulated cells expressing a fluorescent protein (e.g. GFP) were mixed with unlabeled wildtype cells at a known ratio. The percentage of labeled cells was calculated for the basal and suprabasal layers and compared to the predicted to determine effects on stratification.

Modeling human epidermis with epidermal equivalent raft cultures

In order to assess the potential role of DSMs at later developmental stages of stratified epithelium, we utilized an organotypic epidermal equivalent raft culture model. (A) NHEKs are plated on top of collagen plugs seeded with fibroblasts. This plug is then lifted to an air-liquid interface to induce the process of epidermal stratification. (B) The raft cultures exhibit morphology very similar to human skin, with all four layers.

Evidence of a mechanical gradient in the epidermis

Using two-photon laser ablation in live epidermal equivalent raft cultures, we showed that the initial displacement post ablation is greater in the upper layers (magenta) of the culture compared with the lower layers (green). Moreover, the basal layer (blue) transitions from an outward expansion upon ablation to a collapse, suggesting a transition from tension to compression. Together, these data indicate there is a mechanical gradient in the epidermis, with the upper living layers experiencing the most tension. Bar = 5 μ m

Evidence of Dsg1-mediated effects on the mechanical gradient in the epidermis

Three dimensional renderings of whole mount staining of tension-sensitive adherens junction component vinculin (A) and tight junction component ZO1 (B) indicate Dsg1-depletion shifts tension downward into the lower layers. Images are presented with a look up table that depicts z-depth in the indicated colors. The basal layer is in blue and teal, spinous in green and yellow, and granular in red and pink. Right panels show representative staining in each layer. (C) The effect of Dsg1 loss on cell-cell junction localized ZO1 is cell autonomous. (D) In addition, depletion of Dsg1 results in a decrease in the protein expression of tight junction components. Bars = 20 μ m.

The role of ErbB2 in regulating tight junctions

Day 9 epidermal equivalent raft cultures were treated with the ErbB2 specific inhibitor TAK165 for 48 hours prior to harvesting. (A) Inhibiting ErbB2 with TAK165 resulted in a disruption in the polarized distribution of tight junctions. ZO1 localization to cell-cell interfaces was lost in the granular layers and increased in the lower layers. Inhibition of ErbB2 also resulted in a decrease in cortical F-actin staining. (B) TAK165 treatment resulted in a decrease in the protein levels of tight junction components and (C) a decrease in transepidermal electrical resistance (*p=0.006). This suggests that Dsg1 may regulate tight junctions through ErbB2.

Summary

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