Functional consequences of keratin isoform switching during epidermal differentiation
6-month progress report

Objective

Keratin filaments form strong mechanical scaffolds, stabilizing the epidermis.[1] During epidermal differentiation, the composition of keratin filaments shifts, with increased expression of differentiation-associated keratin isoforms relative to basal epidermal keratins.[2] Our overall goal is to determine whether this shift in keratin isoform mixture is merely a marker of epidermal differentiation or functionally contributes to the differentiated cell state. We hypothesize that, independent of the more general mechanical scaffold function, isoform-specific interactions allow differentiation-associated keratins to organize signal transduction cascades driving epidermal differentiation, and that ichthyosis-causing mutations disrupt these signaling pathways.

Progress to date

In the first 6-months of this project period, we have created keratinocyte cell lines engineered with modestly increased expression of keratin 5 (K5), a basal epidermal keratin, or K1, a differentiation-associated keratin.[3] These K5^{high} and K1^{high} cell lines show no evidence of disrupted mechanical integrity, barrier function, or growth behavior, and retain the ability to differentiate when cultured at an air-liquid interface. However, we identified subtle morphological differences in epidermal organoids grown from these cells. In the spinous layers, but not in the basal layer, K1^{high} cells adopted a flatter morphology than K5^{high} cells, suggestive of accelerated differentiation (Figure 1).

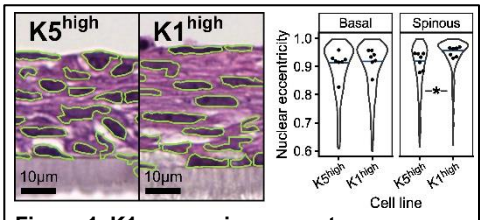

Figure 1. K1 expression promotes differentiated-like cell morphology. Hematoxylin- and eosin-stained sections of epidermal organoid cultures grown from K5^{high} or K1^{high} keratinocytes. Cell nuclei are segmented and nuclear eccentricity calculated for cells in the basal and spinous layers. Higher eccentricity indicates greater elongation.

To further investigate this observation, we grew epidermal organoids from a mosaic population of keratinocytes including both K5^{high} and K1^{high} cells marked by different fluorescent proteins. We then imaged these organoids using 2-photon microscopy to capture three-dimensional sections extending through the entire organoid depth (Figure 2, left). Using a custom computer vision pipeline, we segmented cells within the volume, "unwrapped" the basal and spinous layers[4], and quantified the proportion of K5^{high} and K1^{high} cells present in each layer (Figure 2, center). Compared to K5^{high} cells, K1^{high} cells were systematically enriched in the upper spinous layers of the cultures (Figure 2, right). Since K1 expression in the K1^{high} cell line is engineered, not simply induced by the normal process of epidermal differentiation, this result indicates that K1 in fact drives cell behaviors that are part of the epidermal differentiation program, and does not merely reflect a differentiated cell state.

To investigate the effect of ichthyosis-causing mutations on K1 function, we engineered a keratinocyte cell line expressing K1^{L161P}, a causal variant of epidermolytic ichthyosis[5], along with wild-type K1. Expression of K1^{L161P} resulted in significant disruption of the keratin filament cytoskeleton (Figure 3). In our initial attempts, K1^{L161P} expression prevented the growth of epidermal organoids, which rely on tight barrier formation to maintain the air-liquid interface that stimulates differentiation. As outlined below, we plan to attempt strategies to mitigate or overcome K1^{L161P}-mediated filament network disruption.

Plans for the next 6-months and long-term outlook

Given the striking enrichment of K1^{high} cells in the upper spinous layers of mosaic epidermal cultures, we are proceeding to test for K1-specific interactions that could explain this behavior. We are preparing cell lines

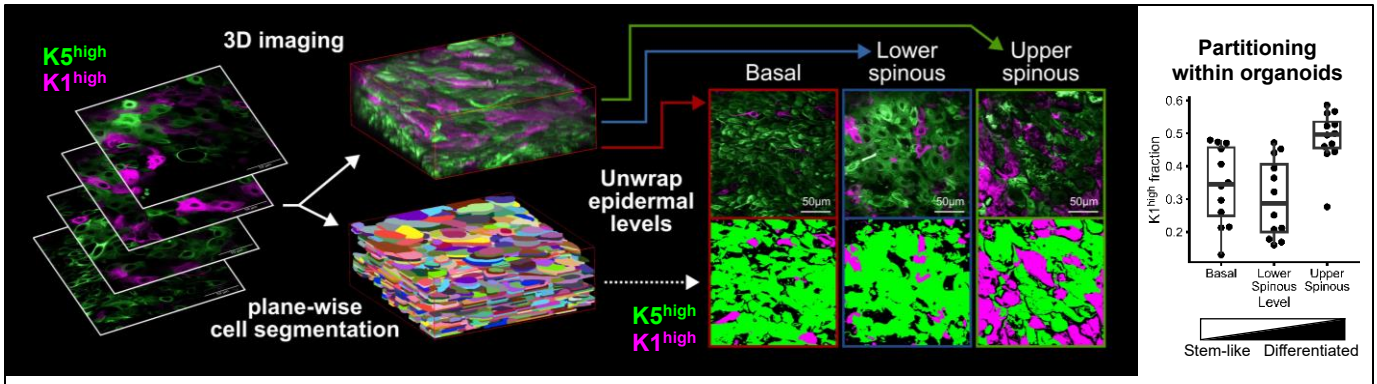

Figure 2. K1 expression drives cell partitioning into upper organoid layers. In epidermal organoids grown from mixtures of K5^{high} and K1^{high} cells, K1^{high} cells accumulate in the upper layers, associated with a differentiated stratified epithelial cell state.

expressing K5 or K1 fused to a promiscuous biotin ligase, allowing us to isolate keratin-associated proteins and identify them by mass spectrometry.[6] By comparing $K1^{high}$ to $K5^{high}$ cell lines, this proximity biotin labeling assay will allow us to identify keratin isoform-specific interactions, including those that might organize signaling pathways driving differentiated cell behavior specifically when K1 expression is increased.

Disruption of keratin filaments by the $K1^{L161P}$ mutant complicates our plan to test the influence of ichthyosis-causing mutations on keratin filament interacting proteins, since any interactions altered by $K1^{L161P}$ expression might be due to keratin filament disruption in general, rather than an isoform-specific effect per se. We will attempt two strategies to overcome this complication. First, we will use fluorescence-activated cell sorting to select for cells expressing lower levels of $K1^{L161P}$. If low expression preserves keratin filament structure, a $K1^{L161P}$ construct can be used directly for proximity biotin labeling. As an alternative approach, we can co-express equal levels of $K1^{L161P}$ in $K1^{high}$ or $K5^{high}$ cells, expecting equivalent filament network disruption in both lines. Proximity biotin labeling will then allow us to compare K1 and K5 interacting proteins in the setting of filament disruption ($K1^{L161P}$ co-expressing cells) versus intact filaments (wild-type background). Although indirect, this approach would allow us to infer which keratin interactions depend on intact filaments and which interactions depend on specific isoforms, potentially distinguishing mechanical and non-mechanical mechanisms of ichthyosis-causing mutations, an important distinction which existing approaches have typically been unable to address.

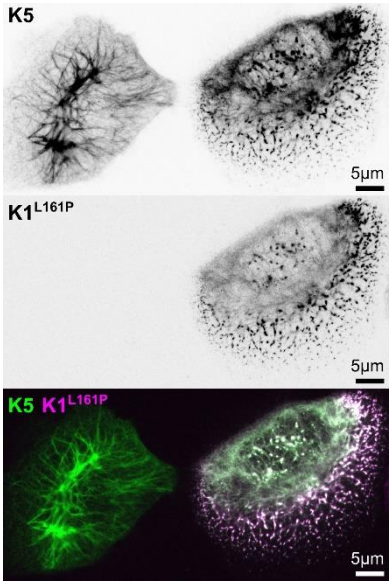

Figure 3. Ichthyosis-causing mutation $K1^{L161P}$ disrupts the keratin filament network. Two keratinocytes expressing fluorescently labeled K5. The cell on the right co-expresses $K1^{L161P}$, while the cell on the left does not.

Ultimately, by identifying isoform-specific keratin interactions that are disrupted in ichthyosis, these studies aim to better define the fundamental signaling networks that control epidermal structure and function. Even if the direct effect of ichthyosis-causing keratin mutations is on the keratin mechanical scaffold, the keratin signaling scaffold may reveal potential therapeutic targets to compensate for the mechanical barrier defect in ichthyosis.

Lay summary

Proteins called keratins form scaffolds within skin cells, allowing the skin to resist mechanical stress. Mutations in keratin genes can disrupt this scaffold and cause certain forms of ichthyosis. Our goal is to understand why different keratins are expressed in different layers of the skin, how these different keratins shape cell behavior beyond their mechanical function, and whether ichthyosis-causing mutations also disrupt these non-mechanical functions. Our preliminary data so far show that different keratin proteins directly contribute to maturation of the skin barrier, and we are currently searching for the molecular interactions underlying this behavior. Understanding the molecular interactions specific to different keratin proteins given us an important window into the molecular signals controlling skin barrier formation and may reveal entirely novel therapeutic targets for ichthyosis and other diseases where the skin barrier is disrupted.

References

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