Cell Reports
Preview

More Than the Sum of Its Parts: Single-Cell Transcriptomics Reveals Epidermal Cell States

Ka-Wai Mok,[1,3] Nivedita Saxena,[1,2,3] and Michael Rendl[1,2,*]
[1]Black Family Stem Cell Institute, Department of Cell, Developmental and Regenerative Biology, Department of Dermatology, Icahn School of Medicine at Mount Sinai, NY 10029, USA
[2]Graduate School of Biomedical Sciences, Icahn School of Medicine at Mount Sinai, NY 10029, USA
[3]Co-first author
*Correspondence: michael.rendl@mssm.edu
https://doi.org/10.1016/j.celrep.2018.10.041

Compared to mouse models, less is known about human epidermal cell states and differentiation. In this issue of *Cell Reports*, Cheng et al. (2018) dissect the cell states and heterogeneity in human epidermis with large-scale transcriptomics of 92,889 single epidermal cells from normal and inflamed skin.

Understanding epidermal development and cellular diversity is paramount for elucidating how skin pathologies arise. The paradigm of epithelial lineage specification and differentiation begins early in embryogenesis with a single layer of epidermal progenitors, which differentiate through basal, proliferative keratinocytes upward to form a protective layer with barrier function (Blanpain and Fuchs, 2009). Epidermal progenitors also give rise to skin appendages such as hair follicles (Gonzales and Fuchs, 2017). Even in well-characterized mouse models, there is debate about whether one or two distinct basal stem cell populations together with downstream progenitors function to maintain the epidermis throughout life (Rognoni and Watt, 2018), and yet, much less is known in humans. While keratinocytes along the differentiation trajectory have been classified morphologically and with specific markers, questions regarding the (1) heterogeneity among each population, (2) hierarchical relationships between populations, and (3) existence of rare populations and cell types without specific locations remain unanswered. Adding complexity to the generalized scheme of epidermal differentiation are the different functional requirements of skin epithelium between anatomical sites. It stands to reason that state-specific and anatomical site-specific cellular differences are accomplished through exquisitely regulated transcriptional programs. The recent breakthrough in single-cell RNA sequencing (Heitman et al., 2018) now enables resolving cellular heterogeneity, ordering cells along a developmental trajectory, and discovering rare subpopulations (Grün et al., 2016; Saunders et al., 2018). In this issue of *Cell Reports*, Cheng et al. (2018) employed this powerful technique to tease apart the differences in human epithelial differentiation and cellular heterogeneity in three anatomical sites—scalp, trunk, and foreskin. Additionally, to elucidate the pathological changes in inflamed epidermis, the authors compared single-cell transcriptomes of psoriatic truncal epidermis and healthy epidermis. For high transcriptomic resolution, they performed large-scale sequencing of 92,889 individual epidermal cells to define human skin epithelial cell states, putative epidermal differentiation trajectories, and region-specific molecular signatures of both non-inflamed and inflamed skin (Figure 1).

Clustering analyses revealed many expected cell states conserved across scalp, trunk, and foreskin epidermal isolates, including those of basal and differentiating spinous and granular epidermal layers, as well as of epithelial cells associated with hair follicles (termed "follicular"). Interestingly, the authors identified three additional populations they termed "WNTI", "mitotic", and "channel". The WNTI cluster is enriched in WNT signaling antagonists and is likely comprised of stem-cell-containing hair follicle outer bulge cells, while the mitotic cluster is enriched in DNA synthesis and proliferation genes. The authors also describe the channel cluster, which is enriched in ion and mitochondrial transcripts, as a previously undescribed human epidermal cell state. Further study is needed to validate this population and fully characterize its role. Pseudotime analyses reveal four distinct putative differentiation paths, all beginning with basal keratinocyte clusters. They positively identified the well-characterized upward differentiation of basal keratinocytes. The remaining three differentiation trajectories pass through the mitotic cluster, and separately end in the channel, follicular, or WNTI clusters. While many of these cell states can be found in spatially restricted patterns, the authors found mitotic and the newly described channel cells sporadically interspersed in basal and suprabasal layers. In the case of the mitotic cluster, they suggest that keratinocytes retain proliferative capacity well into the suprabasal layer.

Additional basal cell subclustering showed site-specific cellular heterogeneity: basal1 is predominant in scalp and truncal epidermis, basal2 is the major cluster in foreskin and psoriasis-derived samples, and basal3 is found exclusively in foreskin. Intriguingly, the emergence of basal2 in psoriatic truncal samples

implies a dysregulated transcriptional program, which occurs endogenously in neonatal foreskin, underscoring the importance of spatially restricted gene regulation. However, despite the power of *in silico* discovery of cell subpopulations with single-cell transcriptomic analyses, the potential existence of stem cell and progenitor subtypes within the basal cells of human epidermis could not be resolved.

Unsurprisingly, psoriatic inflammation dramatically changes the transcriptional program of epidermal cells, including global upregulation of S100 genes and enrichment of mitotic and channel cell types. Epidermis-resident immune cells from all psoriatic samples showed aberrant enrichment of myeloid dendritic and T cells, suggesting their involvement in psoriatic skin pathological features. Interestingly, hallmarks of inflammation were also found in healthy scalp and foreskin. The authors speculate that enrichment of inflammatory genes and T cell subpopulations in scalp may be relevant for the pathogenesis of lymphocyte-mediated alopecias and that inflammation marker enrichment in foreskin could represent neonatal or genital-specific immunosurveillance. These data invite many follow-up analyses for uncovering how inflammation disrupts the homeostasis in truncal epidermis and how aberrant enrichment of immune cells contributes to psoriatic rashes.

Altogether this study characterized human epidermal cell states across different anatomical regions and psoriatic skin (Figure 1). Importantly, it opens the door for yet more questions, particularly related to the capacity of basal keratinocytes to execute distinct differentiation programs and the recruitment of immune cells to psoriatic sites. Other studies have attempted to resolve the former question in mouse models (Joost et al., 2016), but there are limitations to how well current *in silico* modeling reflects *in vivo* processes. A cognate large-scale single-cell RNA sequencing study of the dermis from healthy and psoriatic skin may be key to answering the second question. Overall, Cheng et al. (2018) have generated valuable human epidermal transcriptomic data, accessible at an online database, with profound implications for a better understanding of human epidermal differentiation and the changes in inflamed skin. With the emergence of single-cell transcriptomics to define previously unknown diversity in cell states and types, larger scale analyses—including combined independent studies—may provide crucial insights to understanding intercellular relationships.

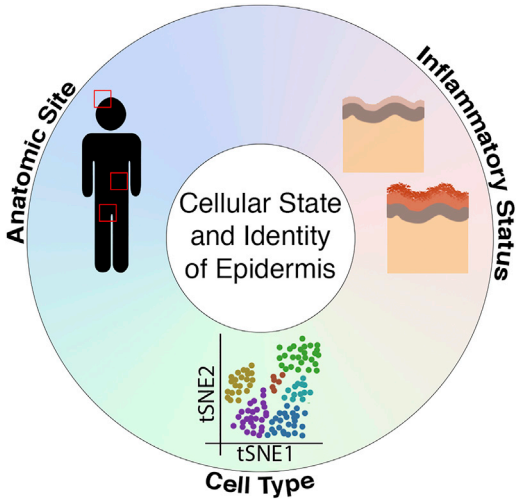

Figure 1. Cell Type, Anatomic Site, and Inflammatory Status Determine Epidermal Cell States and Identities
Large-scale RNA sequencing of 92,889 human epidermal cells from three anatomic sites and inflamed and non-inflamed donors elucidates epidermal cell states, putative differentiation relationships, and unique immune infiltration in psoriatic skin.

REFERENCES

Blanpain, C., and Fuchs, E. (2009). Epidermal homeostasis: a balancing act of stem cells in the skin. Nat. Rev. Mol. Cell Biol. *10*, 207–217.

Cheng, J.B., Sedgewick, A., Finnegan, A.I., Harirchian, P., Lee, J., Kwon, S., Fassett, M.S., Golovato, J., Gray, M., Ghadially, R., et al. (2018). Transcriptional programming of normal and inflamed human epidermis at a single-cell resolution. Cell Rep. *25*, this issue, 871–883.

Gonzales, K.A.U., and Fuchs, E. (2017). Skin and Its Regenerative Powers: An Alliance between Stem Cells and Their Niche. Dev. Cell *43*, 387–401.

Grün, D., Muraro, M.J., Boisset, J.C., Wiebrands, K., Lyubimova, A., Dharmadhikari, G., van den Born, M., van Es, J., Jansen, E., Clevers, H., et al. (2016). De Novo Prediction of Stem Cell Identity using Single-Cell Transcriptome Data. Cell Stem Cell *19*, 266–277.

Heitman, N., Saxena, N., and Rendl, M. (2018). Advancing insights into stem cell niche complexities with next-generation technologies. Curr. Opin. Cell Biol. *55*, 87–95.

Joost, S., Zeisel, A., Jacob, T., Sun, X., La Manno, G., Lönnerberg, P., Linnarsson, S., and Kasper, M. (2016). Single-Cell Transcriptomics Reveals that Differentiation and Spatial Signatures Shape Epidermal and Hair Follicle Heterogeneity. Cell Syst. *3*, 221–237.e9.

Rognoni, E., and Watt, F.M. (2018). Skin Cell Heterogeneity in Development, Wound Healing, and Cancer. Trends Cell Biol. *28*, 709–722.

Saunders, A., Macosko, E.Z., Wysoker, A., Goldman, M., Krienen, F.M., de Rivera, H., Bien, E., Baum, M., Bortolin, L., Wang, S., et al. (2018). Molecular Diversity and Specializations among the Cells of the Adult Mouse Brain. Cell *174*, 1015–1030.e16.