Mammalian skin cell biology: At the interface between laboratory and clinic

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Mammalian skin research represents the convergence of three complementary disciplines: cell biology, mouse genetics, and dermatology. The skin provides a paradigm for current research in cell adhesion, inflammation, and tissue stem cells. Here, I discuss recent insights into the cell biology of skin. Single-cell analysis has revealed that human epidermal stem cells are heterogeneous and differentiate in response to multiple extrinsic signals. Live-cell imaging, optogenetics, and cell ablation experiments show skin cells to be remarkably dynamic. High-throughput, genome-wide approaches have yielded unprecedented insights into the circuitry that controls epidermal stem cell fate. Last, integrative biological analysis of human skin disorders has revealed unexpected functions for elements of the skin that were previously considered purely structural.

Skin research has made spectacular progress over the past 30 years (Box 1). In 1975, the ability to culture cells efficiently from biopsies of human epidermis, the outer covering of the skin, was reported (1). This quickly opened up opportunities to expand cell sheets for transplantation onto burn victims, to characterize genes that are differentially expressed in different epidermal layers, and to analyze tissue assembly in cell culture (2). Cloning the genes that encode epidermal keratins led to a second major advance: Gene promoters could drive transgene expression in specific layers of the skin and, subsequently, perform targeted gene knockouts and lineage analysis in mice (3).

Even with the power of the in vitro and in vivo laboratory-based approaches, skin research would not be in its current vibrant state had it not been for the major contributions of the dermatology community. Eminent clinicians in the early 1980s taught scientists the fundamentals of skin structure and function and called attention to rare skin conditions, such as Epidermolysis bullosa, that at the time were of unknown etiology. As a result, the molecular basis of many human genetic skin disorders was quickly determined and validated in mouse models, laying the foundation for ongoing efforts to treat them by means of gene correction and other approaches (4).

Here, I highlight recent advances in our understanding of skin cell biology. A variety of technologies are illuminating cellular heterogeneity, the extrinsic and intrinsic controls that regulate cell behavior and tissue architecture, and the surprising role of structural elements of the epidermis in regulating skin function.

Skin architecture

Mammalian skin forms the outer covering of the body and consists of two major layers (Fig. 1).

Fig. 1. Mouse back skin. Markers of different epidermal stem cell populations (LGR6, LRIG1, PLET1, GLI1, LGR5, and CD34) are shown. LGR6 and LRIG1 are expressed in the hair follicle isthmus, whereas CD34 and LGR5 are bulge markers. The three dermal layers (boxed) are the reticular dermis, papillary dermis, and hypodermis/white adipose tissue. The dermal papilla and arrector pili muscle constitute two specialized populations of dermal mesenchymal cells. The hair is shown in the resting phase of the hair growth cycle. [Redrawn from (60).]
in the hair follicle and, on contraction, causes the hair follicles to become erect.

Although epidermal epithelial cells (keratinocytes) and dermal fibroblasts are the most abundant cell types in the skin, there are several other key cell types that are either permanent residents of the tissue or traffic through the skin. These include the cells of the peripheral nervous system (7) and blood vessels, melanocytes (8), and cells of the innate and adaptive immune system (9).

**Single-cell approaches to skin cell biology**

Cell behavior is regulated by a combination of intrinsic and extrinsic mechanisms. Local extrinsic signals are provided by the cellular microenvironment, or niche, and include interactions with neighboring cells, secreted factors, extracellular matrix (ECM) proteins, physical parameters such as tissue stiffness, and environmental conditions such as hypoxia (10). The ability to isolate and culture single stem cells from human epidermis allows analysis of stem cell–niche interactions at the single-cell level (Fig. 2). One approach is to capture cells on ECM-coated micropatterned islands and direct them to adopt specific shapes (11). Another is to seed cells on hydrogels that differ in bulk stiffness or on composite substrates containing gold nanoparticles that change the way that ECM proteins are anchored to the substrate and thereby influence cell attachment (12). In both cases, activator protein 1 (AP1) factors are activated to execute the terminal differentiation program, but the signal transduction pathways are different. It remains unclear which of the alternative pathways—depending on serum response factor and the other on extracellular signal-regulated kinase (ERK) mitogen-activated protein kinase (MAPK)—operates in vivo, and whether other pathways—in particular, the Hippo pathway, which is mechanosensitive and active in regulating epidermal differentiation (13, 14)—are also involved.

The same type of reductionist approach has provided new insights into how individual cells assemble into a multilayered epithelium. As few as 10 cells are sufficient to form a stratified epidermis, a process that requires actin polymerization and assembly of two of the major classes of epidermal intercellular adhesive junction: adherens junctions and desmosomes (15, 16). Cells can assemble an epidermis even when there are discontinuities in the underlying ECM (15, 16) by forming multicellular bridges held together by intercellular adhesions that are under tension (16). The ability of keratinocytes to form these bridges may play a role in wound healing (16).

There is clear evidence for extensive interactions between different types of adhesive junction and different cytoskeletal elements within the epidermis. For example, cadherin-mediated adhesions modulate forces transmitted to the ECM so that keratinocytes in a cohesive colony localize traction forces to the colony periphery (17) and the desmosomal plaque protein desmoplakin regulates assembly and function of gap junctions (18). Understanding the dynamics of these interactions has been facilitated by mathematical modeling, as in the case of the impact of actin and keratin filaments on keratinocyte cell spreading (19) and how epidermal stem cells self-organize within stratified cell sheets (17, 20).

There has also been recent progress in modeling dermal fibroblast–niche interactions. Recent in vitro approaches have elucidated reciprocal signaling between dermal subsets and keratinocytes, such as identifying soluble factors secreted by keratinocytes that promote adipocyte differentiation and fibroblast factors that stimulate keratinocyte differentiation (21, 22). In addition, the keratinocyte ECM protein nephroplakin promotes differentiation of a subset of fibroblasts into APM cells in vitro (23). Both epithelium and dermis have been reconstituted by directed differentiation of human iPS cells (24), which opens up a new approach for understanding tissue organization and also for disease modeling.

To date, stem cell characterization has relied largely on enrichment of cell populations with specific markers. Single-cell global gene expression profiling provides much higher resolution and the potential to understand how much cell-to-cell variation is stochastic versus functionally important (25). In cultured human keratinocytes, single-cell global gene expression profiling has revealed cell-to-cell variation in the relative abundance of transcripts of two previously reported markers of human epidermal stem cells: the Notch ligand Delta-like 1 (DLL1) and the epidermal growth factor receptor antagonist LRIG1 (26). Cells that express high levels of DLL1 also have elevated expression of genes associated with endocytosis, integrin-mediated adhesion, and receptor tyrosine kinase signaling, and there was some evidence that expression of these genes is not independent regulated (26). The two cell states may be reinforced by virtue of their influence on how keratinocytes interact with the microenvironment. For example, one of the genes up-regulated in cells with high levels of DLL1 is caveolin-1, which is known to couple β1 integrin, Notch, and receptor tyrosine kinase signaling. The desmosomal cadherin Desmoglein-2 associates with caveolin-1, an interaction that is believed to regulate proliferation (27).

**Cell behavior in the context of the intact tissue**

The way epidermal stem cells behave under steady-state conditions can be quite different from how they behave after tissue damage or upon isolation and transplantation (6). This conclusion is based, in part, on extensive lineage tracing of the progeny of different mouse epidermal stem cell populations (Fig. 1). Most recently, lineage-tracing has also been performed in the dermis (28–31). The results indicate that the fibroblasts in different dermal regions (Fig. 1) arise from different lineages during embryonic development and can be modulated by epidermal Wnt signaling (30, 31). Bone marrow–derived cells do not appear to contribute to dermal mesenchyme (28, 31). Dermal fibroblast subpopulations express different genes at different stages of development (31).

Because skin is on the surface of the body, cell behavior can be analyzed noninvasively. Serial optical sections from the skin of anesthetized mice obtained by using two-photon laser scanning microscopy (32) have revealed coordinated cell movements during hair follicle growth. With laser-induced cell-ablation of fluorescently labeled dermal papilla cells, the importance of the dermal papilla for initiation of hair growth has been confirmed (32). Conversely, after hair follicle stem cell ablation, neighboring keratinocytes repopulate the niche, allowing hair follicle growth to proceed (7, 33).

Optogenetic tools have been used to resolve a longstanding controversy about how the epidermal mechanosensory cells, called Merkel cells, communicate with nerve cells. By stimulating Merkel cells that express a light-sensitive hyperpolarizing
proton pump, it has been established that Merkel cells form a functional, excitatory connection with sensory neurons in the skin (34).

High-throughput/genome-wide approaches

Single-cell studies are complemented by genome-wide approaches to skin biology. Cultured human keratinocytes have previously been used to screen compound libraries for drugs that stimulate or inhibit terminal differentiation, and the same assay format has been adapted for high-throughput small interfering RNA (siRNA) and short hairpin RNA (shRNA) screens. A screen of more than 300 chromatin regulatory genes (35) identified a network of five chromatin factors that regulate genes involved in keratinocyte-ECM interactions and revealed how intrinsic controls of gene expression affect stem cell–niche interactions (35). A further application of high-throughput approaches is a screen of more than 9000 recombinant proteins for direct binding to the long noncoding RNA terminal differentiation-induced noncoding RNA (TINCR), which plays a role in regulating keratinocyte terminal differentiation (36). This led to identification of Staufen1 protein, which in concert with TINCR stabilizes a subset of mRNAs required for epidermal differentiation.

High-throughput approaches are also being used to knock out epidermal genes in the mouse. Ultrasound-guided in utero infection introduces fluorescently labeled lentiviral vectors into mouse embryos, resulting in efficient, selective, and stable transduction of the epidermis. This approach has been used to screen short hairpin RNA libraries for genes that confer a selective growth advantage or disadvantage on keratinocytes in embryonic and postnatal life and to identify genes that modulate epidermal responses to oncogenic H-Ras (37, 38).

Whole-mount imaging of mouse tail epidermis has been used for a large-scale screen of more than 500 knockout mouse mutants via confocal microscopy (39). Roughly 10% of mutants had an epidermal phenotype, several of which mapped to known human genetic conditions. Some mutant genes were expressed in the skin, whereas others were not, indicating systemic effects that could not have been found by selectively targeting the epidermis.

In keeping with the observation that gene deletion can have direct or indirect effects on skin function, integrative biology approaches are being used to explore disease mechanisms in skin conditions that affect more than one cell type. For example, the benign skin condition psoriasis is characterized by epidermal hyperproliferation, disturbed differentiation and tissue architecture, and a dermal inflammatory infiltrate (40). By integrating transcriptomic data sets with data from biological models such as mouse knockouts and human psoriatic skin xenografts on mice, it has been possible both to identify, and validate, IL-22 as a new target in the treatment of psoriasis.

New functions for structural proteins

A key function of the interfollicular epidermis is to act as a protective interface between the body and the external environment, and it contains several architectural elements that enable it to fulfill this function (Fig. 3). The basal layer of the epidermis is anchored to the basement membrane by cell-extracellular matrix receptors, including the β1 integrins and α6β4, which are found in focal adhesions and hemidesmosomal junctions, respectively (41). Integrins are down-regulated with the onset of terminal differentiation, and this is accompanied by changes in intercellular adhesion, notably down-regulated expression of P-cadherin, increased expression of E-cadherin, and changes in the number and composition of desmosomal junctions (42). In addition, the keratin filaments change in composition with the onset of terminal differentiation (43). Last, a structure known as the cornified envelope replaces the plasma membrane in the outermost epidermal layers and consists of insoluble, transglutaminase–cross-linked proteins and lipids (44).

All of these elements of the epidermis play active roles in regulating skin function, which might not have been anticipated from their role in maintaining skin integrity. For example, integrins not only mediate adhesion to the basement membrane but also control initiation of terminal differentiation (41). Misexpression of integrins in the suprabasal layers of hyperproliferative epidermis is linked to up-regulation of ERK MAPK signaling, altered cancer susceptibility, and inflammation. Differentiating epidermal cells in which ERK MAPK signaling is activated can recruit cells in the underlying basal layer to become hyperproliferative and can promote wound-induced tumor formation via signaling to macrophages and T cells (45). Suprabasal epidermal expression of α6β4 integrin is a feature of human skin squamous cell carcinomas and increases susceptibility to chemically induced tumor formation in mice. Suprabasal α6β4 integrin expression stimulates secretion of pro-inflammatory molecules such as CXCL5 and M-CSF and stimulates a protumorigenic skin microenvironment by augmenting the influx of immunosuppressive granular cells during tumor promotion (46).

Proteins that mediate keratinocyte intercellular adhesion also play an active role in regulating proliferation and differentiation. Intercellular adhesion and ECM adhesion are closely coupled. The desmosome protein plakophilin 2 affects cell migration by modulating focal adhesion dynamics and integrin protein expression (47), coupling actomyosin remodeling to desmosomal plaque assembly via RhoA (48). Kazrin, a cytoplasmic protein that binds the desmosomal protein periplakin, also regulates cell shape, cytoskeletal organization, and terminal differentiation via Rho-dependent and–independent mechanisms (49). Epidermal ablation of α-catenin, a key cytoplasmic component of adherens junctions, selectively induces apoptosis in suprabasal differentiating keratinocytes, altering ECM adhesion and growth factor signaling in the underlying basal layer (50). In humans, mutations in integrins and desmosomal components are associated with a variety of diseases (4). Intriguingly, Desmoglein 1 deficiency has recently been linked to severe dermatitis, multiple allergies, and metabolic wasting in humans (51).

![Fig. 3. Structural components of the interfollicular epidermis.](Image)

Each component of human epidermis is listed, together with its structural and signaling properties. The location of each component is indicated by brackets. [Redrawn from (41).]
linked to defective stratum corneum formation (55). Recent studies indicate a link between the epidermal barrier and skin cancer. Studies in a mouse model of atopic dermatitis, in which three epidermal barrier proteins have been deleted—envoplakin, periplakin, and involucrin—show a high resistance to developing benign tumors (56). The mechanism is believed to involve at least two elements. One is a reduction in the transit time of keratinocytes through the epidermis, which has previously been shown to be tumor-suppressive in oncogene-driven skin cancers (39). The second is an exacerbated inflammatory response to the phorbol ester 12-

**Conclusion**

Skin cell research benefits from the integration of complementary technologies and disciplines. As we learn more about every corner of the cell biology of the tissue, we are gaining a wider appreciation about how skin function is regulated and how it may be possible to intervene to treat a variety of skin conditions.

**Future directions**

Future research in skin biology will see an increasing emphasis on holistic approaches, combining in vitro and in vivo data and data from mouse models and clinical material. There will be increasing use of computational biology to interrogate existing publicly available databases, such as genome-wide chromatin immunoprecipitation–sequencing and gene expression data sets, to validate and extend conclusions from individual screens (35). Second, by collating information about phenotypes in multiple tissues for single-cell datasets, there may be possible to intervene to treat a variety of skin conditions.

**References and Notes**

58. S. Demetri et al., Cancer Cell 22, 494–505 (2012).

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