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Making an epidermis

Maranke I. Koster

Department of Dermatology and Charles C. Gates Regenerative Medicine and Stem Cell Biology Program, University of Colorado Denver, Aurora, CO

Abstract

The skin functions as a barrier protecting the body from dehydration and environmental insults. This barrier function is mainly provided by the outermost layer of the skin, the epidermis. The epidermis is maintained by epidermal stem cells which reside in the basal layer and which generate daughter cells that move upwards towards the surface of the skin. During this journey, keratinocytes undergo a series of biochemical and morphological changes that result in the formation of the various layers of the epidermis. Eventually, these cells turn into the outermost layer of dead cornified cells that are sloughed into the environment. This review summarizes our current understanding of the mechanisms that control proliferation and differentiation of epidermal stem cells, and thus addresses fundamental processes that control epidermal morphogenesis and function.

Keywords

stem cell; epidermis; p63

The epidermis is a stratified epithelium which regenerates continuously throughout the life of the organism. In humans, it is estimated that the epidermis turns over every 40–56 days^{1,2}, whereas in mice the estimated epidermal turnover time is 8–10 days³. This constant turnover of the epidermis is mediated by epidermal stem cells, which reside in the basal layer of the epidermis⁴. The progeny of epidermal stem cells, termed transit amplifying (TA) cells, also reside in the basal layer and undergo a few rounds of cell division before they initiate terminal differentiation. During this process, TA cells permanently withdraw from the cell cycle, initiate expression of epidermal terminal differentiation markers, and move suprabasally. Ultimately, this terminal differentiation program results in the formation of corneocytes, keratinocytes which have completed the terminal differentiation program and which have lost their nucleus and cytoplasmic organelles. Within the epidermis, stem cells and their differentiated progeny are organized into columns named epidermal proliferation units. Such units were first proposed to exist over 30 years ago, and more recently their existence was confirmed by genetically tracing the progeny of single epidermal stem cells^{5,6}.

Stem cells and the TA cells they generate are both critical for the continued regeneration of the epidermis. Although numerous genes whose expression is differentially expressed between epidermal stem cells and TA cells have been identified using global gene expression profiling, genes that are exclusively expressed in epidermal stem cells have not been identified^{7,8}. Therefore, mice with genetic alterations in epidermal stem cells, but not in TA cells, cannot be generated, thus precluding the *in vivo* analysis of genes involved in epidermal stem cell maintenance.

Although relatively little is known about the regulation of epidermal stem cells, considerable progress has been made in understanding the mechanisms that control TA cell proliferation and differentiation. Within the epidermis, TA cells are located within the basal layer where they undergo proliferation. Although the precise number of cell divisions that TA cells undergo has not been determined, they eventually withdraw from the cell cycle and initiate terminal differentiation. To ensure normal epidermal differentiation and turnover, this balance between proliferation and differentiation is tightly controlled. One protein which is critical for various aspects of TA cell regulation is p63, a transcription factor which is expressed as at least six different isoforms generated as a result of alternative promoter use and differential splicing. The predominantly expressed p63 isoform in postnatal epidermis, $\Delta Np63\alpha$, can directly induce expression of keratin 14 (K14), a keratin expressed in TA cells, but not in differentiating keratinocytes⁹. Together with the finding that, $\Delta Np63\alpha$ is important for maintaining the proliferative state of TA cells¹⁰, these data suggest that $\Delta Np63\alpha$ is involved in maintaining TA cells and in preventing their premature onset of terminal differentiation.

In addition to maintaining the epidermal basal layer in an undifferentiated, proliferating state, TA cells also regulate the switch from proliferation to differentiation. During this switch, TA cells permanently withdraw from the cell cycle and initiate expression of markers of terminal differentiation. Intriguingly, $\Delta Np63\alpha$ is also involved in this process as demonstrated by the phenotype of mice with reduced $\Delta Np63$ expression in the epidermis. In these mice, epidermal keratinocytes proliferate excessively while failing to properly produce the differentiated layers of the epidermis¹¹. In addition, a very similar skin phenotype is observed in patients with the skin fragility disorder AEC (Ankyloblepharon ectodermal dysplasia and clefting), an ectodermal dysplasia caused by impaired $\Delta Np63\alpha$ function¹². Variations of this phenotype are also observed in mice with defects in Notch signaling, mice with a mutation in 14-3-3 σ , and mice with a germline deletion of IKK α , Ovol1, or IRF6¹³. The partial phenocopy of mice with these various genetic defects suggests that genetic interactions exist between components of these signaling pathways. Indeed, we demonstrated that $\Delta Np63\alpha$ directly induces IKK α , a protein which is required for cell cycle withdrawal, a prerequisite for epidermal terminal differentiation^{11,14}. Furthermore, $\Delta Np63\alpha$ synergizes with Notch signaling components to induce expression of keratin 1 (K1), a marker of differentiating keratinocytes¹⁵. Intriguingly, IRF6 deficient mice, 14-3-3 σ mutant mice, and IKK α deficient mice have virtually indistinguishable phenotypes. Although genetic interactions between IRF6 and 14-3-3 σ have been identified, the precise relationship between IRF6 and 14-3-3 σ , and between these proteins and IKK α , remains to be determined.

In addition to interfollicular stem cells, the epidermis contains at least two other stem cell populations: hair follicle stem cells and sebaceous gland stem cells¹⁶. Of these, hair follicle stem cells have received considerably more attention, partially due to their defined location within the hair follicle. Hair follicle stem cells reside in the bulge region, a morphologically discernible area of the hair follicle located in the midportion of the follicle at the arrector pili muscle attachment site¹⁷. One gene that is expressed at high levels in the bulge region is keratin 15 (K15)¹⁸. K15 expression, however, is also detected in the interfollicular epidermis, albeit at lower levels. Even though K15 expression is not restricted to the bulge, a fragment of the K15 promoter was serendipitously found to be exclusively expressed in the hair follicle bulge in adult mice¹⁸. By using this fragment of the K15 promoter, transgenic mice designed to ablate, downregulate, or overexpress genes specifically in bulge keratinocyte stem cells can be generated. These mouse models will be valuable tools for exploring the regulation of bulge stem cells.

Under homeostatic conditions, interfollicular and hair follicle stem cells only contribute to their respective tissues of origin. However, transplantation experiments and lineage tracing experiments have demonstrated that, in response to injury, bulge-derived keratinocytes

participate in the repair of the interfollicular epidermis^{19,20}. Within the healed epidermis, bulge-derived cells only survive transiently and they are rapidly replaced by progeny of interfollicular stem cells. Consistent with these findings, cutaneous wounds heal with a significant delay in mice which lack hair follicles owing to the absence of hair follicle stem cells²¹. However, wound healing ultimately takes place, presumably due to the activation of interfollicular epidermal stem cells. In addition to the ability of bulge stem cells to contribute to wound healing, interfollicular keratinocytes, presumably the stem cells, can also contribute to *de novo* hair follicle formation, which occurs in the center of large excisional wounds²².

In summary, even though stem cells are critical to the differentiation and regeneration of the epidermis, much needs to be learned about the genetic pathways that control their proliferation and differentiation potential.

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