Basal cells in the epidermis and epidermal differentiation

Raghvendra Singh (✉ raghvend@iitk.ac.in)

Indian Institute of Technology Kanpur

Research Article

Keywords: Epidermal stem cells, Basal cells, Epidermal differentiation, Stem cells

Posted Date: June 29th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-668420/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Basal cells in the epidermis and epidermal differentiation

Raghvendra Singh*

Department of Chemical Engineering, Indian Institute of Technology Kanpur, Kanpur
208016, India

***Correspondence to:**

Raghvendra Singh, Ph.D.

Assistant Professor

Department of Chemical Engineering

Indian Institute of Technology Kanpur

Kanpur, India, 208016

Tel: 91-512-259 7605

Fax: 91-512-259 0104

Email: raghvend@iitk.ac.in

Abstract

A definite identification of epidermal stem cells is not known and the mechanism of epidermal differentiation is not fully understood. Toward both of these quests, considerable information is available from the research on lineage tracing and clonal growth analysis in the basal layer of the epidermis, on the hair follicle and interfollicular epidermal stem cells, and on Wnt signaling along with its role in developmental patterning and cell differentiation. In this paper, literature on the aforementioned research has been collated and analyzed. In addition, models of basal layer cellular composition and epidermal differentiation have been presented.

Introduction

Epidermis self-renews and maintains homeostasis autonomously without influx of hair follicle (HF) cells[1]. On the other hand, during wound healing, HF stem cells (HFSCs), residing in the bulge, and LGR6+ and Lrig1+ cells, residing in the upper isthmus, migrate to the wound[1-3]. Notably, bulge cells are eliminated from the epidermis several weeks after the wound occurs and are responsible for acute wound healing[1]. Like every other tissue, the epidermis is hypothesized to have its own stem cells. However, a definite identification of epidermal stem cells remains elusive.

The epidermis consists of a basal layer, adhering to a basement membrane, few layers of differentiated cells, and a cornified envelope of dead cells. Epidermal stem cells, requiring a specific niche and interaction with the extracellular matrix, reside in the basal layer. Basal cells are columnar while differentiated cells form sheets and are called squamous cells.

A classical model of epidermal stem cell differentiation exists[4]. This two-compartment stem cell model consists of slowly dividing stem cells (SCs) and fast dividing non-stem cell progenies, called the transit-amplifying cells(TAs)[5], which divide few times before undergoing a program of differentiation[5]. Thus, in the two-compartment model, a single stem cell, surrounded by few transit-amplifying cells, lies beneath few layers of differentiated cells, forming a self-limiting epidermal structure[5]. The stem cell compartment expresses higher levels of $\alpha 2\beta 1$, $\alpha 3\beta 1$, and $\alpha 6\beta 4$ integrins and is patterned on the basement membrane[6] [7]. The patterning may not be affected by the dermis and may be autoregulated[6].

However, a recent analysis of clone size distribution did not support the SC/TA hypothesis[8]. According to this study, if the SC/TA hypothesis were correct, the clone size distribution must become time-independent. In contrast, the study found a continuous increase in clone size with time in the epidermis[8]. To analyze the observed clone size

distribution, the study modeled that a stem cell has three stochastic fates when it undergoes mitosis. It can produce two undifferentiated cells or two cells that will go through terminal differentiation or one cell of each fate. Their [8] stochastic analysis at long time scales showed that the average number of basal cells in a clone increases linearly with time, a conclusion inconsistent with the idea of long-lived cycling stem cells[8]. Based on the above inconsistency, the study concludes that a single progenitor cell compartment maintains epidermis during homeostasis[8]. Nonetheless, the study acknowledges a caveat that a small population of quiescent stem cells, which may be very active in wound healing, would be undetectable in it[8]. However, how a single progenitor cell population maintains the epidermis is not known from experiments. Further, although mathematically a single progenitor cell population may be able to explain certain properties of clonal growth, it may not be sufficient to provide chemical cues required to maintain differentiated layers of the epidermis. Furthermore, since the above model is not applicable to wound healing and wound healing requires interaction between the interfollicular epidermis (IFE) and HF, the one progenitor cell compartment model may not be the complete model of the epidermis.

Is there a requirement for discrete SC and TA populations?

Multiple markers of IFE stem cells have been suggested. These include high levels of $\beta 1$ and $\alpha 6$ integrins, low levels of CD71, high levels of Delta1, low levels of desmoglein, and low levels of EGFR1[6,9-14]. Based on the expression of different markers, multiple studies suggest that there may be a “gradual degree of stemness” in the basal layer[9,14-16]. However, single-cell transcriptomics identified four states of basal cells[17] [18].

During homeostasis when basal and differentiated compartments are fully established, there may not be a need for discrete SC and TA populations as suggested by Clayton et. al [8]. However, if there is a gradual degree of stemness in the basal layer, cells will have an equal and high probability to change into cells of a different stemness. Therefore, in wound healing,

basal cells in nearby IFE may be sufficient to heal the wound. However, during healing LGR6 and Lrig1 cells from the upper isthmus migrate to the wound[1-3], suggesting that these cells have special roles in the basal layer.

To understand the complete model of the epidermis, here, I put together the details available on stem cells, which can generate IFE and HF as well as on the role of Wnt signaling in proliferation, differentiation, and patterning.

First, to reveal the cell types in the basal layer, we list the details available on stem cells in the IFE and present a stochastic model of basal layer cell composition.

IFE and HF stem cells

Recently, the *lgr5* gene, which marks HF stem cells, was identified [19]. LGR5 cells contribute to all hair lineages except IFE and sebaceous gland (SG) [20] [19]. Subsequently, the *lgr6* gene, which marks the stem cells residing above the hair follicle bulge, was identified [20]. LGR6 cells expressed none of the known bulge stem cell markers[20]. They contributed to all lineages of skin including IFE and SG[20]. Thus, LGR6 marks the most primitive epidermal stem cells[20]. Further, HF cycling by LGR5 cells requires Wnt signaling [20]. On the other hand, the role of Wnt signaling in IFE is not clear. Since LGR6+ and LGR5+ cells are two distinct stem cell populations in the hair, the information above suggests that LGR6 stem cells seed the SG and IFE whereas LGR5 stem cells seed the HF. In addition, recently, Axin2, a Wnt target gene, expressing stem cells in IFE has been identified[21]. Notably, Axin2 cells contribute to wound healing without the need for quiescent stem cells [21]. Further, the Axin2+ stem cells produce both Wnt ligands and long-range Wnt inhibitors[21]. Although Wnt signaling has a role in both LGR6 and Axin2 cells, the relationship between the two cells in IFE is not clear.

Stem cell quiescence

In homeostasis and repair of a tissue, stem cell quiescence, proliferation, and differentiation play important roles. In HFSCs, quiescence is controlled by NFATc1[22], which through BMP signaling represses CDK4, maintaining quiescence[22]. On the other hand, premature activation of stem cells caused by suppression of NFATc1 signaling causes precocious follicular growth[22]. Similar to NFATc1 in HF, Lrig1 is responsible for the quiescence of IFE stem cells[14].

LGR6 and Lrig1 mark stem cells of IFE

Lrig1⁺ cells are the quiescent stem cells in the basal layer of the epidermis[14]. In HF, they are found in the junctional region near the sebaceous gland and infundibulum [5,23]. In IFE, Lrig1 expressing cells were found at a specific location at the junction of dermis and epidermis, called rete ridge[14]. These cells also express high levels of β 1 integrin [14]. Other studies claim that Lrig1 is expressed throughout the basal layer[23,24]. However, cells expressing high levels of Lrig1 also have high levels of β 1 integrin[23,24].

Lrig1 is a negative feedback regulator of EGFR signaling [25]and negatively regulates the myc promoter[14]. On the other hand, Lrig1 is induced by myc[23]. This negative feedback loop may be responsible for the low level of myc in the basal cells. Since myc causes differentiation of epidermal stem cells [26-28] and Lrig1 inhibits their proliferation, Lrig1 acts as a brake against proliferation and differentiation. Further, Lrig1⁺ cells express high levels of α 6/ β 4 integrins [14,23] while myc reduces the expression of α 6/ β 4 integrins [29]. Furthermore, expression of myc2 in the basal layer reduced expression of β 1 integrin[30].

LGR6 stem cells are also found in the basal layer of IFE in addition to SG and isthmus [31]. Further, LGR6⁺ but not Lrig1⁺ cells in the epidermis are capable of forming new hair follicles[32]. Thus, LGR6 cells are more primitive stem cells than Lrig1 cells. Further, LGR6 cells also express α 6 integrins [33].

With the above information, since single-cell transcriptomics identified four states of basal cells[17] [18], I present a four-cell-type model of the basal layer.

Results and discussion

Stochastic model of the basal layer

We model that the interconversions among the 4 cell types (Table 1) during mitosis happen stochastically in the basal layer. Further, we assume that the probability of conversion of a favorable process, i.e. conversion of LGR6(+) or Lrig1(+) cell to LGR6(-) or Lrig1(-) cell, respectively, is p while that of the reverse, less favorable process is σp , where σ is the probability of basal cells expressing each of the two markers, LGR6 and Lrig1. A compartment containing n cell types will have $\left[\frac{n(n-1)}{2} + n\right]$ stochastic conversion reactions for each cell type, amounting to 10 reactions for each of the four cell types in the basal layer.

Table 2 A and B lists all the reactions occurring in the basal layer and their probabilities.

From Table 2 A and B, we calculate the propensity of conversion of each type of cell if conversion reactions occur stochastically during epidermal homeostasis. The propensities of conversions are:

$$\begin{aligned}\Delta A &= p^2(5 + \sigma + 3\sigma^2 + \sigma^3); & \Delta B &= p^2(12 + 4\sigma - 3\sigma^2 + \sigma^3 - \sigma^4); & \Delta C &= p^2(-5 + 4\sigma + 7\sigma^2 + 2\sigma^3 + 2\sigma^4); \\ \Delta D &= p^2(5 + \sigma + 3\sigma^2 + \sigma^3)\end{aligned}$$

Since at homeostasis the fractions of different cell types will distribute according to their propensity of conversion, the proportions of different cell types are given as:

$$Fraction A = \frac{5 + \sigma + 3\sigma^2 + \sigma^3}{17 + 10\sigma + 10\sigma^2 + 5\sigma^3 + \sigma^4} \quad (1)$$

$$Fraction B = \frac{12 + 4\sigma - 3\sigma^2 + \sigma^3 - \sigma^4}{17 + 10\sigma + 10\sigma^2 + 5\sigma^3 + \sigma^4} \quad (2)$$

$$Fraction C = \frac{-5 + 4\sigma + 7\sigma^2 + 2\sigma^3 + 2\sigma^4}{17 + 10\sigma + 10\sigma^2 + 5\sigma^3 + \sigma^4} \quad (3)$$

$$Fraction D = \frac{5 + \sigma + 3\sigma^2 + \sigma^3}{17 + 10\sigma + 10\sigma^2 + 5\sigma^3 + \sigma^4} \quad (4)$$

For example for $\sigma=0.7$: %A=24.3, %B=43.5, %C=7.8, and %D=24.3

SC/TA model of the basal layer of IFE

Since cell type C (Table 1) expresses both stem cell markers and maybe quiescent, we characterize it as the major stem cell in the basal layer. Further, its population in the basal layer is stochastically the least (see the section above). Interestingly, Lrig1+ cells do not express any of the bulge stem cell markers[19,23,34,35] like the LGR6 cells[20]. On the other hand, LGR6 cells can generate all skin lineages including Lrig1+ cells[20] and Lrig1 cells express high levels of Blimp1, a known marker of LGR6[36], suggesting an overlap between the two markers[3,23]. Further, Blimp1 negatively regulates myc directly[37] in addition to the negative regulation of myc by Lrig1. Thus, through these double repressions, the LGR6(+)/Lrig1(+) cells may be highly resistant to differentiation. In contrast, LGR6(-)/Lrig1(-) cells, cell type B (Table 1), have been characterized as the major TA cells since they are proliferative and express none of the two markers. Further, stochastically their population is the largest. On the other hand, the LGR6(+)/Lrig1(-) cells, the cells labeled as A (Table 1), although express the stem cell marker, are proliferative and may differentiate directly. Thus, these cells may constitute another type of TA cells population and we label them as the minor TA cells 2, the second major TA cells in the basal layer. Interestingly, proliferating LGR6+ cells were found to express Axin2[38]. Thus, LGR6+ cells and Axin2+ cells may be the same and Axin2 cells have been shown to differentiate directly[21]. In contrast, the LGR6(-)/Lrig1(+), type D, cells have been labeled as the minor TA cells 3 because they are nonproliferative. However, notwithstanding their quiescence in the basal layer, LGR6(-)/Lrig1(+) cells can upregulate myc and differentiate in the upper layers of the epidermis. However, differentiation of Lrig1+ epidermal basal cells remains to be shown although it has been shown in meibomian gland epithelial cells in the eyelid, where progenitor cells express Lrig1 while differentiated cells suppress Lrig1 and express DNase2 instead[39]. Interestingly, $\alpha 6\beta 4$ integrins are found on surfaces of some cells in the

immediate suprabasal region of the epidermis[40], suggesting the existence of minor TA-related cells in the suprabasal region.

Major TA cells must convert to the major SCs with a minimum probability

Since σp is the probability that LGR6(-) or Lrig1(-) cells change into LGR6(+) and Lrig1(+) cells while p is the probability of the reverse process, σ should not approach unity. For σ in the range 0.56-1, the percentage of the major stem cells (type C cells) varies from 0%-23.3% according to equation 3. This puts a limit on the difference between the major TA (type B cells) and the major stem cells (type C cells). The major TA cells should convert to major SCs with a minimum probability, $CP_{\min} = \sigma_{\min}^2 p = 0.31p$, in the basal layer, where, $\sigma_{\min} = 0.56$. Thus, although major TA cells are significantly different from the major SCs, they may not be so different that major TA cells cannot convert to major SCs, otherwise the major SC population in the basal layer will be exhausted.

Since the environment of each layer can be assumed to be responsible for maintaining individual layers, the minimum limit on the probability of conversion of major TA cells to major SCs also underlines the importance of maintaining the difference between the basal layer and the suprabasal layers in a fundamental manner. This may be the reason that basal cells are columnar so that if the environment from the upper layer seeps in the basal layer, the cells exposed to such environment move up consistently. In contrast, the differentiation of TA cells in the basal layer may reduce the conversion probability below $CP_{\min}$, exhausting the stem cells, destroying the homeostasis.

Evidence in support of the discrete SC/TA hypothesis is stronger than that in support of the gradual stemness hypothesis

Next, we calculated the conversion propensities for the basal layer having 8 cell types (Supplementary information S) and compared them with the basal layer having 4 cell types. Table 3 shows the minimum value of σ for the basal layer having different numbers of cell

types. If types of cells in the basal layer were more than 4, the minimum σ will progressively approach 1 (Table 3), making all conversions have a high probability, which is the case with the gradual degree of stemness hypothesis. However, $\sigma_{\min}$ approaching 1 is impractical from the biological point of view because the probability of conversion of a nonstem cell to a stem cell cannot be as high as that of a stem cell to a nonstem cell progeny. Further, the gradual stemness hypothesis may create an unstable basal layer in which any physical perturbation, reducing the conversion probability of major TA to major SC below the minimum allowable probability, may exhaust the major SC population. In addition, the gradual stemness hypothesis may not require LGR6+ and Lrig1+ from the upper isthmus to migrate to the wound and is, therefore, unrealistic. Thus, the gradual stemness hypothesis, which is similar to a single type of progenitor cell conclusion drawn by Clayton et. al [8], has less support than the discrete SC/TA hypothesis. Further, in invalidating SC/TA model, Clayton et. al [8] assumed that TA cells produced from SCs are differentiated (Clayton et. al supplementary information), which may be invalid since the basal layer may not contain the differentiated cells.

There is strong evidence in support for a 4-cell type model of the basal layer

If there were only 2 or 3 types of cells in the basal layer (Supplementary information S), the minimum allowable probability of conversion of LGR6(-) or Lrig1(-) cells to LGR6(+) and Lrig1(+) is 0 (Table 3). Thus in these cases, the TA cells could be completely different from SCs and there was no requirement for the basal layer environment to be completely different from that of the suprabasal layers other than the niche required for the SC because cell differentiation was allowed even in the basal layer. The minimum number of cell types for which there is an explicit requirement for the basal layer to be different from suprabasal layers, i.e. to have a nonzero $\sigma_{\min}$, is 4 (Table 3). Further, for the number of cell types to be 4, the $\sigma_{\min}$ may not be unreasonably high (Table 3) and most importantly single-cell

transcriptomics identified four states of the basal cells[17] [18]. Thus, the evidence in support of a 4-cell-type basal layer model is significantly strong.

For $\sigma=0.7$ and the 4-cell type model, the percentage of major SC is 7.8%, which is in the range observed previously[41,42]. For σ in the range 0.56-1, the percentage of Lrig1+ cells (%C+%D) varies from 27% to 51.3% which explains the presence of these cells throughout the basal layer[23,24]. Further, according to the 4-cell type model, the majority of basal cells (i.e. LGR6+ and/or Lrig1+) may express $\alpha 6$ integrins as has been observed previously[40].

The model above presents the composition of the basal layer. To present a model of differentiation of TA cells in the upper layers of IFE, I put together information on Wnt signaling enhancers and antagonists, their combinatorial expression in patterning, and the role of Wnt inhibitor Dkk in cancer and cell differentiation.

Wnt signaling is controlled by feedback loops

LGR4, LGR5, and LGR6 receptors along with their soluble ligands, R-spondins, are enhancers of Wnt signaling [43]. R-spondins can bind to the three receptors, which are facultative Wnt receptor components that enhance the Wnt signal [44]. Reducing LGR5 reduces β -catenin translocation to the nucleus and expression of two Wnt target genes c-myc and cyclin D [45]. LGR5 associates with Wnt coreceptors Frizzled/Lrp [44,46] and potentiates Wnt signaling by enhancing Lrp6 phosphorylation[47]. In addition to the direct effect, the LGR5/R-spondin complex neutralizes the two E3 ligases, Rnf43 and Znf3, which negatively regulate the Wnt receptors [43]. Moreover, Rnf43 and Znf3 are Wnt target genes, forming a negative feedback regulation of Wnt signaling[43]. This negative feedback loop may block Wnt signaling, which can be rescued by the action of LGR5/R-spondins on Rnf43 and Znf3. In addition to Rnf3/Znf3, Dickkopf (Dkk)1, an inhibitor of Wnt signaling is a Wnt target gene, creating another negative feedback loop[48]. Thus, enhancers, negative feedback regulators, and anti-negative feedback regulators modulate Wnt signaling and may

create a spatial distribution of its strength in a context-specific manner. Interestingly, negative feedback loops may create Turing instability, which can generate patterns in development[49].

Combinatorial expression of Wnt and its antagonists in patterning

Wnt signaling inhibits anterior development in *Xenopus*, zebrafish, and mice [50]. The combinatorial expression of Wnt and its antagonist Dkk1 generates the spatial signaling strength required for axial patterning including induction of head[50-52]. Similarly, endocytosis of Dkk1 generates the right amount of spatial Wnt antagonism, which is important for the gastrulation of embryos[53]. In addition, strong Dkk4 expression in the epidermis at discrete locations before hair placode formation has been observed and combinatorial Wnt and Dkk expression are important for hair follicle spacing[54] through a reaction-diffusion mechanism involving Turing instability. Moreover, negative feedback regulation of Wnt by Dkk2 is sufficient to explain sensory organ size constancy in zebrafish[55]. Thus, Wnt/Dkk combination has been found to play an important role in multiple developmental patterning.

Dickkopf (Dkk) in cell differentiation and cancer

Dkk1 represses the growth of colon cancer[56] and it has been implicated in $1\alpha,25$ -dihydroxy vitamin D3 induced differentiation of colon cancer cells[57]. Further, it has been shown to cause cardiovascular ES cells differentiation[58]. Furthermore, Dkk-1 and -2 have been shown to mediate osteoblast differentiation[59] [60,61]. Similarly, oxysterol-induced osteogenic differentiation of bone marrow stromal cells is mediated by Dkk1[62]. Interestingly, Dkk3 expression is localized in the upper layer at the interface of the upper spinous layer and granular layer of IFE[63,64], suggesting a role of Dkk3 in epidermal differentiation.

Autocrine vs paracrine Wnt signaling

Axin2 expressing IFE stem cells secrete Wnt ligands that act in an autocrine manner[21]. I hypothesized that these cells are LGR6+ stem cells in the epidermis. In HF, the highest activity of Wnt signaling was observed in the precursor cells that produce hair shaft i.e. just before the differentiation of TA cells in HF[65]. Presumably, these precursor cells are the progenies of LGR5+ HFSCs. Interestingly, in HF generation, epidermal Wnt ligands are required for the dermal Wnt signaling and dermal Wnt signaling is required for the patterned upregulation of epidermal Wnt signaling[66], forming a positive feedback loop. This loop is critical for the initiation of hair follicle placodes in the skin[66]. Thus, hair shaft precursor cells, the TA cells in HF, may receive Wnt ligands in a paracrine manner from the outer root sheath. In paracrine Wnt activation, gradation of Wnt strength can be achieved merely by the distance of the Wnt ligand source, which supports the finding that Wnt signaling strength is the highest in hair shaft precursor cells, residing close to the outer root sheath [65]. In contrast, in IFE, besides diffusion of Wnt ligands from the basal layer, autocrine Wnt signaling is also present[21]. Thus, differentiation of IFE TA cells may also require spatial distribution of Wnt inhibitors such as Dkk to suppress the autocrine Wnt signaling[21]. Interestingly, Dkk3 has been found to accumulate in the upper layers of the epidermis[21,63,64].

Here, I present the model [21] of the epidermal differentiation and combine it with the HF.

A combined model of HF and differentiation program of IFE

In this model, in IFE, stem cells produce both ligands and inhibitors of Wnt pathway[21]. While a higher concentration of Wnt ligand is found in the basal layers, a higher concentration of Wnt inhibitors is found in the suprabasal layers[21,63,64]. On the other hand, high, stabilized β -catenin turned committed IFE cells to HF stem cells and caused hair tumors[67]. Thus, HFSCs, through LGR5 and paracrine Wnt ligands may have a higher

strength of Wnt signaling than IFE LGR6+ cells, which may use autocrine Wnt signaling along with expression of Wnt inhibitors. In this model, IFESC switches from LGR6 to LGR5 receptor, changing to HFSC while Wnt inhibitors along with Wnt ligands generate the graded strength of Wnt signaling, causing TA cell differentiation in the epidermis (Fig. 1).

Conflict of Interest

The author declares that he has no conflict of interest.

References

1. Ito M, Liu Y, Yang Z, Nguyen J, Liang F, et al. (2005) Stem cells in the hair follicle bulge contribute to wound repair but not to homeostasis of the epidermis. *Nat Med* 11: 1351-1354.
2. Dekoninck S, Blanpain C (2019) Stem cell dynamics, migration and plasticity during wound healing. *Nat Cell Biol* 21: 18-24.
3. Page ME, Lombard P, Ng F, Gottgens B, Jensen KB (2013) The epidermis comprises autonomous compartments maintained by distinct stem cell populations. *Cell Stem Cell* 13: 471-482.
4. Potten CS (1981) Cell replacement in epidermis (keratopoiesis) via discrete units of proliferation. *Int Rev Cytol* 69: 271-318.
5. Watt FM, Jensen KB (2009) Epidermal stem cell diversity and quiescence. *EMBO Mol Med* 1: 260-267.
6. Jones PH, Harper S, Watt FM (1995) Stem cell patterning and fate in human epidermis. *Cell* 80: 83-93.
7. Watt FM (1998) Epidermal stem cells: markers, patterning and the control of stem cell fate. *Philos Trans R Soc Lond B Biol Sci* 353: 831-837.
8. Clayton E, Doupe DP, Klein AM, Winton DJ, Simons BD, et al. (2007) A single type of progenitor cell maintains normal epidermis. *Nature* 446: 185-189.
9. Jones PH, Watt FM (1993) Separation of human epidermal stem cells from transit amplifying cells on the basis of differences in integrin function and expression. *Cell* 73: 713-724.
10. Li A, Simmons PJ, Kaur P (1998) Identification and isolation of candidate human keratinocyte stem cells based on cell surface phenotype. *Proc Natl Acad Sci U S A* 95: 3902-3907.

11. Lowell S, Jones P, Le Roux I, Dunne J, Watt FM (2000) Stimulation of human epidermal differentiation by delta-notch signalling at the boundaries of stem-cell clusters. *Curr Biol* 10: 491-500.
12. Wan H, Stone MG, Simpson C, Reynolds LE, Marshall JF, et al. (2003) Desmosomal proteins, including desmoglein 3, serve as novel negative markers for epidermal stem cell-containing population of keratinocytes. *J Cell Sci* 116: 4239-4248.
13. Fortunel NO, Hatzfeld JA, Rosemary PA, Ferraris C, Monier MN, et al. (2003) Long-term expansion of human functional epidermal precursor cells: promotion of extensive amplification by low TGF-beta1 concentrations. *J Cell Sci* 116: 4043-4052.
14. Jensen KB, Watt FM (2006) Single-cell expression profiling of human epidermal stem and transit-amplifying cells: *Lrig1* is a regulator of stem cell quiescence. *Proc Natl Acad Sci U S A* 103: 11958-11963.
15. Niemann C, Watt FM (2002) Designer skin: lineage commitment in postnatal epidermis. *Trends Cell Biol* 12: 185-192.
16. Potten CS, Loeffler M (1990) Stem cells: attributes, cycles, spirals, pitfalls and uncertainties. Lessons for and from the crypt. *Development* 110: 1001-1020.
17. Wang S, Drummond ML, Guerrero-Juarez CF, Tarapore E, MacLean AL, et al. (2020) Single cell transcriptomics of human epidermis identifies basal stem cell transition states. *Nat Commun* 11: 4239.
18. Haensel D, Jin S, Sun P, Cinco R, Dragan M, et al. (2020) Defining Epidermal Basal Cell States during Skin Homeostasis and Wound Healing Using Single-Cell Transcriptomics. *Cell Rep* 30: 3932-3947 e3936.
19. Jaks V, Barker N, Kasper M, van Es JH, Snippert HJ, et al. (2008) *Lgr5* marks cycling, yet long-lived, hair follicle stem cells. *Nat Genet* 40: 1291-1299.

20. Snippert HJ, Haegebarth A, Kasper M, Jaks V, van Es JH, et al. (2010) Lgr6 marks stem cells in the hair follicle that generate all cell lineages of the skin. *Science* 327: 1385-1389.
21. Lim X, Tan SH, Koh WL, Chau RM, Yan KS, et al. (2013) Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling. *Science* 342: 1226-1230.
22. Horsley V, Aliprantis AO, Polak L, Glimcher LH, Fuchs E (2008) NFATc1 balances quiescence and proliferation of skin stem cells. *Cell* 132: 299-310.
23. Jensen KB, Collins CA, Nascimento E, Tan DW, Frye M, et al. (2009) Lrig1 expression defines a distinct multipotent stem cell population in mammalian epidermis. *Cell Stem Cell* 4: 427-439.
24. Fuchs E (2009) Finding one's niche in the skin. *Cell Stem Cell* 4: 499-502.
25. Wong VW, Stange DE, Page ME, Buczacki S, Wabik A, et al. (2012) Lrig1 controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. *Nat Cell Biol* 14: 401-408.
26. Arnold I, Watt FM (2001) c-Myc activation in transgenic mouse epidermis results in mobilization of stem cells and differentiation of their progeny. *Curr Biol* 11: 558-568.
27. Gandarillas A, Watt FM (1997) c-Myc promotes differentiation of human epidermal stem cells. *Genes Dev* 11: 2869-2882.
28. Watt FM, Frye M, Benitah SA (2008) MYC in mammalian epidermis: how can an oncogene stimulate differentiation? *Nat Rev Cancer* 8: 234-242.
29. Frye M, Gardner C, Li ER, Arnold I, Watt FM (2003) Evidence that Myc activation depletes the epidermal stem cell compartment by modulating adhesive interactions with the local microenvironment. *Development* 130: 2793-2808.
30. Waikel RL, Kawachi Y, Waikel PA, Wang XJ, Roop DR (2001) Deregulated expression of c-Myc depletes epidermal stem cells. *Nat Genet* 28: 165-168.

31. Fullgrabe A, Joost S, Are A, Jacob T, Sivan U, et al. (2015) Dynamics of Lgr6(+) Progenitor Cells in the Hair Follicle, Sebaceous Gland, and Interfollicular Epidermis. *Stem Cell Reports* 5: 843-855.
32. Kretschmar K, Weber C, Driskell RR, Calonje E, Watt FM (2016) Compartmentalized Epidermal Activation of beta-Catenin Differentially Affects Lineage Reprogramming and Underlies Tumor Heterogeneity. *Cell Rep* 14: 269-281.
33. Gunnarsson AP, Christensen R, Li J, Jensen UB (2016) Global gene expression and comparison between multiple populations in the mouse epidermis. *Stem Cell Res* 17: 191-202.
34. Liu Y, Lyle S, Yang Z, Cotsarelis G (2003) Keratin 15 promoter targets putative epithelial stem cells in the hair follicle bulge. *J Invest Dermatol* 121: 963-968.
35. Trempus CS, Morris RJ, Bortner CD, Cotsarelis G, Faircloth RS, et al. (2003) Enrichment for living murine keratinocytes from the hair follicle bulge with the cell surface marker CD34. *J Invest Dermatol* 120: 501-511.
36. Hsu SY, Kudo M, Chen T, Nakabayashi K, Bhalla A, et al. (2000) The three subfamilies of leucine-rich repeat-containing G protein-coupled receptors (LGR): identification of LGR6 and LGR7 and the signaling mechanism for LGR7. *Mol Endocrinol* 14: 1257-1271.
37. Horsley V, O'Carroll D, Tooze R, Ohinata Y, Saitou M, et al. (2006) Blimp1 defines a progenitor population that governs cellular input to the sebaceous gland. *Cell* 126: 597-609.
38. Lee JH, Tammela T, Hofree M, Choi J, Marjanovic ND, et al. (2017) Anatomically and Functionally Distinct Lung Mesenchymal Populations Marked by Lgr5 and Lgr6. *Cell* 170: 1149-1163 e1112.

39. Xie HT, Sullivan DA, Chen D, Hatton MP, Kam WR, et al. (2018) Biomarkers for Progenitor and Differentiated Epithelial Cells in the Human Meibomian Gland. *Stem Cells Transl Med* 7: 887-892.
40. Sonnenberg A, Calafat J, Janssen H, Daams H, van der Raaij-Helmer LM, et al. (1991) Integrin alpha 6/beta 4 complex is located in hemidesmosomes, suggesting a major role in epidermal cell-basement membrane adhesion. *J Cell Biol* 113: 907-917.
41. Alonso L, Fuchs E (2003) Stem cells of the skin epithelium. *Proc Natl Acad Sci U S A* 100 Suppl 1: 11830-11835.
42. Potten CS, Morris RJ (1988) Epithelial stem cells in vivo. *J Cell Sci Suppl* 10: 45-62.
43. de Lau W, Peng WC, Gros P, Clevers H (2014) The R-spondin/Lgr5/Rnf43 module: regulator of Wnt signal strength. *Genes Dev* 28: 305-316.
44. de Lau W, Barker N, Low TY, Koo BK, Li VS, et al. (2011) Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature* 476: 293-297.
45. Hsu HC, Liu YS, Tseng KC, Tan BC, Chen SJ, et al. (2014) LGR5 regulates survival through mitochondria-mediated apoptosis and by targeting the Wnt/beta-catenin signaling pathway in colorectal cancer cells. *Cell Signal* 26: 2333-2342.
46. Carmon KS, Lin Q, Gong X, Thomas A, Liu Q (2012) LGR5 interacts and cointernalizes with Wnt receptors to modulate Wnt/beta-catenin signaling. *Mol Cell Biol* 32: 2054-2064.
47. Carmon KS, Gong X, Lin Q, Thomas A, Liu Q (2011) R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/beta-catenin signaling. *Proc Natl Acad Sci U S A* 108: 11452-11457.
48. Niida A, Hiroko T, Kasai M, Furukawa Y, Nakamura Y, et al. (2004) DKK1, a negative regulator of Wnt signaling, is a target of the beta-catenin/TCF pathway. *Oncogene* 23: 8520-8526.

49. Smith S, Dalchau N (2018) Model reduction enables Turing instability analysis of large reaction - diffusion models. *Journal of the Royal Society Interface* 15.
50. Niehrs C (2006) Function and biological roles of the Dickkopf family of Wnt modulators. *Oncogene* 25: 7469-7481.
51. Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, et al. (1998) Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 391: 357-362.
52. Shinya M, Eschbach C, Clark M, Lehrach H, Furutani-Seiki M (2000) Zebrafish Dkk1, induced by the pre-MBT Wnt signaling, is secreted from the prechordal plate and patterns the anterior neural plate. *Mech Dev* 98: 3-17.
53. Kawamura N, Takaoka K, Hamada H, Hadjantonakis AK, Sun-Wada GH, et al. (2020) Rab7-Mediated Endocytosis Establishes Patterning of Wnt Activity through Inactivation of Dkk Antagonism. *Cell Rep* 31: 107733.
54. Sick S, Reinker S, Timmer J, Schlake T (2006) WNT and DKK determine hair follicle spacing through a reaction-diffusion mechanism. *Science* 314: 1447-1450.
55. Wada H, Ghysen A, Asakawa K, Abe G, Ishitani T, et al. (2013) Wnt/Dkk negative feedback regulates sensory organ size in zebrafish. *Curr Biol* 23: 1559-1565.
56. Gonzalez-Sancho JM, Aguilera O, Garcia JM, Pendas-Franco N, Pena C, et al. (2005) The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is downregulated in human colon cancer. *Oncogene* 24: 1098-1103.
57. Aguilera O, Pena C, Garcia JM, Larriba MJ, Ordonez-Moran P, et al. (2007) The Wnt antagonist DICKKOPF-1 gene is induced by 1 α ,25-dihydroxyvitamin D3 associated to the differentiation of human colon cancer cells. *Carcinogenesis* 28: 1877-1884.

58. David R, Brenner C, Stieber J, Schwarz F, Brunner S, et al. (2008) MesP1 drives vertebrate cardiovascular differentiation through Dkk-1-mediated blockade of Wnt-signalling. *Nat Cell Biol* 10: 338-345.
59. Fujita K, Janz S (2007) Attenuation of WNT signaling by DKK-1 and -2 regulates BMP2-induced osteoblast differentiation and expression of OPG, RANKL and M-CSF. *Mol Cancer* 6: 71.
60. van der Horst G, van der Werf SM, Farih-Sips H, van Bezooijen RL, Lowik CW, et al. (2005) Downregulation of Wnt signaling by increased expression of Dickkopf-1 and -2 is a prerequisite for late-stage osteoblast differentiation of KS483 cells. *J Bone Miner Res* 20: 1867-1877.
61. Li X, Liu P, Liu W, Maye P, Zhang J, et al. (2005) Dkk2 has a role in terminal osteoblast differentiation and mineralized matrix formation. *Nat Genet* 37: 945-952.
62. Amantea CM, Kim WK, Meliton V, Tetradis S, Parhami F (2008) Oxysterol-induced osteogenic differentiation of marrow stromal cells is regulated by Dkk-1 inhibitable and PI3-kinase mediated signaling. *J Cell Biochem* 105: 424-436.
63. Kataoka K, Du G, Maehara N, Murata H, Sakaguchi M, et al. (2012) Expression pattern of REIC/Dkk-3 in mouse squamous epithelia. *Clin Exp Dermatol* 37: 428-431.
64. Du G, Kataoka K, Sakaguchi M, Abarzua F, Than SS, et al. (2011) Expression of REIC/Dkk-3 in normal and hyperproliferative epidermis. *Exp Dermatol* 20: 273-277.
65. DasGupta R, Fuchs E (1999) Multiple roles for activated LEF/TCF transcription complexes during hair follicle development and differentiation. *Development* 126: 4557-4568.
66. Chen D, Jarrell A, Guo C, Lang R, Atit R (2012) Dermal beta-catenin activity in response to epidermal Wnt ligands is required for fibroblast proliferation and hair follicle initiation. *Development* 139: 1522-1533.

67. Gat U, DasGupta R, Degenstein L, Fuchs E (1998) De Novo hair follicle morphogenesis and hair tumors in mice expressing a truncated beta-catenin in skin. *Cell* 95: 605-614.

Table Legends

Table 1: SC/TA cells in the basal layer of the epidermis

Table 2: Conversion reactions and probabilities of conversion of SC/TA cells in the basal layer for the 4-cell type model

Table 3: Minimum value of the probability that the basal cells express either LGR6 or Lrig1 markers, σ , for the different number of types of SC/TA cells in the basal layer

Figure Legends

Figure 1: A combined model of HF and differentiation program of the epidermis. LGR6 cells convert to LGR5 cells, which seed the HF. LGR6 cells establish the differentiated epidermis with the help of Wnt ligands and inhibitors.

Tables

Table 1

Marker	Cell label	Characteristics
LGR6(+)/Lrig1(-)	A	Minor TA cell 2
LGR6(-)/Lrig1(-)	B	Major TA cell
LGR6(+)/Lrig1(+)	C	Major Stem cell, quiescent
LGR6(-)/Lrig1(+)	D	Minor TA cell 3, quiescent

Table 2

A	Conversion	Probability	Conversion	Probability
	$A \rightarrow A+A$	p^2	$B \rightarrow A+A$	$\sigma^2 p^2$
	$A \rightarrow B+B$	p^2	$B \rightarrow B+B$	p^2
	$A \rightarrow C+C$	$\sigma^2 p^2$	$B \rightarrow C+C$	$\sigma^4 p^2$
	$A \rightarrow D+D$	$\sigma^2 p^2$	$B \rightarrow D+D$	$\sigma^2 p^2$
	$A \rightarrow A+B$	p^2	$B \rightarrow A+B$	σp^2
	$A \rightarrow A+C$	σp^2	$B \rightarrow A+C$	$\sigma^3 p^2$
	$A \rightarrow A+D$	σp^2	$B \rightarrow A+D$	$\sigma^2 p^2$
	$A \rightarrow B+C$	σp^2	$B \rightarrow B+C$	$\sigma^2 p^2$
	$A \rightarrow B+D$	σp^2	$B \rightarrow B+D$	σp^2
	$A \rightarrow C+D$	$\sigma^2 p^2$	$B \rightarrow C+D$	$\sigma^3 p^2$

B	Conversion	Probability	Conversion	Probability
	$C \rightarrow A+A$	p^2	$D \rightarrow A+A$	$\sigma^2 p^2$
	$C \rightarrow B+B$	p^2	$D \rightarrow B+B$	p^2
	$C \rightarrow C+C$	p^2	$D \rightarrow C+C$	$\sigma^2 p^2$
	$C \rightarrow D+D$	p^2	$D \rightarrow D+D$	p^2
	$C \rightarrow A+B$	p^2	$D \rightarrow A+B$	σp^2
	$C \rightarrow A+C$	p^2	$D \rightarrow A+C$	$\sigma^2 p^2$
	$C \rightarrow A+D$	p^2	$D \rightarrow A+D$	σp^2
	$C \rightarrow B+C$	p^2	$D \rightarrow B+C$	σp^2
	$C \rightarrow B+D$	p^2	$D \rightarrow B+D$	p^2
	$C \rightarrow C+D$	p^2	$D \rightarrow C+D$	σp^2

Table 3

Number of types of cells in the basal layer	$\sigma_{\min}$
2	0
3	0
4	0.56
8	0.70

Figure 1

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryinformation.pdf](#)