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Basal Cells in the Epidermis and Epidermal Differentiation

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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 the interfollicular epidermal stem cells, and on Wnt signaling along with its role in the developmental patterning and cell differentiation. In this paper, literature on the aforementioned research has been collated and analyzed. In addition, models of the basal layer cellular composition and the epidermal differentiation have been presented.

Keywords Epidermis · Epidermal stem cells · Basal cells in the epidermis · Basal keratinocytes · Epidermal differentiation · Stochastic epidermis · Wnt signaling · LGR5 · LGR6 · Lrig1 · Hair follicle · Hair follicle stem cells

Introduction

Epidermis self-renews and maintains homeostasis autonomously without the influx of the 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, the 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 the 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 the differentiated cells form sheets and are called squamous cells.

A classical model of epidermal stem cell differentiation exists [4]. This two-compartment 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 the 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 the 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 the 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. The stochastic analysis of the study [8] at long time scales showed that the average number of the basal cells in a clone increases linearly with time, a conclusion contradictory to the idea of the long-lived self-renewing stem cells [8]. Based on the above inconsistency, the study concludes that a single progenitor cell compartment maintains the 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

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epidermis is not known from experiments. Further, although mathematically a single progenitor cell population may be able to explain certain properties of the clonal growth, it may not be sufficient to provide the chemical cues required to maintain the 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 the 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 the IFE stem cells have been suggested. These include high levels of β1 and α6 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 the basal cells [17, 18].

During the homeostasis when the basal and the differentiated compartments are fully established, there may not be a need for the 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 the 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, we put together the details available on the stem cells, which can generate the IFE and the 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 the stem cells in the IFE and present a stochastic model of the basal layer cell composition.

IFE and HF Stem Cells

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

Stem Cell Quiescence

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

LGR6 and Lrig1 Mark Stem Cells of the IFE

Lrig1+ cells are the quiescent stem cells in the basal layer of the epidermis [14]. In the HF, they are found in the junctional region near the sebaceous gland and infundibulum [5, 23]. In the IFE, Lrig1 expressing cells were found at a specific location at the junction of the dermis and epidermis, called rete ridge [14]. These cells also express high levels of β1 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 β1 integrin [23, 24].

Lrig1 is a negative feedback regulator of EGFR signaling [25] and negatively regulates 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 the epidermal stem cells [26–28] and Lrig1 inhibits their proliferation, Lrig1 acts as a brake against proliferation and differentiation (Fig. 1). Further, Lrig1+ cells express high levels of $\alpha6\beta4$ integrins [14, 23] while myc reduces the expression of $\alpha6\beta4$ integrins [29]. Furthermore, expression of myc2 in the basal layer reduced the expression of β1 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, the LGR6 cells are more primitive stem cells than the Lrig1 cells. Further, like the Lrig1 cells, the LGR6 cells express α6 integrins [33].

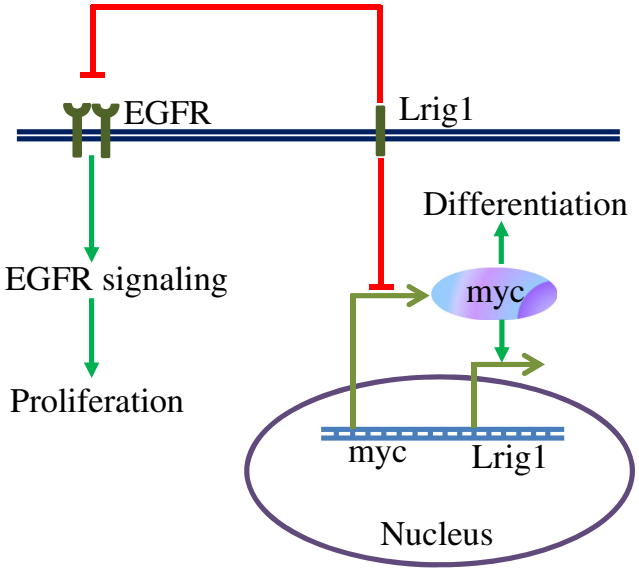

Fig. 1 Regulation of proliferation and differentiation by Lrig1. Lrig1 negatively regulates EGFR, inhibiting the proliferation of cells. Lrig1 and myc are linked in a negative feedback loop, inhibiting the differentiation of the cells. Positive regulation has been represented by an arrow while negative regulation has been shown by a line (a minus sign)

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

Stochastic Model of the Basal Layer

We model that the interconversions among the 4 cell types (Table 1) during the 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, the less favorable process, is σp, where σ is the probability of the basal cells expressing either 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 2a and b lists all the reactions occurring in the basal layer and their probabilities.

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

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 Conversion reactions and probabilities of conversion of the SC/TA cells in the basal layer for the 4-cell type model. **a** Conversion reactions of the LGR6+/Lrig1- (the cell type labeled as A) and the LGR6-/Lrig1- (the cell type labeled as B) cells **b** Conversion reactions of the LGR6+/Lrig 1+ (the cell type labeled as C) and the LGR-/Lrig1+ (the cell type labeled as D) cells

Conversion	Probability	Conversion	Probability
A➔A+A	p^2	B➔A+A	σ^2p^2
A➔B+B	p^2	B➔B+B	p^2
A➔C+C	σ^2p^2	B➔C+C	σ^4p^2
A➔D+D	σ^2p^2	B➔D+D	σ^2p^2
A➔A+B	p^2	B➔A+B	σp^2
A➔A+C	σp^2	B➔A+C	σ^3p^2
A➔A+D	σp^2	B➔A+D	σ^2p^2
A➔B+C	σp^2	B➔B+C	σ^2p^2
A➔B+D	σp^2	B➔B+D	σp^2
A➔C+D	σ^2p^2	B➔C+D	σ^3p^2
C➔A+A	p^2	D➔A+A	σ^2p^2
C➔B+B	p^2	D➔B+B	p^2
C➔C+C	p^2	D➔C+C	σ^2p^2
C➔D+D	p^2	D➔D+D	p^2
C➔A+B	p^2	D➔A+B	σp^2
C➔C+A	p^2	D➔A+C	σ^2p^2
C➔A+D	p^2	D➔A+D	σp^2
C➔B+C	p^2	D➔B+C	σp^2
C➔B+D	p^2	D➔B+D	p^2
C➔C+D	p^2	D➔C+D	σp^2

From Table 2a and b, we calculate the propensity of conversion of each type of the basal cells if the conversion reactions occur stochastically during the epidermal homeostasis. The propensities of conversions are:

$$\Delta A = p^2\left(5+\sigma+3\sigma^2+\sigma^3\right);$$
$$\Delta B = p^2\left(12+4\sigma-3\sigma^2+\sigma^3-\sigma^4\right);$$
$$\Delta C = p^2\left(-5+4\sigma+7\sigma^2+2\sigma^3+2\sigma^4\right);$$
$$\Delta D = p^2\left(5+\sigma+3\sigma^2+\sigma^3\right)$$

Since at the 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 σ = 0.7: %A = 24.3, %B = 43.5, %C = 7.8, and %D = 24.3.

SC/TA Model of the Basal Layer of the IFE

Since the cell type C (Table 1) expresses both stem cell markers and may be 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, the LGR6 cells can generate all skin lineages including the 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 (Fig. 2a). In contrast, the LGR6(−)/Lrig1(−) cells, the cell type B (Table 1), have been characterized as the major TA cells since they are proliferative and can differentiate (Fig. 2b). 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 (Fig. 2c). Thus, these cells may constitute another type of the TA cell population and we label them as the minor TA cell 2, the second major TA cell in the basal layer. Interestingly, proliferating LGR6+ cells were found to express Axin2 [38]. Thus, the LGR6+ cells and the Axin2+ cells may be the same and the Axin2 cells have been shown to differentiate directly [21]. In contrast, the LGR6(−)/Lrig1(+) cells, the type D cells, have been labeled as the minor TA cell 3 because they are nonproliferative (Fig. 2d). However, notwithstanding their quiescence in the basal layer, the LGR6(−)/Lrig1(+) cells can upregulate myc and differentiate in the upper layers of the epidermis. Yet, the differentiation of the Lrig1+ epidermal basal cells remains to be shown although it has been shown in the meibomian gland epithelial cells in the eyelid, where the progenitor cells express Lrig1 while the differentiated cells suppress Lrig1 and express DNase2 instead [39]. Interestingly, $\alpha 6\beta 4$ integrins are found on the surfaces of some cells in the immediate suprabasal region of the epidermis [40], suggesting the existence of the 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 the LGR6(−) or the Lrig1(−) cells change into LGR6(+) and Lrig1(+) cells while p is the

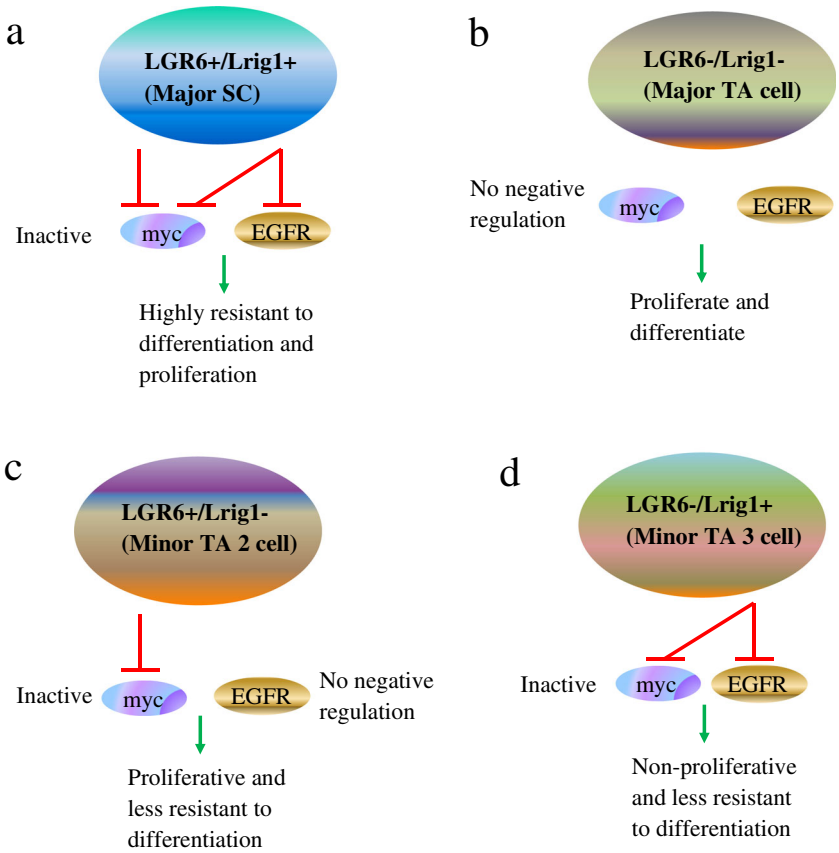

Fig. 2 Properties of the four types of cells in the basal layer. The property of proliferation and differentiation of the four types of the basal cells have been shown (**a**) the major stem cell (**b**) the major transit-amplifying cell (**c**) the minor transit-amplifying cell 2 (**d**) the minor transit-amplifying cell 3

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 (the type C cells) varies from 0%–23.3% according to eq. 3. This puts a limit on the difference between the major TA (the type B cells) and the major stem cells (the type C cells). The major TA cells should convert to the 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 the major TA cells are significantly different from the major SCs, they may not be so different that the major TA cells cannot convert to the 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 the individual layers, the minimum limit on the probability of conversion of the major TA cells to the 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 the 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 the 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 minimum value of σ, corresponding to the zero value of the fraction of the major stem cell, for the basal layer having 8 cell types (Supplementary information S) and compared it with the basal layer having 4 cell types. Table 3 shows the minimum value of σ for the basal layer having different numbers of the cell types. If the 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, σ_{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 the major TA cells to major SCs below the minimum allowable probability, may exhaust the major SC population. In addition, the gradual stemness hypothesis may not require the LGR6+ and Lrig1+ cells 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 the TA cells produced from the SCs commit to the terminal differentiation in the basal layer before moving up, which may be invalid since the basal layer may not contain the committed cells.

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. Fractions of the different cell types in the basal layer, consisting of either 2 types of cells or 3 types of cells or 4 types of cells or 8 types of cells, have been calculated based on their propensities of conversion. The minimum value of σ corresponds to the fractions when the fraction of the major stem cell becomes zero

Numbers of types of cells in the basal layer	σ_{min}
2	0
3	0
4	0.56
8	0.70

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 the LGR6(−) or Lrig1(−) cells to LGR6(+) and Lrig1(+) is 0 (Table 3). Thus in these cases, the TA cells could be completely different from the 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 the 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 the suprabasal layers, i.e. to have a nonzero σ_{min}, is 4 (Table 3). Further, for the number of cell types to be 4, the σ_{min} may not be unreasonably high (Table 3) and, most importantly, the 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 σ = 0.7 and the 4-cell type model, the percentage of the 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 (% of type C cells + % of type D cells) 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 the basal cells (i.e. LGR6+ and/or Lrig1+) may express α6 integrins as has been observed previously [40].

The model above presents the composition of the basal layer.

Epidermal Differentiation

To present a model of the differentiation of TA cells in the upper layers of the IFE, we put together information on Wnt signaling enhancers and antagonists, their combinatorial expression in patterning, and the role of the 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 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 the Wnt coreceptors Frizzled/Lrp [44, 46] and potentiates Wnt signaling by enhancing Lrp6 phosphorylation [47] (Fig. 3). In addition to the direct effect, the LGR5/R-spondin complex neutralizes the two E3 ligases, Rnf43 and Znrf3, which negatively regulate Wnt receptors [43] (Fig. 3). Moreover, Rnf43 and Znrf3 are Wnt target genes, forming a negative feedback regulation of the Wnt signaling [43] (Fig. 3). This negative feedback loop may block Wnt signaling, which can be rescued by the action of LGR5/R-spondins on Rnf43 and Znrf3 (Fig. 3). In addition to Rnf3/Znrf3, Dickkopf (Dkk)1, an inhibitor of Wnt signaling is a Wnt target gene, creating another negative feedback loop [48] (Fig. 3). Thus, enhancers, negative feedback regulators, and anti-negative feedback regulators modulate the Wnt signaling and may create a spatial distribution of its strength in a context-specific manner.

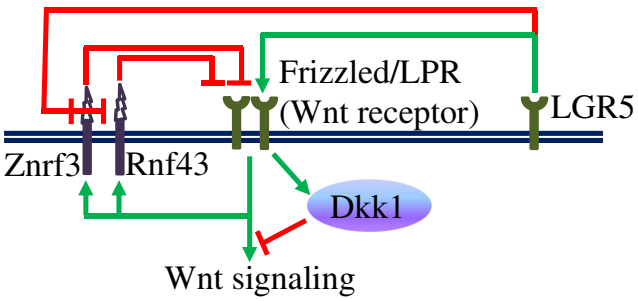

Fig. 3 Wnt pathway feedback loops. Wnt receptors (Frizzled/LPR) are linked to two E3 ligases (Znrf3/Rnf43) in a negative feedback loop. LGR5 potentiates Wnt signaling and negatively affects Znrf3/Rnf43, antagonizing the effect of the negative feedback loop. Further, Wnt receptors (Frizzled/LPR) are linked to Wnt inhibitor Dkk1 in another negative feedback loop. Positive regulation has been shown with an arrow while negative regulation has been shown with a line (a minus sign)

Interestingly, negative feedback loops may create Turing instability, which can generate patterns in the 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 the axial patterning including the 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 the combinatorial Wnt and Dkk expression are important for the 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 1α,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 [59–61]. Similarly, oxysterol-induced osteogenic differentiation of the 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 the IFE [63, 64], suggesting a role of Dkk3 in the epidermal differentiation.

Autocrine Vs. Paracrine Wnt Signaling

The Axin2 expressing IFE stem cells secrete Wnt ligands that act in an autocrine manner [21]. We hypothesized that these cells are the LGR6+ stem cells in the epidermis. In the HF, the highest activity of Wnt signaling was observed in the precursor cells that produce the hair shaft, i. e. just before the differentiation of the TA cells in the HF [65]. Presumably, these precursor cells are the progenies of the LGR5+ HFSCs. Interestingly, in the HF generation, epidermal Wnt ligands are required for the dermal Wnt signaling and the dermal Wnt signaling is required for the patterned upregulation of the epidermal Wnt signaling [66], forming a positive feedback loop. This loop is critical for the initiation of the hair follicle placodes in the skin [66]. Thus, the hair shaft precursor cells, the TA cells in the HF, may receive Wnt ligands in a paracrine

manner from the outer root sheath. In the paracrine Wnt activation, gradation of the Wnt strength can be achieved merely by the distance of the Wnt ligand source, which supports the finding that the Wnt signaling strength is the highest in the hair shaft precursor cells, residing close to the outer root sheath [65]. In contrast, in the IFE, besides diffusion of the Wnt ligands from the basal layer, the autocrine Wnt signaling is also present [21]. Thus, the differentiation of the IFE TA cells may also require the spatial distribution of the 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, we present the model [21] of the epidermal differentiation and combine it with the HF.

A Combined Model of the HF and the Differentiation Program of the IFE

In this model, in the IFE, stem cells produce both ligands and inhibitors of the 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 the committed IFE cells to HF stem cells and caused hair tumors [67]. Thus, the HFSCs, through LGR5 and paracrine Wnt ligands may have a higher strength of Wnt signaling than the IFE LGR6+ cells, which may use the autocrine Wnt

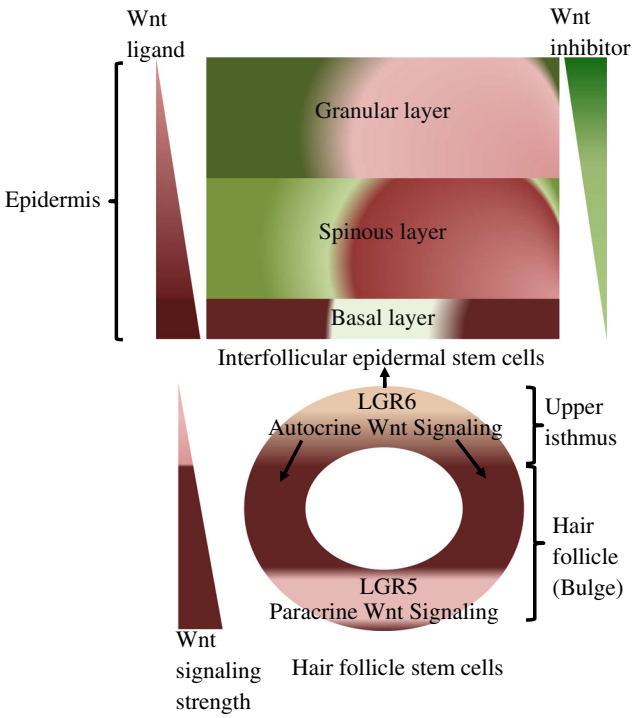

Fig. 4 A combined model of the HF and the differentiation program of the epidermis. LGR6 cells convert to LGR5 cells, which seed the HF. LGR6 cells establish the differentiated epidermis with the help of Wnt ligands and inhibitors

signaling along with the expression of Wnt inhibitors. In this model, IFESC switches from the LGR6 to LGR5 receptor, changing to HFSC, while Wnt inhibitors along with the Wnt ligands generate the graded strength of Wnt signaling in the IFE, causing the TA cell differentiation in the epidermis (Fig. 4).

Conclusion

In this paper, we have collated and analyzed the literature on the epidermal and the hair follicle stem cells, the Wnt signaling, and the role of the Wnt inhibitor Dickkopf in cell differentiation and cancer. Further, we have presented a model in which there are 4-types of the basal cells. The types of basal cells include a major stem cell, a major transit-amplifying cell, and two minor transit-amplifying cells. The four types of the basal cells are in a stochastic equilibrium, maintaining the homeostasis of the epidermis. The model shows that the major TA cell must convert to the major SC with a minimum probability otherwise the major SC population will be exhausted. Further, we discussed that while the paracrine Wnt signaling may be important for the hair follicle, autocrine Wnt signaling may have a role in the epidermal differentiation. Moreover, the epidermal cells differentiate due to a programmed expression of the Wnt ligands and inhibitors. Wnt signaling is controlled by the feedback loops and feedback regulation may provide the right amount of Wnt signaling strength, controlling basal cells' homeostasis, their proliferation and migration in the wound healing, and their differentiation to form a stratified epidermis. Furthermore, while the LGR5 cells seed the hair follicle, LGR6 cells are the most primitive epidermal stem cells.

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Availability of Data and Material All data are available in the manuscript and the supplementary information files.

Code Availability Not applicable.

Authors' Contributions RS performed the literature search, data analysis, and model development, and wrote the manuscript.

Declarations

Conflict of Interest The author declares that he has no conflict of interest.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent for Publication The author agrees to publish the manuscript.

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