Chapter 3

Mechanism of Hair Loss from the Point of View of Epidermal Cell Polarity

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/66735

Abstract

The epidermis and hair follicle epithelium have a polarized stratified architecture, and epidermal homeostasis is maintained by stem cell and progenitor populations present in the basal layer of the interfollicular epidermis and in different compartments of the hair follicle. Atypical protein kinase Cs (aPKCs—a subgroup of the PKC family) are localized to tight junctions and regulate the apico-basal epithelial polarity in simple epithelia. In the stratified epidermis, aPKCs are expressed in the basal layer and are implicated in the regulation of oriented cell division by localizing to the apical pole of basal cells during mitosis. Mutant mice harboring epidermis-specific deletion of aPKCλ showed progressive hair loss, abnormal hair cycling, an increase of asymmetric cell division in the epidermis and hair follicles, and a gradual decrease in the hair follicle stem cell (HFSC) population. Lineage tracing analysis has demonstrated that mutant HFSCs lose their stemness and become more committed proliferating progenitors. Moreover, the expressions of quiescence-inducing factors (Bmp6 and Fgf18) were suppressed in the mutant hair follicles. These results clarify a novel function of aPKCλ in maintaining the quiescence of HFSCs and suggest that epidermal cell polarity is a new clue to understanding the pathogenesis of hair loss.

Keywords: cell polarity, atypical protein kinase C, hair follicle stem cell, quiescence, alopecia, hair cycle, conditional knockout mouse

1. Introduction

The stimulus-induced turnover of membrane lipids is an important event during cell signaling. The protein kinase C (PKC) family is a group of serine/threonine kinases that mediate intracellular signaling activated by growth factor receptors, G-protein-coupled

receptors, and tyrosine kinase receptors through lipid-derived secondary messengers [1]. The PKC family members share a highly conserved carboxy-terminal kinase domain, and differences in their requirements for lipids and calcium for activation are attributed to structural differences in the amino-terminal regulatory domain [2]. In mammals, the PKC family is composed of the following three structurally and functionally distinct subgroups: conventional PKCs (cPKC; α, βI/II, and γ), novel PKCs (nPKC; δ, ε, η, and θ), and atypical PKCs (aPKC; ζ and ι/λ: λ in mice) (**Figure 1**) [2, 3]. The cPKCs have a prototypic regulatory domain consisting of the following two conserved regions: C1 and C2. The C1 region serves as a binding site for diacylglycerol (DAG) and phospholipids, whereas the C2 region serves as a binding site for calcium. The C1 domain also acts as a target for tumor-promoting phorbol esters [4]. The nPKCs are similarly activated by DAG, phospholipids, and phorbol esters, but are not activated by calcium because of the lack of calcium-binding loops in the C2-like region [2]. The aPKCs have an atypical C1 domain and do not depend on DAG or calcium for activation [5, 6]. The activity of aPKCs is primarily regulated by protein-protein interactions through a Phox/Bem1 (PB1) domain located at the amino-terminus, which interacts with other PB1 domain-containing proteins, such as PAR 6 (see below) [7, 8].

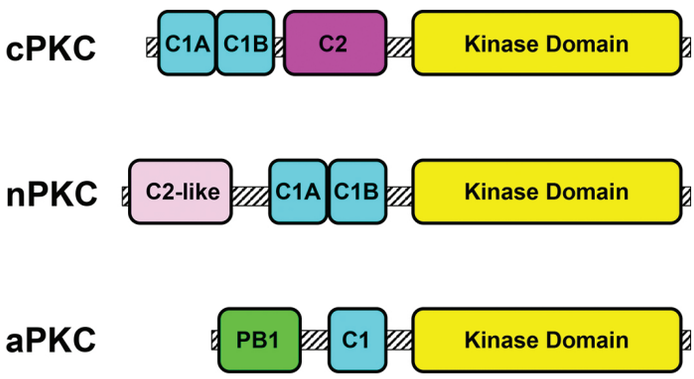

Figure 1. Schematic presentation of the PKC family. In mammals, the PKC family is composed of the following three structurally and functionally distinct subgroups: conventional PKCs (cPKCs; α, βI/II, and γ), novel PKCs (nPKCs; δ, ε, η, and θ), and atypical PKCs (aPKCs; ζ and ι/λ: λ in mice). aPKC (a regulator of epithelial cell polarity) lacks the prototypic regulatory domain composed of C1 and C2 regions.

Among the PKC family members, aPKCs play essential roles in establishing epithelial cell polarity by interacting with partition-defective (Par) proteins [9, 10]. The par genes were first identified in genetic screening for regulators of asymmetric division in the early embryo of *Caenorhabditis elegans* (*C. elegans*) [11, 12]. Par3 (a mammalian ortholog of *C. elegans* par-3) has been rediscovered as an aPKC-interacting protein [13]. Depletion of PKC-3 (an aPKC ortholog in *C. elegans*) using RNA interference results in a phenotype similar to that of par-3 and par-6 mutants [14, 15], leading to the discovery of physical and functional interactions among PKC-3, par-3, and par-6. Both Par3 and Par6 are PDZ domain-containing proteins and act as scaffold proteins. Indeed, Par6 and aPKCs form a stable heterodimer through their respective PB1 domains [7, 8]. Subsequently, Par6 serves as an adaptor protein for Rho family GTPases, Rac1, and Cdc42, in the activation of the Par6-aPKC heterodimer [16, 17]. The active form of Par6-aPKC, in turn, binds to Par3, which is mediated by the kinase domain of aPKCs and the

PDZ domain of Par6. This aPKC-Par3-Par6 ternary complex is evolutionarily conserved and is implicated in a variety of cell polarity events [9, 10, 18].

Although in mammalian simple epithelia, the aPKC-Par complex is located at tight junctions and regulates apicobasal epithelial polarity, in the stratified epidermis, tight junctions are present only in the stratum granulosum [19, 20]. The aPKCs are expressed in the multilayered epidermis; aPKCλ, a predominant aPKC isoform in the epidermis, is distributed throughout the epidermis, whereas aPKCζ is present in basal cells [21]. These distributions suggest that the aPKCs have unidentified functions in the stratified epidermis, where epithelial polarity is established across different cell layers, and proliferation and differentiation are strictly regulated.

To clarify the functions of aPKCs in the stratified epidermis, two groups, including my group, have generated mutant mice harboring epidermis-specific deletion of aPKC [22, 23], using the transgenic mouse line expressing Cre recombinase under the control of the keratin 5 (K5) or keratin 14 (K14) promoter [24, 25]. These mice show essentially the same phenotypes: progressive hair loss, abnormal hair cycling, hyperplasia of the epidermis and sebaceous gland, loss of the hair follicle stem cell (HFSC) quiescence, and a gradual decrease in HFSC in population. In this article, I discuss how these various phenotypes are related with one another and present the mechanism of hair loss from the point of view of epidermal cell polarity.

2. Progressive hair loss

Whole-body inactivation of aPKCλ results in embryonic lethality, which hampers further examination of the role of the aPKCλ-Par complex in epidermal homeostasis. To overcome this problem, two groups, including my group, generated mutant mice with epidermis-specific loss of aPKCλ using K5-Cre or K14-Cre mice [22, 23]. Although, in a strict sense, the distribution of K14-Cre transgene activity differs from that of K5-Cre transgene activity in the epidermis and hair follicle [25, 26], both mutant mice showed similar skin phenotypes. Thus, hereafter, when referring to findings common to both conditional knockout (cKO) mice, the term mutant mice or aPKCλ cKO mice is used, and when referring to findings obtained in the mutant mice associated with K5-Cre or K14-Cre individually, the term K5-cKO or K14-cKO mice is used, respectively.

Although aPKCλ cKO mice showed no gross anomalies at birth, they were easily distinguished from their control counterparts by the thinning of pledge hair from around postnatal day (P) 14 onward. The hair loss was progressive, and one-year-old mutant mice exhibited total alopecia (**Figure 2**) [22, 23]. The vibrissae were also shortened or were lost in the mutant mice [23].

K14-cKO mice showed impaired hair morphology. Scanning electron microscopy demonstrated that the regular cuticle pattern was lost in the mutant hair shafts. Consistent with this finding, the expressions of hair keratins in the inner root sheath (K28), cuticle (K35, K82, K85), cortex (K35, K81, K85), medulla (K28, K6, K75), and companion layer (K75, K6) were severely reduced [22].

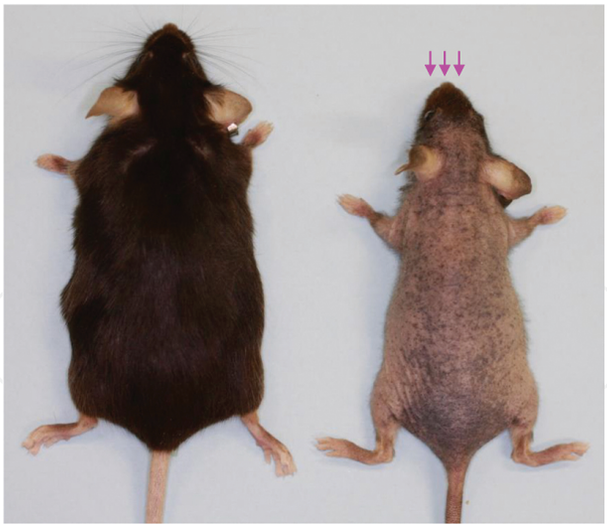

Figure 2. Hair loss phenotype of mutant mice. Macroscopic presentation of a one-year-old control (left) and a K5-cKO mouse (right). Note that the vibrissae were lost in the mutant mouse (arrows).

3. Abnormal hair cycling

During postnatal morphogenesis stages (P0–P16), the hair cycling of mutant hair follicles appeared to proceed normally, although the mutant integument became histologically noticeable with a thickened interfollicular epidermis (IFE) and enlarged sebaceous glands [22, 23]. However, entry into the first postnatal telogen (resting phase, normally starts around P18) was delayed in the mutant hair follicles. At P28, when control hair follicles entered into the first anagen (growth phase), they still had a long epithelial strand, a characteristic structure of catagen (regression phase) [27], and were positive for placental cadherin (P-cadherin), a marker for the epithelial strand. As a result, the start of the first anagen was delayed until P37 in the K5-cKO mice. In the K14-KO mice, the percentages of hair follicles that properly entered into catagen, telogen, and anagen were significantly reduced.

Moreover, mutant hair follicles were morphologically abnormal. They exhibited hyperkeratotic plugs and cyst-like structures with an expanded infundibulum and isthmus. Strikingly, these severely deformed mutant hair follicles regrew and entered into the second anagen much later than the control hair follicles. However, the mutant hair follicles in anagen did not proceed further into the second catagen or telogen. Instead, they started to degenerate, as revealed by the shrinking hair bulbs and reduced expressions of Ki67 and Lef1 [23]. In one-year-old mutant mice, the number of hair follicles was severely diminished.

Fibroblast growth factor 18 (Fgf18) is expressed in hair follicles and colocalized with keratin 15 (K15) and CD34 [28, 29], both of which are expressed in the bulge region at telogen. Fgf18 shows a cyclic expression pattern in hair follicles; its levels are low in anagen and high throughout telogen [29]. In mutant mice with epidermis-specific loss of Fgf18, the length of telogen was short, resulting in rapid succession of hair cycling [29]. Interestingly, the expres-

sion of Fgf18 was severely suppressed in the K5-cKO mice during hair morphogenesis and hair development [23]. Although precise molecular mechanisms associated with abnormal hair cycling in cKO mice remain to be elucidated, these results suggest that aPKCλ controls hair follicle cycling through Fgf18 signaling.

4. Hyperplasia of the epidermis and sebaceous gland

In aPKCλ cKO mice, the IFE and sebaceous glands were affected [22, 23]. The thickness of the IFE of the dorsal skin increased in the mutant mice, as revealed by significant expansion of the expression domain for loricrin (a marker for terminal differentiation) and keratin 10 (K10, a marker for spinous cells). Moreover, the sebaceous glands were enlarged in the cKO mice. Accordingly, immunostaining for adipose differentiation-related protein (ADFP, a marker for the surface of lipid droplets) and stearoyl-CoA desaturase 1 (SCD1, a marker for mature sebocytes), and Nile red staining showed remarkable increases in the sebaceous glands of the mutant mice.

Importantly, the expression domain of Lrig1 was expanded in the mutant mice [22, 23]. Lrig1 marks the junctional zone between the infundibulum and the sebaceous gland, and Lrig1-expressing cells contribute to the IFE and sebaceous glands [30]. Thus, this bipotent activity of Lrig1 is thought to be implicated in hyperplasia of these tissues.

5. Dysregulation of HFSC marker expression

The hair follicle at telogen is composed of several compartments, including the interfollicular epidermis, infundibulum, isthmus, bulge, and hair germ. Recent studies have identified multiple new stem cell and progenitor populations in each compartment, which exhibits unique marker expression profiles (**Figure 3**) [31, 32].

5.1. Bulge

In the skin, label-retaining cells (LRCs) having a highly proliferative activity reside in the bulge region of the hair follicle [33], and LRCs in the bulge are multipotent [34, 35]. Since the first identification of K15 as a bulge marker [36], several factors have been demonstrated to be expressed in the bulge region at telogen, such as CD34 [37], leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) [38], and transcriptional factors Sox9 [39, 40], Tcf3 [41], Lhx2 [42, 43], and nuclear factor of activated T cells c1 (Nfatc1) [44]. CD34, a hematopoietic stem and progenitor cell marker, colocalizes with LRCs and K15 expression in the bulge region [37]. Lgr5 marks the lower bulge and secondary hair germ during telogen, and contributes to all hair lineages, but not to the epidermis and sebaceous glands [38]. Sox9-expressing cells also contribute to all skin epithelial lineages [39, 40]. Tcf3 and Tcf4 are downstream targets of Wnt signaling, and in Tcf3/Tcf4-null mice, hair follicle formation was initiated, but further development was severely impaired [45]. Lhx2 maintains the HFSC

character downstream of Wnt and Shh signaling [43]. Nfatc1 colocalizes with other bulge stem cell markers, including CD34, Sox9, Tcf3, and Lhx2 [44]. Nfatc1 mediates HFSC quiescence by transcriptionally suppressing cyclin-dependent kinase 4 (CDK4) expression upstream of BMP4 signaling [44].

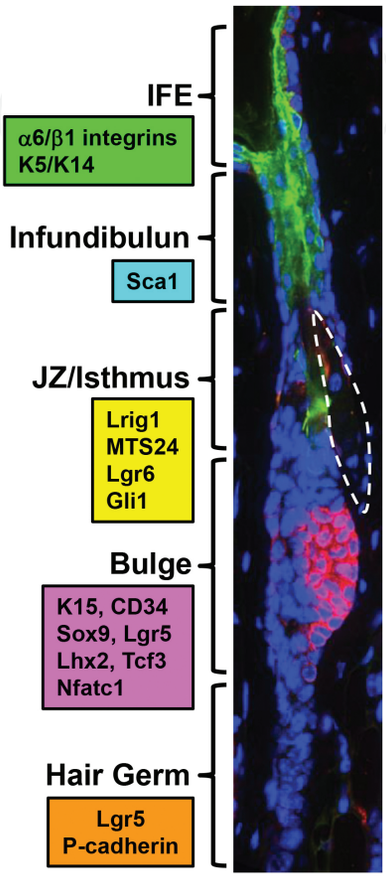

Figure 3. Distinct stem cell populations in the hair follicle. The hair follicle is divided into the interfollicular epidermis (IFE), infundibulum, junctional zone (JZ)/isthmus, sebaceous gland, bulge, and hair germ. The expressions of proposed stem cell markers at telogen are indicated. Note that the expression domains of these markers dynamically change during hair cycling. A murine hair follicle in telogen is immunostained for keratin 10 (a marker for suprabasal cells, green) and keratin 15 (a marker for bulge stem cells, red). Nuclei are counterstained with 4′, 6-diamidino-2-phenylindole. The dotted area indicates the sebaceous gland.

Comparison of the expression profiles of these bulge stem cell markers between the early and late stages in mutant mice demonstrated that the expressions of these markers diminished at later stages or were mislocalized to areas outside the bulge [22, 23]. These results indicate that aPKCλ regulates the expression and localization of HFSCs.

5.2. Junctional zone/isthmus

Markers that recognize distinct cell populations of the upper (junctional zone) and lower bulge regions have been identified. As mentioned above, Lrig1 is a marker for the junctional zone/isthmus, which is located between the sebaceous gland and the bulge [30]. Lrig1-expressing

cells have a bipotent activity in the steady state, and give rise to the IFE and sebaceous glands [30]. The cell-surface glycoprotein MTS24 is another marker for the isthmus/junctional zone [46]. MTS24-expressing cells do not express CD34 or keratin 15, and LRCs are infrequently observed among them. Lgr6 is expressed in a distinct population between the upper K5- and CD34-expressing cells and lower MTS24- and Lrig1-expressing cells in the bulge region [47]. Although prenatal Lgr6-positive cells contribute to the IFE, sebaceous glands, and hair follicles, the contribution to the hair follicles diminishes with age. In mutant hair follicles, the expression domains for Lrig1 and MTS24 were considerably expanded.

6. Depletion of HFSCs

HFSCs can be identified as α6-integrin and CD34 double-positive cells with fluorescence-activated cell sorting (FACS) analysis [48]. Consistent with the decrease in the expression of the bulge stem cell markers at later stages, quantitative FACS analysis demonstrated that the number of α6-integrin/CD34 double-positive cells gradually decreased in aPKCλ cKO mice as the mice aged [22, 23]. Moreover, LRCs in BrdU pulse-chase labeling experiments were severely reduced in the bulge region of the mutant hair follicles [22, 23]. Conversely, the number of Lrig1- or MTS24-expressiong cells was gradually increased [22]. These results indicate that in aPKCλ cKO mice a decrease in quiescent HFSCs is accompanied by an increase of progenitor cells committed to the junctional zone/isthmus and infundibulum.

7. Loss of HFSC quiescence

Mutant mice analyses have identified intrinsic and paracrine mechanisms to clarify the loss of HFSC quiescence caused by epidermis-specific inactivation of aPKCλ.

7.1. Intrinsic mechanism: oriented cell division

Oriented cell division is crucial for tissue morphogenesis and homeostasis [49, 50]. Basal cells in the epidermis show the following two types of cell division: symmetric cell division (SCD) and asymmetric cell division (ACD). SCD, in which alignment of the mitotic spindle is parallel to the basement membrane, results in two equivalent daughter cells, whereas ACD, in which alignment of the mitotic spindle is perpendicular to the basement membrane, results in two daughter cells with different fates (one basal cell and one more differentiation-committed suprabasal cell) (**Figure 4**).

In the epidermis, a balance between SCD and ACD is important for coordinated proliferation and differentiation. A shift from SCD to ACD in basal cells of the developing epidermis coincides with the onset of stratification [51]. At embryonic day 12.5 (E12.5), most of the murine epidermis is single-layered, and the majority of cell divisions (>90%) are symmetric, whereas at E14.5–18.5, more than 70% of cell divisions are perpendicular to the basement membrane [51]. ACD promotes Notch signaling, leading to epidermal differentiation [52]. During SCD

and ACD, the aPKC-Par complex localizes at the apical surface in a β1-integrin- and α-catenin-dependent manner [51]. Thus, in the β1-integrin KO and α-catenin KO epidermis, apical localization of the aPKC and Par3-LGN-inscuteable complex is abolished. Niessen et al. demonstrate that epidermal loss of aPKCλ induced a shift toward ACD not only in the IFE but also in the bulge stem cells and the junctional zone/isthmus region, leading to an expansion of progenitor cell populations committed to epidermal cell fate [22].

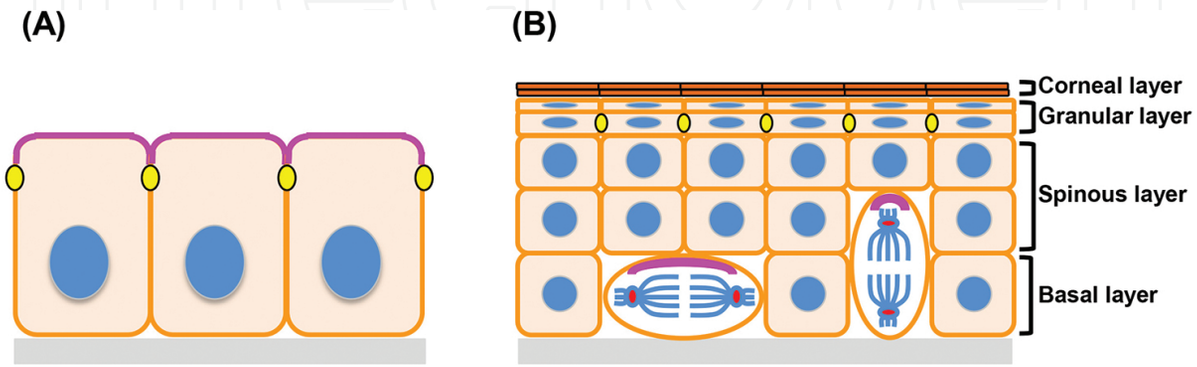

Figure 4. Localization of aPKC in the simple epithelium and multilayered epidermis. (A) In the simple epithelium, aPKC forms a ternary complex with Par3 and Par6 and is localized to the apical surface of the cell and tight junctions (yellow circles). (B) In the stratified epidermis, tight junctions are only present in the granular layer. During symmetric cell division, in which alignment of the mitotic spindle is parallel to the basement membrane (gray), and during asymmetric cell division, in which alignment of the mitotic spindle is perpendicular to the basement membrane, aPKC (pink) is localized to the apical surface of the cell.

Lineage tracing analysis using Lgr5-Cre mice confirmed that epidermal loss of aPKCλ changes the fate of a bulge stem cell to an epidermal lineage [22]. In telogen, Lgr5-positive cells resided in the lower bulge and hair germ, as described above. During anagen, Lgr5 progeny exclusively contributed to down-growing hair follicles, whereas upon loss of aPKCλ, Lgr5-positive cells contributed to the upper junctional zone/isthmus and the IFE, as well as the lower-growing hair follicles. Consistent with this the expression domains for Lig1 and MTS24 increased. These findings indicate that aPKCλ regulates oriented cell division and thereby controls epidermal stem cell behavior and cell fate decisions.

7.2. Paracrine mechanism

HFSCs remain quiescent during telogen. Near the end of telogen, the HFSCs become activated to elicit the growth phase of the hair cycle. Basically, Bmp signaling induces quiescence and Wnt signaling activates HFSCs. However, the molecular mechanism underlying the cyclic inhibition and activation of HFSCs has recently started to be elucidated. Three types of Bmps from different sources induce quiescence in HFSCs: Bmp2 from subcutaneous adipocytes, Bmp4 from dermal fibroblasts [53], and Bmp6 from the inner layer of the bulge (**Figure 5**) [54]. Bmp antagonism is one of the key concepts to understand morphogenesis. In the telogen to anagen transition, the dermal papilla secretes HFSC-activating factors, Fgf7, Fgf10, Wnts, and

Tgfβ2 [55], and Bmp inhibitors, such as noggin [53, 56], overcome the inhibitory effects of Bmps to activate HFSCs (**Figure 5**) [57].

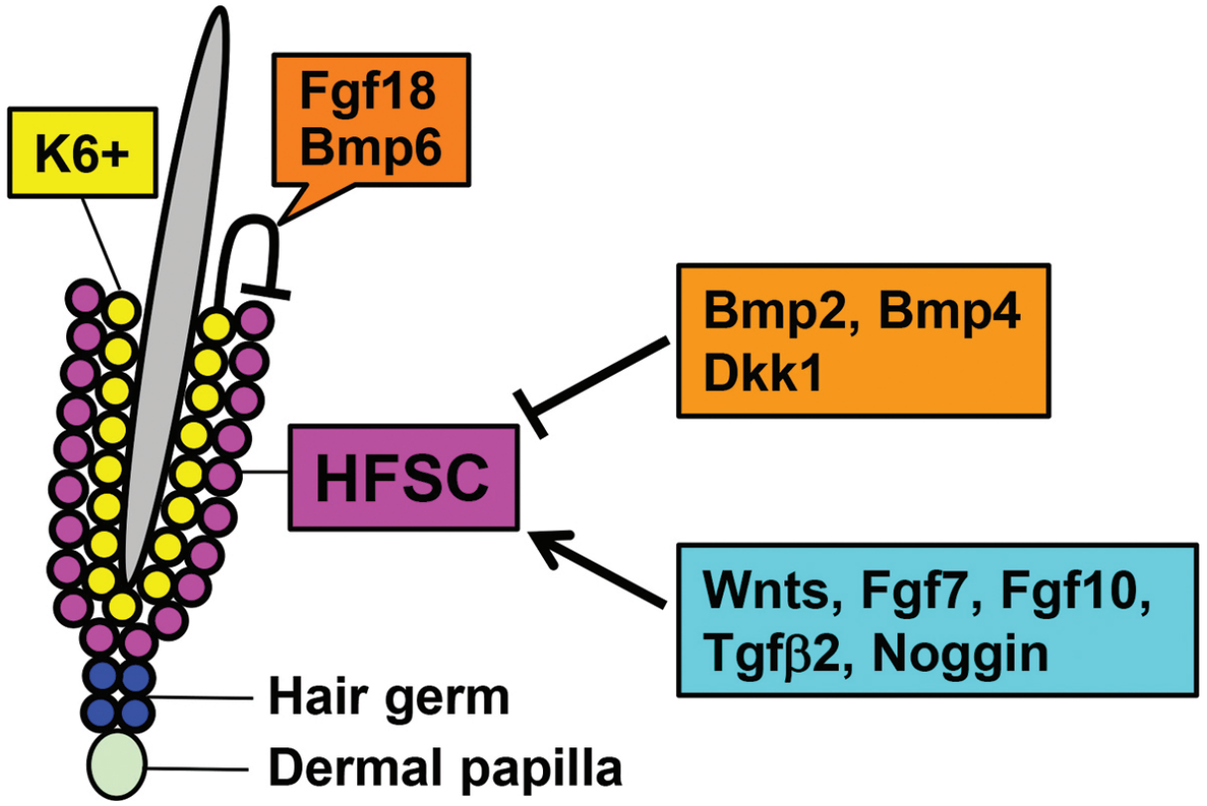

Figure 5. Current model for the maintenance of HFSC quiescence. A hair follicle at telogen is presented. K6-positive cells mark the inner layer of the bulge, from which quiescence-inducing Bmp6 and Fgf18 are produced. The HFSCs are kept quiescent by Bmp2 from subcutaneous adipocytes and Bmp4 from dermal fibroblasts. At the telogen to anagen transition, HFSC-activating factors, such as Fgf7, Fgf10, Wnts, Noggin (Bmp antagonist), and Tgfβ2, antagonize the inhibitory effects of quiescence-inducing factors.

The inner layer of the bulge has attracted attention as a source of the quiescence-inducing factors Bmp6 and Fgf18 [54]. Although induction of K6 expression is closely associated with hyperproliferative conditions [58, 59], such as psoriasis and squamous cell carcinoma, a recent study has clarified that during telogen, K6 is strongly expressed in the inner layer of the bulge and K6-positive bulge cells secrete Bmp4 and Fgf18 to inhibit proliferation of the CD34-positive outer bulge stem cells [54]. Thus, upon the ablation of K6-positive bulge cells, HFSCs become prematurely activated and enter a new cycle of hair growth [54]. In the K5-cKO mice, K6 expression in the inner bulge cells was abolished, and the expressions of Bmp6 and Fgf18 were suppressed at the mRNA and protein levels [23], suggesting that aPKCλ regulates HFSC quiescence upstream of Bmp6 and Fgf18. Moreover, because K6-positive bulge cells are also involved in intercellular junctions that anchor the old hair shaft [54], a decrease in the expression of K6 in the inner layer of the mutant bulge may be involved in the falling-off of hair shafts in mutant hair follicles.

8. The roles of other components of the aPKC-Par complex in hair loss

8.1. aPKC

In the epidermis, aPKCζ, another isoform of aPKC, localizes in the cytoplasm and nucleus of basal cells, although its expression level is much lower than that of aPKCλ in the skin of newborns and adults (40-fold and 10-fold, respectively) [21]. Although the activity of aPKCζ in epithelial polarity in vitro is distinguishable from that of aPKCλ, mice lacking aPKCζ at the whole-body level were viable and showed no obvious skin phenotypes [60]. This may be attributed to the low expression of aPKCζ in the epidermis, and aPKCλ may compensate for the loss of aPKCζ. However, these results do not exclude the possibility that aPKCλ and aPKCζ synergistically regulate epithelial cell polarity, oriented cell division, epidermal differentiation, and HFSC maintenance. Indeed, the combined deletion of the aPKCλ/ι and aPKCζ isoforms in podocytes leads to defective glomerular maturation with incomplete capillary formation and mesangiolysis, and causes severe proteinuria and perinatal death [61]. Thus, studies on mutant mice with simultaneous epidermal inactivation of aPKCλ and aPKCζ would help provide further information on the synergism between the two.

8.2. Junctional aPKC vs non-junctional aPKC

Because aPKCs are localized to tight junctions, in aPKCλ cKO mice, the aPKCλ-Par6-Par3 complex at tight junctions was supposed to be absent or impaired in the granular layer. However, in the mutant mice, the overall multilayered architecture of the epidermis appeared to be normal, or rather hyperplasic [22, 23], suggesting that in contrast to simple epithelia, junctional aPKCλ is dispensable for establishing the polarity of the stratified epidermis, and that aPKCλ localized to the apical surface of basal cells during mitosis is more critical for maintaining epidermal homeostasis. Par6 and aPKCs form a stable heterodimer through their respective Phox/Bem1 (PB1) domains [7, 8], and aPKC-mediated phosphorylation is required for the dissociation of Par3 from the ternary complex. Thus, analysis of the dynamics of Par6 and Par3 or the Par6-Par3 complex in the absence of aPKCλ is helpful to understand the role of junctional aPKCλ.

The difference between junctional and non-junctional aPKC has been demonstrated by two-step chemical skin carcinogenesis experiments using epidermis-specific Par3 knockouts [62]. The epidermal loss of Par3 reduced papilloma formation and promoted keratoacanthoma formation, indicating that Par3 acts as a tumor promoter for papilloma and as a tumor suppressor for keratoacanthoma. In the absence of Par3, the aPKC-Par6 complex localized to the cytoplasm [62]. These results imply that the junctional aPKC-Par6 complex with Par3 is involved in papilloma formation, whereas the non-junctional, cytoplasmic aPKC-Par6 complex without Par3 is involved in keratoacanthoma formation.

8.3. Par proteins

The role of other components of the aPKC-Par complex (Par3 and Par6) in HFSC maintenance is unknown. Par3 is expressed throughout the interfollicular epidermis and the hair follicles,

and it interacts with aPKCλ to colocalize at keratinocyte tight junctions [62]. However, no hair abnormalities were described in epidermis-specific Par3-deleted mice [62]. During SCD and ACD in the basal layer, Par3 was localized to the apical surface of the cell as a component of the Par3-LGN-inscuteable complex [51]. It would be useful to examine whether a shift from SCD to ACD in the hair follicle and subsequent HFSC depletion occurs in Par3-deficient mice as seen in aPKCλ cKO mice. Additionally, it would be interesting to investigate whether Par6 knockouts show similar phenotypes to those of aPKCλ knockouts. To the best of my knowledge, Par6 knockouts have not yet been reported. The presence of three isoforms of Par6 in mammals might make it difficult to reveal the phenotypes of Par6 inactivation.

9. aPKC and aging

Is alopecia observed in aPKCλ cKO mice relevant to human diseases? As few inflammatory cells were present around the hair follicles of mutant mice, the mutant mice model is unlikely to be a disease model for alopecia areata, which involves perifollicular T cell infiltration and autoimmune responses to hair antigens. Progressive hair loss in aPKCλ cKO mice was similar to alopecia observed in collagen XVII (COL17A1/BP180/BPAG2, a structural component of the hemidesmosome) knockout mice and aged mice. In humans, COL17A1 deficiency causes a subtype of congenital junctional epidermolysis bullosa [63]. The patients also show premature hair loss (alopecia) with hair follicle atrophy [64, 65], suggesting that COL17A1 plays a role in hair follicle homeostasis. Consistent with this finding, *Col17a1*-deficient mice also show premature hair loss [66]. Mouse Col17a1 is preferentially localized along the dermal-epidermal junction of bulge keratinocytes, and loss of Col17a1 prevents the expression of HFSC markers, such as K15, CD34, and α6-integrin [67]. Similar to aPKCλ cKO mice, *Col17a1*-deficient mice showed progressive hair loss, hair follicles in sustained anagen, HFSC depletion, and deficient stemness of the HFSC population [67], indicating that Col17a1 is essential for HFSC maintenance. In addition, *Col17a1*-deficient HFSCs coexpress K15 and K1 in the bulge, and show increased K1 expression in the upper junctional zone and IFE, implying that the fate of HFSCs changes to epidermal differentiation.

A recent study clarified that accumulation of DNA damage in HFSCs leads to proteolysis of COL17A1 that triggers HFSC aging [68]. Importantly, aged HFSCs lose their stem cell signature and commit to epidermal differentiation, and they are finally eliminated from the epidermis [68]. The progressive depletion of HFSCs and the cell fate change observed in aPKC cKO mice are similar to aged mice. Thus, it would be interesting to examine whether the expression of Col17a1 is decreased in mutant mice and whether aPKCλ is involved in the induction and maintenance of Col17a1.

10. Concluding remarks

Analyses of mutant mice with epidermal loss of aPKCλ have clarified a novel function of aPKCλ in HFSC maintenance. aPKCλ influences HFSC maintenance through the regulation

of oriented cell division among HFSCs in the bulge (intrinsic mechanism) and the regulation of the expression of quiescence-inducing Fgf18 and Bmp6 (paracrine mechanism). Identification and evaluation of the downstream effectors of aPKCλ in HFSC maintenance will provide further insight into the mechanism of hair loss.

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