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Hairy tale of signaling in hair follicle development and cycling

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Abstract

Hair follicle is an appendage from the vertebrate skin epithelium, and is critical for environmental sensing, animal appearance, and body heat maintenance. Hair follicles arise from the embryonic ectoderm and regenerate cyclically during adult life. Distinct morphological and functional stages from development through homeostasis have been extensively studied for the past decades to dissect the critical molecular mechanisms. Accumulating work suggests that different signaling cascades, such as Wnt, Bmp, Shh, and Notch, together with specific combinations of transcription factors are at work at different stages. Here we provide a comprehensive review of mouse genetics studies, which include lineage tracing along with knockout and over-expression of core genes from key signaling pathways, to paint an updated view of the molecular regulatory network that govern each stage of hair follicle development and adult cycling.

Keywords

Signaling pathway; Wnt; Bmp; Shh; Notch; transcription factor; hair follicle; stem cell; morphogenesis; hair cycle; homeostasis; cancer

1. Introduction to general notions of cell and tissue signaling

Proper tissue architecture and function depends upon each cell in the body faithfully fulfilling the purpose of its existence. The harmony among complex signaling networks within and between cells must be strictly maintained in order to prevent developmental defects and fatal diseases. Skin homeostasis is essential for survival, and protects the organism against various environmental assaults such as UV irradiation, microbes, harmful chemicals, etc [1]. Mammalian skin epithelium carries hair follicle (HF) appendages important for overall skin homeostasis, sensory purposes, maintaining body temperature, and establishing proper appearance. Moreover, HF is an ideal mammalian model system to understand more general principles of intra- and inter- cell signaling pathway communication that may apply to other tissues. HF possesses distinct characteristics that allow in depth dissection of molecular signaling. First, synchronous, distinct and dramatic morphological changes allow researchers to easily identify well-characterized HF stages of development or homeostasis [1, 2]. Secondly, hair follicle stem cells (HFSCs) of the bulge, which give rise to all HF lineages and maintain homeostasis, are well characterized [3, 4]. HFSCs dynamic behavior of self-renewal, differentiation, and quiescence, is essential for the HF proliferative and growth cycle [5, 6]. Lastly, stem, progenitor, and terminally

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differentiated cells display specific HF location and molecular marker expression [4], which allows their identification, targeting and isolation for deeper exploration.

The signals that govern skin and HF cell proliferation and differentiation during development and homeostasis have been intensively explored in the past decades [7]. Several transcription factors such as Runx1 [8, 9], Tcf3/4 [10], NFATc1 [11], Lhx2 [12], Sox9 [13], Tbx1 [14], p63 [15, 16], Dlx3 [17], Lef1 [18], Msx2/Foxn1 [19], Gli [20, 21], Gata3 [22] and etc, play critical roles in HFSC activation, self-renewal, differentiation, and cycling by modulating several key signaling pathways, which include Wnt, Bmp, Shh, and Notch. We begin by underlying basic molecular principles for each pathway, and next we review how different combinations of these pathways regulate HF development and cycling.

1.1. Wnt signaling

Wnt molecules act as secreted, morphogenetic signals, which bind to Frizzled or LRP receptors [23–25]. When Wnt is absent, Axin/GSK-3 β /APC kinase complex phosphorylates serine/threonine residues of cytoplasmic β -catenin, leading to ubiquitin-mediated degradation. Upon Wnt ligand-binding to receptors, Dishevelled binds to the intra-cellular domains of the receptors, sequestering and allowing LRP-mediated phosphorylation of Axin/GSK-3 β /APC complex, which inhibits phosphorylation and ubiquitin-mediated degradation of β -catenin [26]. Excess of cytoplasmic un-phosphorylated β -catenin translocate into the nucleus, where it interacts with Lef/Tcf transcription factors [18]. Wnt transcriptional targets include the Wnt molecules themselves, Eda, Fgfs, Keratins, Lef1, Movo1, Foxn1, Msx2, Follistatin, Tgf β 2, Cyclin D1 and other cell cycle related genes [26]. Dkk1 is an inhibitor of LRP [27]. β -catenin also interacts with cadherins (E-cadherin, α -catenin) at the plasma membrane and with APC [28]. Mutation of serine/threonine residues stabilizes β -catenin, mimicking an increased Wnt signal. Recently, LGRs, which are seven transmembrane proteins, were shown to mark stem cell (SC) populations [29], to physically interact with Frizzled/LRP receptors, and to act in R-spondin-mediated Wnt signaling [26, 30, 31].

1.2. BMP signaling

Bone Morphogenetic Protein (BMP) signaling consists of two superfamilies of ligands: (a) the BMP family comprising BMPs, GDFs, and MIS; and (b) the Transforming Growth Factor (TGF) β family comprising TGF β I, II, III, Activin, and Nodal. BMP ligands have higher affinity to type I BMP receptors, while TGF β family ligands have higher affinity to type II receptors. Type I and II receptors carry an N-terminal ligand-binding domain, a transmembrane domain, and a C-terminal serine/threonine kinase domain [32, 33]. TGF β /Activin ligands seem to first bind to type II receptors resulting in a conformational change that enables type I receptors to bind to the complex. Upon activation, receptors can directly phosphorylate R-Smad which releases R-Smad for its binding to Co-Smad (Smad4) and for further localization into the nucleus where it binds the DNA and regulates transcription along with other co-factors [32]. R-Smads (Smad 2/3 for TGF β and Smad 1/5/8 for BMP family) are normally bound to type I receptor in an inactive state. The BMP-related ligands can be bound and inhibited by inhibitors such as Noggin or Follistatin [34].

1.3. Shh signaling

The mammalian Hedgehog family consists of three secreted proteins: Sonic Hedgehog (Shh), Indian Hedgehog and Desert Hedgehog [35]. Patched1, a twelve-span transmembrane protein that acts as a Shh antagonist, normally represses Smoothened (Smo), a seven-span transmembrane co-receptor. Shh signaling inhibits Patched1 thereby relieving the repression of Smo, which can now activate Gli proteins which serv as transcriptional effectors [36].

Each Gli gene (Gli1, 2, and 3) has varying expression patterns in different time and location with differential impact on tissue development and physiology [37–39].

1.4. Notch signaling

Notch is a single-span transmembrane protein, originally identified in *Drosophila* [40], with four family members Notch 1, 2, 3, and 4 identified in mammals. The extracellular domain of Notch carries EGF-like repeats and is activated upon ligand-binding (Delta or Jagged) from neighboring cells. The activation signal triggers ADAM, a metalloproteinase, to cleave Notch before the transmembrane domain and release the intracellular domain from the membrane. The ADAM cleaved product of Notch acts as a substrate for γ -secretase, which cleaves and subsequently releases NICD (Notch Intra-Cellular Domain) for its translocation into the nucleus, where it forms a complex with CBF1/RBPj κ /Su(H)/Lag-1 for transcriptional activation [40].

1.5. Other signaling pathways

Epidermal Growth Factors (EGFs) and TGF α bind to EGF-Receptors (EGFRs), and this binding triggers dimerization of the receptors and auto-phosphorylation of tyrosine residues, leading to transcriptional activation through Src-Homology proteins, ultimately resulting in cell proliferation [41, 42]. In particular, EGF molecules promote robust keratinocyte proliferation [43]. FGF ligands signal through FGF receptors that are tyrosine-kinases and regulate various cellular processes such as proliferation, migration, and differentiation [44].

1.6. Hair follicle specification, generation, and maintenance during development and skin homeostasis

Skin epithelium arises from the single layer of ectoderm that surrounds the embryo body during embryonic development [1]. Cells of the basal layer (BL) produce highly keratinized terminally differentiated cells of the suprabasal layers that form the interfollicular epidermis. Starting around E14.5, epithelial-mesenchymal signaling triggers BL cells of the epithelium to aggregate, forming a hair placode (Figure 1) [45, 46], which proliferate and form a hair germ (HG). The mesenchymal cells residing below start to condensate, and as the HG becomes a “peg”, they become engulfed in the epithelium forming the “dermal papilla” (DP). Soon after, the highly proliferative cells at the bottom of the peg become matrix (Mx) cells, and start producing inner layers, terminally differentiated HF lineages, forming a bulbous peg (Figure 2). Meanwhile, cells on one side of the upper outer root sheath (ORS) cluster, forming a bulge structure that would later contain HFSCs. The sebaceous gland (SG) is also generated from the epithelial HF cells [47]. Melanocytes are a separate lineage that originates from the neural crest, resides in the bulge and Mx, and produces melanin that incorporates into the differentiated hair shaft for color. The HF compartmentalization and differentiation lasts up to ~2.5 weeks after mouse birth [1, 34].

After a period of hair growth known as anagen, cells in the lower HF portion undergo apoptosis and HF regresses during the catagen phase, which brings the DP in close proximity to the bulge (Figure 3). Around ~PD21 HF enters quiescence or rest (telogen), but is ready to re-activate upon signaling from the DP and the environment. During adult homeostasis, HF undergoes cycles of anagen, catagen and telogen, referred to as “hair cycle” [5]. HFSCs reside in the bulge and are the source that fuels all HF lineages [3, 48, 49]. At telogen, upon activation signals, some bulge SCs migrate out into HG where they lose SC characteristics and become progenitors that start to divide rapidly [6]. The remaining bulge cells divide ~3 $\times$ during one hair cycle to replenish their SC pool [50, 51]. Rapid proliferation of progenitors (or Transit-Amplifying (TA) cells) results in formation of Mx cells, which give rise to differentiated HF lineages, similar to the anagen of morphogenesis [2]. In catagen, cells in the lower HF die again, followed by rearrangement

of the upper and mid ORS cells around the new club hair and the germ, respectively [52]. Upon injury conditions such as hair plucking, waxing, and wounding, HFSCs are activated and produce not only HF but also contribute to epidermal regeneration [1].

Around the HF, other organs and tissue structures aid in HF homeostasis. The Arrector Pili Muscle (APM) provides the physical force in HF erection necessary for body temperature and appearance [53]. Recent evidence suggests that Nephronectin, a Wnt-target gene, is enriched in the bulge and this subset of bulge cells serve as a niche for muscle cells. Also, vascularization regulated by VEGF is essential for creating a nurturing environment around the HF [54, 55]. In addition, surrounding adipocytes are important for HF cycling, in which PDGF signaling plays a role [56].

Aberrant signaling is associated with various skin conditions such as pilomatricoma, BCCs, hamartoma, etc [20, 21, 36, 57–68], indicating the importance of maintaining the balance of signaling cascades. A comprehensive review underlining the relevance to human disease has been recently published [69]. Perhaps the best understood is the aberrations that result from perturbation of the Shh signaling cascade. Components of Shh signaling cascade such as Patched1, Gli1, Smo, and Shh are expressed at high level in many basal cell carcinomas (BCCs), which are tumors of the skin epithelium, and over-expression of individual molecules resulted in BCC-like tumor formation [35, 58–60, 64, 65]. Here, we classify these important signaling pathways based on the stage of HF remodeling upon which they act and suggest areas that require further exploration.

2. Signaling during hair follicle development

2.1. Initiation of hair follicle morphogenesis

Wnt signaling is critical for HF induction [24]. Wnt10a, Wnt10b, nuclear β -catenin and Lef1 are expressed in the hair placode epithelium [18, 70, 71], while Wnt5a is expressed in the mesenchyme right below the placode [72]. Targeted deletion of β -catenin in the epithelium via the Keratin 14 promoter active in the BL and ORS inhibited hair placode formation [73], and down-regulation of Lef1, Bmp2, Bmp4, Bmp7, Shh, and Patched1. Similarly, over-expression of Dkk1 resulted in absence of β -catenin, Wnt10b, Lef1, Edar, Bmp2, Bmp4, Gli1, and K17, and lack of hair placode [27]. Conversely, constitutively active β -catenin induced precocious placode formation and multiple HG budding out from the original follicle [67, 74, 75]. B-catenin and hedgehog signal strength can specify the number and location of HFs in adult epidermis without recruitment of bulge SCs.

Lef1 skin knockout reduced the HF numbers [18] and whisker follicle induction, but expression of Lef1 in the dermis at E11.5 rescued this phenotype [76], suggesting a critical role for mesenchymal Lef1. Transgenic Wnt reporter mice containing multiple Lef/Tcf binding sites (TOPGAL) showed activity in the placode as well as in the underlying mesenchyme, which correlated with Lef1 protein localization [77]. Other Wnt-related transcription factors, Tcf3 and 4 are also in the placode, but fade away as HF undergoes further down-growth [78]. Double knockout of Tcf3/4 displayed loss of hair and thinning of skin [10].

Aside from Wnt-related molecules, the placode expresses Bmp2, Bmp7, and Shh while the underlying mesenchyme expresses Noggin, Bmp4, Patched1, and Gli1 [27, 72]. Deletion of Noggin reduced epithelial placode density except for the guard hairs. A few placodes that formed displayed cytoplasmic localization of β -catenin, which was rescued by Lef1 over-expression [79, 80]. As epithelial invagination began, the placode down-regulated E-Cadherin and gained expression of P-Cadherin. E-Cadherin failed to be down-regulated in Noggin knockout mice, and this was also rescued by Lef1 [80].

FGF7 and FGF2 over-expression in K14-lineages also reduced HF density, but displayed epidermal hyper-proliferation, suggesting that FGF signaling may promote epidermal instead of HF cell fate choice [81, 82]. While FGF7 null mice did not show a significant phenotype [83], over-expression of FGF7 dominant negative form in skin resulted in defects in HF production and significant reduction in proliferation upon wounding [84]. In summary, high level of Wnt signaling and suppression of BMP and FGF signaling are required for epidermal cell fate choice toward HF cells and allow these cells to aggregate and begin to invaginate to form the hair placode.

2.2. Follicular invagination and maturation

Shh knockout or knockdown showed initial epithelium invagination and mesenchymal condensation, but HFs became developmentally arrested at this stage [85–87]. Shh transcriptional targets (Ptc1, Smo, Gli1 and Gli2) were detected at lower levels, but Wnt and Bmp signaling were unaffected by the Shh knockout, suggesting that Shh signaling is not critical for initial placode formation but is important for HF growth. Gli2 knockout mice showed similar phenotype to the Shh knockout mice [21]. *K14-ΔNβ-catenin* mice showed aberrant expression pattern of Shh, often on both sides of Mx, and this is associated with wavy HFs, suggesting that asymmetric Shh localization in the Mx is critical for follicular polarity [67].

Wnt signaling plays important roles during follicular growth. For instance, Wnt3 expression is confined to differentiating hair medulla [88]. Dvl2, another important player of the Wnt pathway, co-localizes with AE13 positive differentiated cortex layer. *K14-Wnt3* and *K14-Dvl2* transgenesis resulted in shortening of hair shafts. In addition, Wnt10a/Wnt10b are in the IRS precursors while Wnt5a is expressed in the DP and a subset of ORS cells. Wnt11, Wnt3a, and Wnt4 are present in specific HF layers during maturation [72]. Nuclear Lef1 is confined to the tip of the growing follicle and the mesenchyme touching the tip. At birth, Lef1 mRNA is found in the Mx and pre-cortical region, and Lef1 protein nuclear localization becomes strong in the pre-cortex [77]. Interestingly, TOPGAL mice showed significant signal enrichment in the hair cortex. On the other hand, Tcf3 was not expressed in the Mx but in the lower ORS.

Targeted deletion of β-catenin in melanocyte SCs resulted in loss of hair pigmentation, along with reduced proliferation and absence of differentiation markers [89]. Interestingly, induction of β-catenin dominant active form in K15 lineage resulted in expansion of melanocytes, promoting their proliferation. This seemed to be regulated through Edn, a direct Wnt target gene that is highly induced upon β-catenin activation in epithelial cells, and concomitant increased expression of EdnrB in melanocyte SCs.

Bmp signaling is critical for proper HF differentiation. Bmpr1a deletion resulted in abnormal HF formation due to reduced p-Smad1/5/8 levels through which Bmp signaling occurs along with a lack of lineage-specific differentiation markers [90].

In conjunction with common signaling pathways, different hair types (vibrissae, guard, and pelage) seem to employ slightly different signals for HF induction. For instance, EdaA1/EdaR signaling through NF-κB is essential for guard hair specification [91–93]. On the other hand, mutations in ActivinβA or its inhibitor Follistatin results in defects in development of vibrissae and pelage HFs [94–96]. Sox2 is expressed in DP of “some” but not all HF types and is important for the specification of these follicular types [97].

While Mx cells give rise to differentiated HF lineages, one side of upper ORS cells generate a cluster, forming the bulge, which contains adult HFSCs that are essential for adult HF cycling and skin homeostasis. These differential fates seemed to be specified early in the

placode stage, where there is heterogeneity of several molecular markers such as Shh [98], Sox9 [99], Lgr6 [100], Tbx1 [14] and Runx1 [101] expression in all or a subset of cells in the placode. Lineage tracing experiments showed that these placode cells give rise to ORS, bulge, and other HF lineages [45]. More importantly, these factors interact with signaling molecules in regulating proper HF development. For instance, Runx1 expression is detected in a subset of placode cells as well as in mesenchyme in the very early stage of morphogenesis. Runx1 expression becomes very prominent through the growing follicle as well as in DP, and interacts with Lef1 in differential mediation of Wnt signaling for proper emergence and maintenance of HFSCs [101]. Runx1 expressing cells eventually give rise to adult HFSCs, and Runx1 plays additional roles in their proliferation in adulthood [8, 9]. Mature HFs are compartmentalized and can be characterized by specific expression profiles. The Gli1+ region above the bulge and the Lrig1+, Mts24+, Lgr6+, and Blimp1+ region of the isthmus and SG bear SCs, but these do not contribute to long-term HF regeneration [100, 102–105].

In addition to Wnt, Bmp, and Shh signaling, Notch signaling also ensures proper HF differentiation. Notch 1 is expressed in the Mx. Notch2 is not detected, but Notch 3 and Jagged1 are expressed in pre-cortex cells and cuticle layer of IRS [106]. Over-expression of Notch1 Intra-Cellular Domain (NICD) in pre-cortical and cortical cells resulted in hair loss at the end of 1st hair cycle and displayed abnormal differentiation [107, 108]. Loss of Notch1/2 as well as loss of RBPj κ or γ -secretase in epithelium, result in initiation of HF morphogenesis, but shows similar defects of abnormal differentiation followed by hair loss [109–112]. RBPj κ deletion in DP (*CoII-Cre*) also shows disrupted IRS followed by HF degeneration and cyst formation [113]. In addition, Wnt5a, Fgf7, Fgf10 and Noggin are down-regulated in the DP and re-introduction of these factors partially rescued the HF degeneration phenotype. These results suggest that Notch1 signaling controls DP signature molecules that in return signal to the Mx cells to differentiate.

Importantly, Notch signaling works downstream of several pathways. For instance, ectopic expression of constitutively active β -catenin in K14-lineages results in up-regulation of Notch pathway genes such as Jagged1, suggesting a Wnt-dependent regulation of Notch signaling [106, 114, 115]. Msx2 and Foxn1 seem to play redundant roles in regulating Notch1 expression and are important for proper HF differentiation. Interestingly, Bmpr1a deficient mice display reduction in Msx2 and Foxn1 expression in the HF and show similar phenotype to Msx2/Foxn1 double mutant, suggesting that BMP signaling from Mx/DP regulate Msx2/Foxn1-mediated Notch1 expression in proper HF differentiation [19, 90]. Finally, EGF/TGF α molecules are also detected in the hair bulb and IRS and in the absence of TGF α , HFs become wavy and curly, displaying an abnormal differentiation [116, 117].

2.3. Regression and quiescence of mature hair follicle

Around ~PD16-17, the lower portion of the mature HF is destructed by apoptosis (catagen). DP moves upward and comes in close contact to the bulge, and the HF enters telogen phase around ~PD20-21. Interestingly, targeted deletion of β -catenin in the DP (*Corin-Cre*) around PD3 during HF growth phase, results in premature entrance into catagen [118]. Dlx3, a Dlx-related transcription factor, regulates timely HF regression from the epithelial compartment [17]. Dlx3 expression extends from Mx to IRS, and Lef1 regulates its expression. Dlx3 mutant HFs show enhanced proliferation along with delayed regression due to the absence of BMP signaling [17]. FGF5 is expressed in late anagen during morphogenesis, and its deletion delays catagen, resulting in longer hair shafts [119].

In summary, multiple signals including Wnt, Bmp, Shh, Notch, FGF and EGF are at work in proper follicular morphogenesis: growth, lineage specification, and timely regression after maturation. Interestingly, a handful of the same signaling pathways are utilized in both

formation and maturation of the HFs. However, the different combinations of these pathways during a specific time window and cell populations likely program the cells to distinct fates.

3. Signaling in hair follicle cycling during homeostasis and injury

3.1. From quiescence to activation

The 1st hair cycle begins at first telogen (~PD20-21) after HFs finished regression and await activation. HF quiescence is maintained via Bmp signaling [120]. Specifically, Bmp4 is expressed in DP and secondary HG while Noggin, a Bmp inhibitor, is absent in telogen follicle. On the other hand, Bmpr1a is expressed in HG and in SG [121]. In addition, Bmp2/4 levels are highest at telogen. Inhibition of Bmp signaling via Noggin over-expression resulted in precocious anagen entrance upon hair plucking, suggesting a dominant role of Bmp signaling in maintaining quiescence [122]. HFSCs quiescence is also maintained via Bmp regulation of NFATc1, a bulge-specific marker that once lost results in increased proliferation [11]. A recent study also suggests that TGF β signaling is a major pathway regulating the adult hair cycle [123]. TGF β receptor II (TGF β RII) activity and p-Smad2 were detected in the lower bulge and the HG at the transition from telogen to anagen. In addition, late-telogen DP is enriched with TGF β 2 ligand and injection of TGF β 2 ligand in the TGF β RII knockout mice before endogenous TGF β 2 expression in DP failed to activate anagen at a normal time due to a direct regulation of Tmeff1, a transmembrane protein that inhibits BMP signaling [123]. Moreover, the inner layer (K6+) of the bulge expresses significant amounts of Bmp6 and Fgf18, which inhibit the outer bulge stem cell layer (CD34+) proliferation. Fgf18 deletion in Keratin 5 (K5) + cells (BL and ORS) resulted in shorter telogen length before anagen re-entry. In addition, subcutaneous Fgf18 as well as BMP and Wnt inhibitors all induce HF quiescence [124, 125]. Finally, ablation of the inner bulge layer induces bulge cell proliferation, suggesting a paracrine role of Bmp6 and Fgf18 in maintaining bulge SC quiescence [52].

Upon activation, Bmp4 expression in DP and HG decreases while the rest of the skin dermis shows Bmp4 up-regulation [126]. Concomitantly, DP and the growing HG up-regulate Noggin and down-regulate Bmpr1a. Deletion of Bmpr1a in telogen following shaving results in precocious bulge cells proliferation, but no further development of HF. Concomitantly there is loss of CD34 in the bulge as well as Sox9 and Lhx2 expression in the germ [126]. In addition, the bulge shows increased nuclear β -catenin and Lef1, which is mediated by inactivation of GSK-3 β by its increased phosphorylation, potentially driven by PTEN/PI3-K/P-Akt pathway.

Low level of Wnt signaling is also essential for maintaining HF quiescence. *K14- Δ N β -catenin* hemizygous mice displayed precocious anagen activation, revealing a dose-dependence of β -catenin in hair cycle activation [127]. In addition, inducible ectopic expression of activated β -catenin in the second telogen resulted in precocious induction of anagen-like HF structure associating hyper-proliferation as well as induction of *de novo* sites of HF formation [128, 129]. Lef1 is absent in the bulge, but is enriched in HG during telogen. On the other hand, Tcf3/4 are highly enriched in the bulge, originating from the embryonic Tcf3/4 expressing cells of the ORS [78], acting as a repressor of Wnt signaling [10, 130]. Conversely, Wnt10a/Wnt10b are enriched in the bottom of the HG and in DP at telogen-anagen transition [72]. FGF7 expression gradually increases in DP during the long 2nd telogen prior to activation. Moreover, ectopic Fgf7 induction in telogen results in proliferation induction in the HG [131]. Some of the Wnt pathway genes may be regulated by Runx1, which is present in the lower bulge and HG and governs timely HFSCs activation and proliferation [8, 9].

Lgr5, a protein that is enriched in a subset of cells in the lower bulge and the HG in telogen bear multipotency and seem to have Wnt-dependent function. *In vitro* and biochemical assays revealed that LGR proteins bind to frizzled and LRP receptors forming a complex, which can activate canonical Wnt signaling pathway [29–31]. The role of these LGR proteins in HF *in vivo* has not yet been addressed.

Recently, a circadian molecular clock mediated via Bmal1/Clock transcription factors was shown to affect HFSCs proliferation and quiescence through de-regulation of many critical signaling molecules such as Wnt-related (Dkk3, Lef1, Fzd2/3, Sox9, Lhx2, Lgr5, Tcf3) and Bmp-related (Smad7, Ltbps, Lefty) [132]. Wnt, Bmp, and Fgf interactions counterbalance each other inhibitory and activation signals and predispose telogen follicles to be at a “refractory” or “competent” status with respect to anagen entry [7]. The “waves” of some of these signaling molecules traveling as gradients throughout the mouse skin across the body may be the consequence of fluctuations of the components in the circadian clock that regulate signaling pathway genes.

3.2. Growth and differentiation of adult hair follicle

Growth and differentiation of adult HF employs similar signaling as morphogenesis. Mx cells and pre-cortex cells express Wnt3 and Wnt10a/b [72]. These molecules send cascades of signaling to Lef1, which also show enrichment in the Mx and pre-cortical cells [77]. In addition, differentiated layers express many Wnt molecules such as Wnt3a, Wnt4, and Wnt11.

In addition to Wnt signals, a variety of Bmp ligands and receptors are expressed in the Mx cells (Bmpr1a/b, Bmpr2), hair shaft precursors (Bmp2/4), and IRS lineages (Bmp7/8), which allow the TA cells to stop proliferation and enter the terminal differentiation program [120]. Moreover, surrounding the HF, Bmp2/4 are present at low level in the mesenchyme during early anagen. These levels gradually peak at the late anagen and telogen to inhibit proliferation [122].

The extent of bulge SC proliferation during anagen seems to inversely correlate with the level of BMP ligands [6, 50]. During anagen, bulge SCs self-renew to make up for the loss that occurred prior to anagen activation [6]. Extended self-renewal push cells out downward into the ORS region below the bulge, which later move upwards to make a new bulge and HG [52]. Although to a lesser extent than the inner bulge layer (K6+), bulge SCs (CD34+) express Bmp6 and Fgf18 that seems to send the inhibitory signal toward the ORS cells in regulating their proliferation during anagen [131, 133].

Notch signaling is also important for proper adult HF differentiation, as it was for the developing HF during morphogenesis. RBPjk targeted deletion in the bulge (K15+) at 2nd telogen results in normal HF activation and growth, but HFs display abnormal terminal differentiation, and in long-term form cysts containing epidermal lineage keratinocytes [111].

3.3. Destruction and returning back to quiescence in adulthood

Once the proliferative signal fades and inhibitory signals accumulate in the anagen-catagen transition, HF starts to regress from the bottom. TGFβ1 expression is seen in bulge and ORS in early anagen, followed by enrichment in ORS in late anagen [134]. Its expression increases in catagen in the bulge and the epithelial strands, and disappears in telogen. TGFβ1 deletion resulted in delayed catagen entrance, and ectopic TGFβ1 expression induced premature catagen, suggesting its importance in timing the switch from proliferation to regression.

BMP signals start to accumulate from late anagen, last throughout catagen, and peak at telogen [122]. Also, Fgf18 starts to be expressed in catagen and is maintained throughout telogen. Ectopic induction of Fgf18 in anagen shows reduction in proliferation, suggesting its up-regulation during hair cycle may be important for inducing catagen [125]. EGFR expression is detected in the ORS [135]. Dominant negative EGFR showed initial HF formation, but failed to enter catagen and resulted in hair loss over time [136]. Other signaling molecules such as neurotrophins have also revealed their importance in proper regression [137–140].

In summary, Bmp-mediated repression dominantly contributes to adult HF and HFSCs quiescence, and accumulation of Wnt or other signals are necessary to overcome and lead cells into proliferation. HF bulge cells display extra quiescence relative to other HF compartments due to autocrine and paracrine Bmp and Fgf signals that are innate (from the CD34+ bulge cells) and secreted from the surrounding mesenchyme and the inner bulge layer (K6+). Wnt, Bmp, Shh, Notch, and Egf signals are important for proper adult HF growth and differentiation program. Finally, gradual buildup of Bmp and Fgf signals overtake the proliferative signals, induces HF regression followed by quiescence (telogen).

3.4 The curious case of Runx1: timing and localization matters

As described so far, the growth and differentiation of HF during the morphogenesis and hair cycling resemble each other in their cellular and molecular processes. However, a major difference is that while secondary (adult) HG consists of short-lived progenitors, the primary (embryonic) HG contains precursors that would later give rise to long-lived HFSCs. Interestingly, there is strong and transient expression of Runx1 in the embryonic dermis, which molecularly distinguishes embryonic from adult skin. This coincides with another unique, non-cyclic, event in the life of the HF: the birth of adult HFSCs [101]. When Runx1 is knocked out in the mesenchyme during this stage, HF form and go through the first catagen but adult HFSC cells convert their fates toward SG later on in life. Strikingly, Runx1 regulates autocrine and paracrine Wnt signaling in opposing direction in the dermis versus epidermis, thereby creating gradients of signaling in the embryonic skin. Thus, loss of Runx1 in the mesenchyme at the embryonic stage revealed the importance of Wnt signaling in the embryonic environment to nurture the nascent adult HFSCs to mature well, to shape up their molecular makeup and to endow them with long life and multipotency.

4. Conclusions and future perspectives

In this review, we summarized our current understandings of signaling pathways that play essential roles in governing the development and cycling of HF. Mouse genetics allowed researchers to discover the tip of the iceberg in the molecular networks that are participating in the greater purpose of tissue development and homeostasis. Perturbation in these pathways leads to disease and some of the signals unveiled here are already making their useful way to the clinic. As with most human disease, those affecting skin are likely subtly affecting multiple genes and are difficult to model by gene knockout. The generation of induced pluripotent SCs (iPS) [141] and the ability to differentiate them to keratinocyte [142] combined with high throughput sequencing might allow us not only to identify those gene variations that are possibly associated with disease, but also to model these diseases by regenerating skin and hair follicles from human iPS in immune-compromised mice. Advances in transgenic mouse technology allow us to relatively easily study single gene mutations [143] and may in the future be adapted to rapidly create multiple mutations that can mimic specific traits discovered in human disease.

The advances within the field have begun to uncover the veil of skin biology, pointing the researchers to the areas of further exploration. How are signals sent from dermis, adipocytes,

blood vessels, and inner bulge cells regulated? To what degree do paracrine and autocrine signals that surround or are innate influence the HF status? Are there more signaling pathways waiting to be discovered? What is the degree of redundancy across different activation or inhibitory signals? How can these signaling networks be used or manipulated in medical purpose of treating patients with skin diseases or wounds? Answers to these questions will bring insights in discovering what remains below the tip of the iceberg of skin biology.

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Highlights

- Wnt, BMP, Shh, and FGF signaling play combinatorial roles in regulating hair biology
- Specific transcription factors modulate this signaling upstream and downstream
- Generally, pathways are utilized in the same manner in morphogenesis and homeostasis
- Mesenchymal Runx1 restricted to the embryo instructs adult stem cell maturation
- Skin biology provides a platform for advances in curing disease

Figure 1.
Signaling in hair follicle morphogenesis and development

Figure 2.
Adult hair follicle signaling from quiescence to activation

Signals	Mutation type	Hair follicle phenotype	Reference
1. Wnt-related			
Wnt3	<i>K14-Wnt3</i>	Reduced hair follicle length, premature catagen onset	Millar (1999)
Wnt7a	<i>K14-Wnt7a</i>	Increased hair follicle neogenesis upon wounding	Ito (2007)
Dvl2	<i>K14-Dvl2</i>	Reduced hair follicle length, premature catagen onset	Millar (1999)
Dkk1	<i>K14-Dkk1</i>	Lack of placode formation	Andl (2002)
	<i>K5rtTA; TRE-Dkk1</i>	Lack of hair follicle neogenesis upon wounding	Ito (2007)
APC	<i>K14-Cre; APC^{fl/fl}</i>	Aberrent hair follicle growth, short, curly whiskers	Kuraguchi (2006)
Lef1 (Transcription factor)	<i>Lef1 -/-</i>	Lack of body hair and whisker follicles	van Genderen (1994)
	<i>K14-Lef1</i>	Clustered hair follicles, irregularly angled shafts	Zhou (1995)
	<i>K14-ΔNLeF1</i>	Suppressed hair follicle differentiation, enhanced sebocyte differentiation	Merrill (2001)
β-catenin	<i>K14-ΔNLeF1</i>	Progressive hair loss and cyst and tumor formation	Niemann (2002)
	<i>K14-ΔNβ-catenin (constitutively active)</i>	De novo hair follicle induction	Gat (1998)
	<i>K14-Cre; β-catenin^{fl/fl}</i>	Lack of placode formation, loss of hair in the first hair cycle	Huelsken (2001)
	<i>K5-S33Yβ-cateninER</i>	Anagen induction and enhanced hair follicle proliferation	Van Mater (2003)
	<i>K14-ΔNβ-cateninER</i>	Anagen induction and de novo hair follicle formation	Lo Celso (2004)
	<i>K14-ΔNβ-catenin (hemizygous)</i>	Precocious bulge stem cell activation	Lowry (2005)
	<i>K14-CreER^m; β-catenin^{fl/fl}</i>	Loss of bulge stem cell quiescence and maintenance	Lowry (2005)
	<i>K14-CreER^m; β-catenin^{fl/fl}</i>	Lack of hair follicle neogenesis upon wounding	Ito (2007)
	<i>K14-Cre; Ctnnb1^{(ex3)fl/+} (Dominant active)</i>	Premature hair follicle placode development, predominant differentiation towards hair shaft, defects in hair follicle development	Zhang (2008)
	<i>Cor-Cre (DP specific); Ctnnb1^{fl/fl}</i>	Reduced hair follicle length and thinning	Enshell-Seijffers (2010)
Tcf3/4 (Transcription factor)	<i>K15-ΔNβ-cateninER</i>	Increased bulge proliferation and expansion	Baker (2010)
	<i>ΔK5ΔNβ-cateninER</i>	Ectopic hair follicle induction In sebaceous gland	Baker (2010)
	<i>K14-ΔNTcf3</i>	Suppressed epidermal differentiation	Merrill (2001)
	<i>K14rtTA; TRE-mycTcf3</i>	Lack of epidermal differentiation and sebaceous gland	Nguyen (2006)
2. BMP/TGFβ-related			
TGFβ1	<i>TGFβ1 -/-</i>	Advanced hair follicle formation, delayed catagen development	Foitzik (1999), Foitzik (2000)
TGFβ2	<i>TGFβ2 -/-</i>	Delay of hair follicle morphogenesis and reduced number of hair follicles	Foitzik (1999)

Signals	Mutation type	Hair follicle phenotype	Reference
TGFβ3	<i>TGFβ3</i> ^{-/-}	No effect	Foitzik (1999)
TGFβRII	<i>K14-Cre; TβRII</i> ^{fl/fl}	Prolonged telogen length	Oshimori (2011)
Bmpr1a	<i>K14-Cre; Bmpr1a</i> ^{fl/fl}	Impaired IRS and hair shaft differentiation	Kobielak (2003)
	<i>K14-CreER^{T2}; Bmpr1a</i> ^{fl/fl}	Impaired postnatal hair follicle differentiation	Andl (2004)
	<i>En1-Cre; Bmpr1a</i> ^{fl/fl}	Lack of hair outgrowth, defects in Matrix cell proliferation and differentiation in morphogenesis and hair cycle	Ming Kwan (2004)
	<i>Emx1-Cre; Bmpr1a</i> ^{fl/fl}	Impaired IRS differentiation, reduced number of hair follicle	Yuhki (2004)
	<i>K14-CreER; Bmpr1a</i> ^{fl/fl}	Loss of quiescent stem cells	Kobielak (2007)
Noggin	<i>Noggin</i> ^{-/-}	Reduction of induced hair placodes	Botchkarev (1999)
	<i>Msx2-Noggin</i>	Impaired hair shaft differentiation	Kulesa (2000)
	<i>K14-Noggin</i>	Reduction of telogen length, increased hair density	Plikus (2004), Plikus (2008)
Activin	<i>actβA</i> ^{-/-} , <i>actβB</i> ^{-/-}	Lack of whisker follicles	Matzuk (1995)
Follistatin	<i>fs</i> ^{-/-}	Mis-oriented hair follicles	Matzuk (1995)
3. Shh-related			
Shh	<i>Shh</i> ^{-/-}	Arrest in hair follicle morphogenesis after placode formation	St-Jacques (1998), Chiang (1999)
	<i>K14-Shh</i>	Normal hair placode formation following massive down-growth and tumor formation	Oro (1997)
	<i>hK14-Shh</i>	Epidermal hyperplasia / loss of epidermal tissue renewal	Adolphe (2004)
Dhh	<i>hK14-Dhh</i>	Epidermal hyperplasia / loss of epidermal tissue renewal	Adolphe (2004)
Smo	<i>K5-SmoM2</i>	Epidermal hyperplasia, but no effect in hair follicle development	Xie (1998)
	<i>ΔK5-M2SMO</i>	Patchy hair loss and progressive alopecia	Grachtchouk (2003)
	<i>K14-CreER; RosaSmoM2 / Shh-CreER; RosaSmoM2 / K15-CrePR; RosaSmoM2</i>	BCC originates not from hair follicle bulge but from interfollicular epidermis and the infundibulum	Youssef (2010)
Gli1,2/3 (Transcription factor)	<i>K14-Gli1</i>	Postnatal hair loss and ulcer development	Oro & Higgins (2003)
	<i>K5-Gli2</i>	BCC-like tumor formation	Grachtchouk (2000)
	<i>Gli2</i> ^{-/-}	Arrest in hair follicle development	Mill (2003)
	<i>K5tTA; TRE-Gli2</i>	BCC-like tumors arising from hair follicles	Hutchin (2005)
Ptch1	<i>Ptch1</i> ^{KO1/+} or <i>Ptch1</i> ^{D11/+}	Anagen dependent tumor formation	Oro & Higgins (2003)
4. Notch-related			
Notch	<i>MHKA1-Notch^{ΔE}</i> (active form)	Abnormal differentiation of the medulla and the cuticle	Lin (2000)
	<i>K5-CreERT; Notch1</i> ^{fl/fl}	Epidermal hyperplasia and spontaneous skin tumors	Nicolas (2003)

Signals	Mutation type	Hair follicle phenotype	Reference
RBPj	<i>Msx2-Cre; Notch1^{fl/fl}/Notch2^{fl/fl}/Notch3^{fl/fl}</i>	Failure to maintain IRS cells, hair follicle cell conversion to epidermal cysts	Pan (2004)
	<i>Ivl-Notch-IC</i>	Delay in IRS differentiation followed by alopecia	Uyttendaele (2004)
	<i>K15-CrePR1; Notch1^{fl/fl}/Notch2^{fl/fl}/Notch3^{-/-}</i>	Bulge stem cell fate conversion to interfollicular epidermis and formation of epidermal cysts	Demehri (2009)
	<i>Nestin-Cre; RBP-J^{fl/fl}</i>	Hair loss, cyst formation, and epidermal hyperkeratinization	Yamamoto (2003)
	<i>K14-Cre; RBP-J^{fl/fl}</i>	Absence of spinous layer, impaired matrix cell differentiation to IRS and hair shaft	Blanpain (2006)
	<i>K15-CrePR1; Rbpj^{fl/fl}</i>	Bulge stem cell fate conversion to interfollicular epidermis and formation of epidermal cysts	Demehri (2009)
	<i>K15-CrePR1; Rbpj^{fl/fl} / K5-Cre; Rbpj^{fl/fl}</i>	Impaired hair follicle differentiation with mesenchymal RBP-jκ deletion	Hu (2010)
γ-Secretase	<i>Tgfb3-Cre; Rbpj^{fl/fl}</i>	Impaired epidermal differentiation	Lin (2011)
	<i>Msx2-Cre; PS1^{fl/fl} / Msx2-Cre; PS2^{fl/fl}</i>	Failure to maintain IRS cells, hair follicle cell conversion to epidermal cysts	Pan (2004)
Jagged1	<i>K5-Cre; Jag1^{fl/fl} / K14-CreER; Jag1^{fl/fl} / K14ΔNβ-cateninER; Jag1^{fl/fl}</i>	Inhibition of the hair growth cycle and cyst formation	Estrach (2006)
O-fucosyltransferase 1	<i>Tgfb3-Cre; Pofut1^{fl/fl}</i>	Disrupted telogen hair follicle, delay in anagen activation	Lin (2011)
5. EGF/FGF-related			
TGFα	<i>Tgfa -/-</i>	Misoriented, misaligned hair follicles	Luetke (1993), Mann (1993)
FGF7	<i>K14-Fgf7</i>	Lack of hair follicle formation	Guo (1993)
FGF5	<i>Fgf5 -/-</i>	Elongated hair follicle	Herbert (1994)
	<i>K5-HERCD-533 (dominant negative)</i>	Short and waved hair follicles and curly whiskers followed by gradual alopecia	Murillas (1995)
Fgfr2	<i>Fgfr2-IIIb -/-</i>	Abnormal hair medullar differentiation	Petiot (2003)
FGF10	<i>Fgf10 -/-</i>	Reduced hair follicle number and abnormal bulb expansion with lack of differentiated hair shaft	Ohuchi (2000)
FGF18	<i>K5-Cre; Fgf18^{fl/fl}</i>	Shortened telogen length	Kimura-Ueki (2012)

6. Transcription factors	Mutation type	Hair follicle phenotype	Reference	Related signals
Runx1	<i>K14-Cre; Runx1^{fl/fl} / β-actinCreER; Runx1^{fl/fl} / Runx1^{fl/Runx1-CreER}</i>	Epithelium: Delayed hair follicle morphogenesis and anagen entry, reduced proliferation; mesenchyme (predominant): abnormal hair follicle morphogenesis and enhanced SG formation	Osorio (2008), Hoi (2010), Osorio (2011)	De-regulates Wnt signaling
Sox9	<i>Y10-Cre; Sox9^{fl/fl}</i>	Lack of external hair, proliferation defects, ORS cells fate change to epidermal	Vidal (2005)	Downstream of Shh signaling
Lhx2	<i>K14-Lhx2 (Tg) or Lhx2^{-/-} (KO)</i>	Tg: Suppression of hair follicle differentiation; KO: Reduction of hair follicle density but no effect on differentiation	Rhee (2006)	De-regulates Wnt and Shh downstream factors
NFATc1	<i>Nfatc1^{-/-} and K14-Cre; Nfatc1^{fl/fl}</i>	Normal hair follicle morphogenesis, premature stem cell activation and hair follicle growth	Horsley (2008)	Downstream of Bmp signaling
Tbx1	<i>K14-Cre; Tbx1^{fl/fl}</i>	Delayed hair cycle entry upon shaving, loss of stem cells upon repeated depilation, reduced proliferative potential	Chen (2012)	Regulates Bmp signaling
Dlx3	<i>K14-Cre; Dlx3^{fl/fl}</i>	Alopecia due to failure of hair shaft and IRS differentiation	Hwang (2008)	Downstream of Wnt signaling
Msx2 / Foxn1	<i>Msx2^{-/-} ; Foxn1^{-/-}</i>	Impaired IRS and medulla differentiation	Cai (2009)	De-regulates Notch signaling
p63	<i>p63^{-/-} or K5tTA; TRE-ΔNp63α</i>	Lack of placode formation, defect in hair follicle stem cells and hair cycling	Mills (1999), Yang (1999), Romano (2010)	De-regulates Wnt signaling
Gata3	<i>Gata3^{-/-}</i>	Failure in the IRS precursors differentiation	Kaufman (2003)	Regulates Wnt signaling