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MINIREVIEWS

Recent progress in hair follicle stem cell markers and their regulatory roles

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Abstract

Hair follicle stem cells (HFSCs) in the bulge are a multipotent adult stem cell population. They can periodically give rise to new HFs and even regenerate the epidermis and sebaceous glands during wound healing. An increasing number of biomarkers have been used to isolate, label, and trace HFSCs in recent years. Considering more detailed data from single-cell transcriptomics technology, we mainly focus on the important HFSC molecular markers and their regulatory roles in this review.

Key Words: Hair follicle stem cells; Bulge; Secondary hair germ; Marker; Single-cell RNA-sequencing

Core Tip: Hair follicle stem cells (HFSCs) in the bulge are a multipotent adult stem cell population. They can periodically give rise to new HFs and even regenerate the epidermis and sebaceous glands during wound healing. An increasing number of biomarkers have been used to isolate, label, and trace HFSCs in recent years. Considering more detailed data from single-cell transcriptomics technology, we mainly focus on the important HFSC molecular markers and their regulatory roles in this review.

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INTRODUCTION

Hair follicle (HF) is an appendage of mammalian skin that develops from the interaction of dermal mesenchymal cells and epithelial cells during embryonic development[1,2]. Postnatal HFs undergo cycles of growth (anagen), regression (catagen), and rest (telogen), namely, the hair cycle[3]. The dysfunction of hair regeneration may lead to several diseases. For example, HFs become miniaturization in androgenic alopecia[4]. HF stem cells (HFSCs) reside in the bulge region near the attachment site of the arrector pili muscle and drive periodical hair regeneration. Usually, the new HFs are oriented from HFSCs. Thus, HFSCs may be useful in treating hair loss-related diseases. The regulation of the activation and quiescence of HFSCs is a critical dimension in this research area[5]. The concentric layers of HFs are composed of the outer root sheath (ORS), inner root sheath (IRS), and hair shaft (HS)[6]. Dermal papilla (DP) is a condensed mesenchymal cell compartment at the base of the hair bulb and is important for the activation of HFSCs[7]. There is a secondary group of cells named the secondary hair germ (HG) that resides between the bulge and the DP and becomes activated as the telogen progresses. Although derived from bulge SCs, HG cells respond more quickly to stimulus signals. During early anagen, there is a two-step activation process. First, HG cells become activated before bulge cells[8]. Second, these cells undergo several self-renewals and proliferate downward to form the ORS. In mature HFs, the ORS extends from the bulge to the matrix (Mx) bottom. Mx cells undergo rapid cell growth and differentiation to form new hairs. During the degenerative phase, the lower keratinocytes of HFs begin to undergo apoptosis, and the residual epithelial strands shrink, which pulls the DP upward. At the transition of catagen to telogen, some mid-zone ORS cells survive and generate HG, while the surviving upper slow-cycling ORS cells eventually form a new bulge[8,9]. The stages of HF cycle and the roles played by critical cell types are summarized in Figure 1.

Since the discovery of slow cycling, label-retaining cells in the outermost layer of the bulge region[10], the characteristics and activity of multipotent HFSCs have been intensively studied in recent decades[11-14]. Various markers have been successfully used for the purification and enrichment of HFSCs. Multiple markers have been used to trace HFSCs, and to some extent, there was some overlap and differences among these marked cell populations. Here, we focus on some important biomarkers of HFSCs and their regulatory roles in HFSCs (Table 1).

LEUKOCYTE DIFFERENTIATION ANTIGEN 34

Leukocyte differentiation antigen 34 (CD34) is a transmembrane glycoprotein initially identified on hematopoietic stem and progenitor cells[15]. The expression of CD34 is substantially localized in the outer layer bulge region and HG in mouse HFs[14,16]. In anagen, CD34 expression levels decrease in HFs[17] and at the most peripheral layer of the ORS between the isthmus and the bulb[18]. At present, CD34 is the most commonly used indicator in tracing or sorting bulge SCs and is often combined with α6-integrin (or CD49f)[16,18]. These isolated cells were characterized as having the ability for long-term self-renewal and pluripotent differentiation[19]. However, CD34 is not found in the bulge of human HFs during catagen or telogen[20-22].

CD200

CD200, formerly known as OX-2, is a type-1 transmembrane glycoprotein that mediates an immunoregulatory signal through binding to the CD200 receptor. CD200+ cells are observed in the bulge region of murine[23] and human HFs[20,22,24,25] and colocalize with keratin 14 (K14). In murine HFs, CD200 expression is relatively uniform throughout the whole HF, except for the Mx, DP, and HS[23]. Deficiency of CD200 in C57BL/6 mice led to significant perifollicular and intrafollicular inflammation in skin grafts and an increase in graft-infiltrating T cells after 14 d[23]. Alopecia areata is a common hair loss disease characterized by cellular autoimmune reactions. In older women with alopecia areata, CD200 expression was downregulated in the affected bugle lesions[26]. These results suggest that CD200 may play a role in attenuating inflammatory reactions and promoting immune tolerance.

K15

K15, an intermediate filament protein, is expressed in the epidermal basal layer and HF bulge in neonatal mice[27]. However, the expression level of K15 significantly decreases at approximately 2 wk of age, while strong expression of K15 is still observed in the bulge cells of mature mouse skin, similar to the human scalp[28]. In addition, all four types of HFs (guard, awl, auchene, and zigzag) show strong staining of K15 protein in the bulge cells of the dorsal skin in mice.

Table 1 Expression profile in postnatal hair cycle and related functions of some hair follicle stem cell markers

	Expression location in telogen	Expression location in anagen	Related functions	Ref.
CD34	Outer layer of bulge and HG (but not found in human HF)	Most peripheral layer of the ORS	Sustain the stemness of HFSCs	[14-22]
CD200	Bulge and HG	Relatively uniform throughout the whole HF, except for the Mx, DP and HS	Sustain the stemness of HFSCs	[20,22-26]
K15	Epidermal basal layer and HF bulge in neonatal mice; still strong in bulge in mature mice skin	ORS, the bulge is the strongest area	Not found	[22,27,28]
K19	Bulge	Mainly the U-ORS and the pb-ORS of HFs, stronger within the pb-ORS versus the bulge	Not found	[27,29-31]
Lgr5	Bulge, stronger in HG	The lower portion of the ORS, not overlapped with CD34	In the telogen-to-anagen transition	[32-38]
Lhx2	Bulge and HG	Through the ORS	Epidermal differentiation, HFSC quiescence, hair anchoring	[31,39-41]
Sox9	Bulge and HG	ORS, stronger in the sub-bulge region	Maintainess and differentiation of HFSCs	[12,31,42,43]
Runx1	Bulge, stronger in HG	The infundibulum, bulge, ORS, Mx and cortex	Regulating anagen initiation and HFSC proliferation	[12,42,44-50]
Axin2	Outer bulge cells	ORS	Maintainess of hair-forming ability	[51-60]
Nfatc1	Bulge	Bulge, overlapped with CD34 and Lhx2	Maintainess of HFSC quiescence	[46,61-65]
Foxp1	Bulge	Bulge, ORS, Mx	Maintainess of HFSC quiescence	[66-70]
Foxc1	Bulge, isthmus, sebaceous glands	Bulge, K6+ inner bulge layer, isthmus, IRS and sebaceous glands	Maintainess of the old bulge	[16,40,71-73]
Msi2	Basal layer of bulge and HG	Basal and suprabasal bulge, ORS and IRS	In the telogen-to-anagen transition	[74-77]

CD34: Leukocyte differentiation antigen 34; CD200: Leukocyte differentiation antigen 200; K15: Keratin 15; K19: Keratin 19; Lgr5: Leucine-rich repeat-containing G protein-coupled receptor 5; Lhx2: Lim-homeodomain transcription factor 2; Sox9: Sex-determining region Y-Box 9; Runx1: Runx family transcription factor 1; Axin2: Axin-related protein 2; Nfatc1: Nuclear factor of activated T cells c1; Foxp1: Forkhead Box P1; Foxc1: Forkhead Box C1; Msi2: Musashi 2; Mx: Matrix; HFSC: Hair follicle stem cell; HS: Hair shaft; HG: Hair germ; DP: Dermal papilla; ORS: Outer root sheath; IRS: Inner root sheath; pb-ORS: Proximal bulb outer root sheath; U-ORS: Upper outer root sheath.

Previously, K5 or K14 promoters were usually used to target epithelial SCs in the skin, which traces not only epidermal basal SCs but also HFSCs and even transient amplifying (TA) cells. Thus, the K15 promoter is presently used to selectively target HFSCs in the bulge[22].

K19

K19, another Keratin protein, is expressed in different stages of the hair cycle[27,29,30]. In anagen, the expression of K19 is mainly restricted to two areas, one in the upper ORS (U-ORS) and the other in the proximal bulb ORS (pb-ORS) of HFs. The K19 staining intensity is stronger in the pb-ORS than in the basal layer of the bulge, and the opposite is true for K15 [31]. K15+ and K19+ cells represent two distinct progenitor cell populations located in the bulge and pb-ORS. These K19+ cells in the pb-ORS are K15- and Ki67+[27,29,31], which indicates that this cell population contains more differentiated TA cells. In catagen, the K19+ region of pb-ORS moves upward with the club hair; eventually, it fuses with the K19+ portion of U-ORS during telogen. These two regions are separated again at the beginning of the next growth period.

LEUCINE-RICH REPEAT-CONTAINING G PROTEIN-COUPLED RECEPTOR 5

Leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) is an orphan seven-pass transmembrane protein located on the cell membrane. Lgr5 is a marker of SCs at the bottom of small intestinal crypts[32,33]. In the E18.5 $Lgr5^{LacZ}$ mouse model, Lgr5+ cells are observed in the ORS of larger HFs. During anagen and catagen, Lgr5-LacZ expression is located in the lower ORS up to Mx around the HFs, while in telogen, the expression of Lgr5 is concentrated in the basal

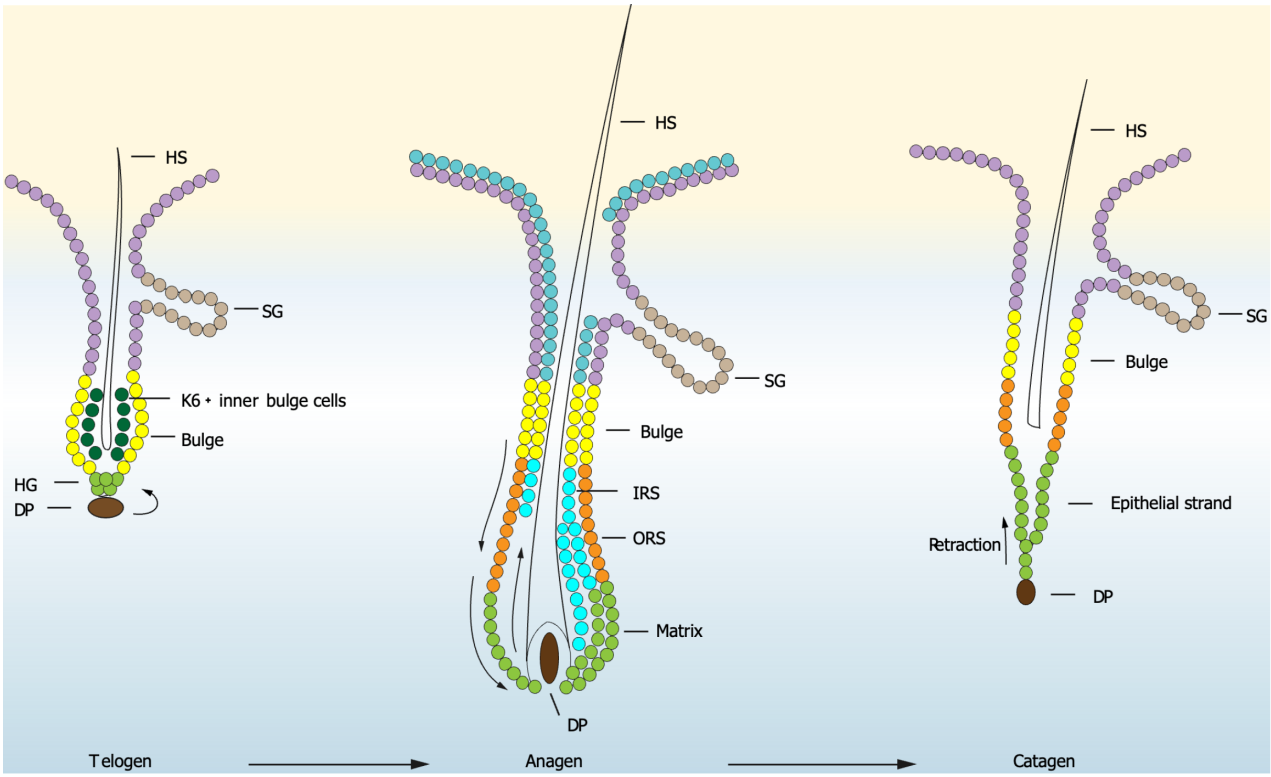

Figure 1 Hair follicle stem cells and the hair cycle. Telogen: The length of the hair follicle (HF) is the shortest. HF stem cells (HFSCs) remain quiescent until dermal papilla (DP) releases a new round of stimulations, which activate the hair germ cells at first, then HFSCs. Anagen: The activated HFSCs proliferate and differentiate, forming the outer root sheath and matrix *via* down growth. After that, the inner root sheath and hair shaft are formed from the matrix. Catagen: The cells in the matrix undergo apoptosis and form an epithelial strand, which retracts the DP. The HF shrinks gradually until entering the next telogen. HS: Hair shaft; SG: Sebaceous gland; HG: Hair germ; DP: Dermal papilla; IRS: Inner root sheath; ORS: Outer root sheath.

cells of the bulge and HG, especially in the HG[34].

Through fluorescence-activated cell sorting (FACS) and flow cytometry, Jaks *et al*[34] analyzed Lgr5-LacZ expression in mouse keratinocytes during telogen and compared it with other known markers. The results showed that approximately 80% of keratinocytes had medium to high levels of a6-integrin expression, whereas Lgr5^{high} cells composed 0.6% of the a6-integrin population. Moreover, 80% of Lgr5+ cells overlapped with the CD34+ cell population in telogen HFs, which constituted only 10% of CD34+ cells. However, CD34+ and Lgr5+ cells, found in the lower ORS, had no overlap in anagen HFs[34,35]. Similarly, K15+ cells showed a partial overlap with Lgr5+ cells in the bulge in telogen but not in anagen[34]. Thus, Lgr5+ cells constitute a particular cell compartment in mouse HFs.

Lgr5+ cells are essential for the telogen-to-anagen transition. Hoeck *et al*[35] utilized diphtheria toxin-mediated Lgr5+ SC ablation in mouse HFs and showed that loss of Lgr5+ cells abolished hair regeneration, but this effect was reversible. During recovery, CD34+ SCs can activate inflammatory response programs and begin to divide. When the SC population was restored, new Lgr5+ cells were generated from CD34+ SCs. This program is dependent on the Wnt signal.

Lgr5 has been identified as a target of the Wnt signaling pathway. R-spondin, a secreted agonist of the canonical Wnt/β-catenin signal, binds to Lgr receptors and stabilizes them[36]. Through intradermal injection of recombinant R-spondin2 protein, the combination of R-spondin and Lgr5 prevents the degradation of β-catenin and improves Wnt signaling[37]. This results in persistent anagen and longer HSs.

After injury, the Lgr5+ bulge cells were sorted and sequenced through lineage tracing. Although the progeny of Lgr5 cells responded and contributed to wound healing, they gradually lost their bulge signature and expressed basal interfollicular epidermis-related genes[38].

LIM-HOMEODOMAIN TRANSCRIPTION FACTOR 2

Lim-homeodomain transcription factor 2 (Lhx2) is a key regulator of bulge SCs[39,40]. During mouse embryogenesis, Lhx2 is first expressed in the early hair placode. As morphogenesis progresses, Lhx2 gradually localizes in the HG and peg precursor cells[40]. At P0, Lhx2 expression is mainly concentrated in the bulge area and is downregulated in the ORS and Mx. At P44, when HFs are in telogen, Lhx2 expression persists in the bulge and secondary HG[41].

Purba *et al*[31] demonstrated that Lhx2+ cells were throughout the ORS, including the basal and adjacent suprabasal layers of occipital scalp HFs in humans, but not in the innermost ORS layers. Lhx2+ bulge cells simultaneously express K15 and CD200. However, the pb-ORS and the sub-bulge cells have brighter fluorescence and more Lhx2+ cell numbers than bulge cells. The Lhx2+ subpopulation is distinct from the sex-determining region Y-Box 9 (Sox9+) cells. Most of the

cells in the sub-bulge and the pb-ORS are either Lhx2+ or Sox9+. Only 17% of these cells are Lhx2 and Sox9 double positive, which reveals that these cells display distinct progenitor cell populations in the ORS. When HFs enter catagen, Lhx2 expression is upregulated in the proximal hair bulb epithelium.

Rhee *et al*[40] reported that Lhx2 remarkably suppressed epidermal differentiation in K14-Lhx2 transgenic mice. However, E16 Lhx2 null embryos displayed a marked reduction in HF density. Lhx2 KO HFs exhibited fewer label-retaining cells, enhanced proliferative activity within the bulge, and shortened telogen. In addition, Lhx2 heterozygous mutations lead to slower wound healing[39]. K14 promoter-mediated conditional knockout of Lhx2 causes the inability to maintain HFSC quiescence and hair anchoring. Therefore, when Lhx2 is absent, the functioning of HFSCs is seriously impaired.

SOX 9

Sox9, a member of the Sox gene family, is characterized by the presence of high mobility group boxes. Sox9 is also an important transcription factor in HFSC biology and has a similar expression pattern to Lhx2 in murine and human HFs [31]. Sox9 expression was first found in the epithelial component of the hair placode. However, the nuclear Sox9 protein is located in the suprabasal cells of the hair placode rather than in the basal layer, which is P-cadherin+ and Lhx2+[42]. At the peg stage, Sox9+ cells are concentrated in an U-ORS region, which exhibits moderate expression of P-cadherin.

In the postnatal hair cycle, Sox9+ cells extend down the ORS and diminish toward the Mx. Cells exhibiting nuclear Sox9 immunoreactivity are mainly distributed in the innermost, differentiated ORS layer and partially distributed in the basal layer. The most apparent Sox9+ region is the sub-bulge region, just below the K15+ bulge compartment, while the Sox9+ population partially overlaps with K15+ cells in the pb-ORS. In catagen, Sox9+ cells are evident in the pb-ORS but not in the bulge area. At P21, Sox9+ cells are concentrated in the CD34+ bulge and HG[12].

Vidal *et al*[42] constructed conditionally targeted Y10:Cre/Sox9(fl/fl) mice, which showed retained atrophic hair coats. Nowak *et al*[12] targeted Sox9 ablation using K14-Cre and further investigated the role of Sox9 in regulating HF morphogenesis and the postnatal hair cycle. Before HF morphogenesis, Sox9 loss leads to the absence of bulge SCs. Although it has no impact on the differentiation of epithelial cells at first, due to the lack of SCs, Mx cells could not be replenished. Eventually, it still affected hair production. To understand whether Sox9 functions in HFSC biology, Kadaja *et al*[43] conditionally targeted Sox9 in the HFSCs of adult mice. Once activated, Sox9-deficient HFSCs differentiate along the epidermal lineage. This differentiation leads to premature termination of the downward growth of the HFs, which is related to the bulge and U-ORS. Genome-wide RNA sequencing profiling and immunofluorescence results further show that Sox9-deficient bulge cells lose SC characteristics. Overall, Sox9 is indispensable for maintaining the identity of HFSCs.

RUNX FAMILY TRANSCRIPTION FACTOR 1

Runx family transcription factor 1 (Runx1), also known as Aml1, is a master regulator of hematopoietic SCs[44]. It regulates the development and homeostasis of multiple tissues. During HF development, Runx1 expression is mainly located in the mesenchyme. At E16.5, X-Gal-positive cells can be detected in the hair placode and germ cells of the Runx1-LacZ mouse embryonic skin, with stronger staining in the upper HF cells[45]. The Runx1 expression pattern is similar to Sox9 and nuclear factor of activated T cells c1 (Nfatc1) in the embryonic epithelium[12,42,46]. The results of lineage tracing experiments showed that cells expressing Runx1 in the embryonic stage contribute to postnatal mesenchymal skin and unique epithelial HF populations.

In the anagen phase, Runx1 is expressed in the infundibulum, bulge, ORS, Mx, and cortex, in contrast to the lower ORS in the catagen phase. There is no expression in the interfollicular epidermis. In the telogen phase, Runx1 is localized to the bulge and HG, and HG cells have the highest expression levels[45]. The Runx1 protein is expressed in the bulge, HS, and IRS of human skin, which is similar to the Runx1 protein expression in mice[47].

To interpret the effect of Runx1 on HFSCs, Osorio *et al*[48] used K14-driven Cre mice to conditionally ablate Runx1 in epithelial cells during morphogenesis. Runx1 ablation mice exhibited blocked hair regeneration due to prolonged telogen. Runx1 disruption affected the activation of HFSCs, including reductions in colony formation and BrdU+ cell numbers during the telogen-anagen transition *in vitro*. If deletion of Runx1 is performed after the end of morphogenesis, it also delays anagen onset[49]. Runx1 loss affects the cell cycle progression of cultured CD34+/α6+-sorted bulge cells by upregulating the cyclin-dependent kinase (CDK) inhibitor Cdkn1a (P21). In addition, it has been reported that Runx1 inhibits the transcription of P21, P27, P57, and P15 in HFSCs *in vivo* and synergistically regulates the resting degree of HFSCs with P21[50]. Therefore, Runx1 may act as a direct regulator of anagen initiation and HFSC proliferation.

AXIN-RELATED PROTEIN 2

Axin-related protein 2 (Axin2), a scaffold protein, can participate in the formation of β-catenin degradation complexes[51-53]. Wnt/β-catenin signaling plays an important role in the development, maintenance, proliferation, and lineage determination of HFSCs[54-58]. As a Wnt target gene, Axin2 is a sensitive Wnt reporter of Wnt/β-catenin signaling in HFs[59]. During embryonic development of the skin epithelium, Axin2-expressing cells are located in the placode and represent

hair cell progenitors. Then, Axin2 expression is gradually lost until the adult HFSC compartment is established, in which Axin2 is expressed again.

By using RNA *in situ* hybridization and Axin2-LacZ mice, it was found that Axin2 is specifically expressed in outer bulge (OB) cells in telogen. Flow cytometry analysis confirmed that these cells exhibited a G0/G1 state. Tracking the fate of these cells and their offspring also showed that Axin2□cells were labeled with long-lived external bulge HFSCs. During the anagen phase, Axin2 in the bulge zone is also continuously expressed[60].

The β-catenin gene was conditionally deleted in Axin2+ cells in Axin2-CreERT2/β-cat$^{\Delta ex2\text{-}6\text{-}fl/fl}$ mutant mice[60]. This deletion led to a decrease in Axin2 mRNA expression levels in the mutant bulge cells. Moreover, Axin2-CreERT2/β-cat$^{\Delta ex2\text{-}6\text{-}fl/fl}$ mutant HFs failed to grow and showed abnormal telogen-like morphology. These results suggest that bulge HFSCs require β-catenin for the expression of Axin2 and to maintain their hair-forming ability.

NFATC1

Nfatc1, a transcription factor, is a member of the NFAT family, which plays key roles in many cellular processes[61,62]. Calcineurin controls the translocation of NFAT proteins from the cytoplasm to the nucleus of activated cells. Using microarray profiling, Nfatc1 was identified as an upregulated gene that can distinguish embryonic hair buds in the epidermis[63,64].

Nuclear Nfatc1 is first detected during late HF morphogenesis. With the maturation of HFs, Nfatc1 expression is also positive in the intermediate segment. In the postanal hair cycle, cells expressing Nfatc1 persist in the bulge not only in growing HFs but also in telogen HFs. Nuclear Nfatc1 is specifically expressed by HFSCs. Compared to other bulge markers, Nfatc1 colocalizes with CD34 and Lhx2 and partially overlaps with Tcf3 and Sox9, with no Nfatc1-positive cells in the lower ORS. Nfatc1 protein and mRNA appear to be specific to bulge cells in both embryonic and adult skin[46].

After transplantation, HFs in the skin of Nfatc1 null mice can form and reenter telogen, but the telogen stage is shortened and the cells subsequently enter anagen early. Therefore, Nfatc1 has an important regulatory effect on the resting state of HFSCs in the bulge. In K14 promoter-driven Nfatc1 knockout mice, HFs could also develop normally, but most of the HFs entered the growth phase at P56, and 75% of the HFs had BrdU-labeled HFs. However, the HFs of wild-type mice are still in telogen until P75[46]. These results suggest that in the absence of Nfatc1, the slow-cycle nature of HFSCs is selectively lost. Horsley *et al*[46] further investigated the effect of Nfatc1 on the cell cycle and found that it downregulated the expression of CDK4 in HFSCs, a key gene in regulating cell cycle progression.

In addition, Keyes *et al*[65] applied Nfatc1 chromatin immunoprecipitation-sequencing analysis and compared age-related signatures. The results showed enrichment of Nfatc1 target genes in FACS-isolated quiescent HFSCs from aged mice. Moreover, when BMP and/or Nfatc1 were inhibited, aged HFSCs showed a lower level of hair regeneration. These results suggest that upregulated Nfatc1 significantly promotes the maintenance of quiescent bulge markers in aged HFSCs.

FORKHEAD BOX P1

The forkhead box (Fox) family contains a group of evolutionarily conserved transcription factors[66], characterized by a forkhead DNA-binding domain, that is involved in numerous functions during development and beyond. As a member of the Fox family, Foxp1 regulates the development of many tissues and is both a transcriptional activator and a transcriptional inhibitor[67-69].

During embryonic development at E13.5, when the epithelium is still a monolayer of ectodermal cells, Foxp1 is uniformly expressed in the epithelium. As the epithelium becomes a multilayer structure, Foxp1 is enriched in the downward-growing HG, which develops into HFs. In postnatal HFs, Foxp1 is continuously expressed in HFSCs of the bulge zone[70]. Foxp1 transcription levels were found to be nearly 3-fold higher in the bulge during telogen than during anagen by real-time polymerase chain reaction analysis. In addition, Foxp1 is also distributed in the ORS and the Mx.

Foxp1 induction and overexpression in keratinocytes using the tetracycline derivative doxycycline system led to proliferation inhibition[70]. Cell cycle analysis showed that Foxp1-induced cells had a higher proportion of cells in the G1 phase and a decrease in the number of cells in the G2 and S phases, which means that the cell cycle was arrested. Foxp1fl/fl; K14-cre (Foxp1 cKO) mice contained 4 types of HFs on dorsal skin, but the number of awl hairs increased, the number of auchene hairs decreased, and the HS length of various hair types became shorter[70]. At P20 and P53, the HFs in the control group were in the quiescent phase, while the HFs in the Foxp1 cKO group had entered the growth phase, indicating that Foxp1 ablation shortened the quiescent phase of HFs. Immunostaining of skin sections showed that HFSCs in the skin of Foxp1 cKO mice were substantially activated and that the number of Ki67-positive cells increased in the bulge region. Furthermore, through gain- and loss-of-function studies, Leishman *et al*[70] proposed that Foxp1 deletion may be caused by its regulation by fibroblast growth factor 18 and CDK inhibitor p57KIP2 expression. Foxp4 is another family member that is similar to Foxp1 in terms of skin expression patterns and cKO phenotypes[69]. Co-immunoprecipitation results showed that there was an interaction between Foxp1 and Foxp4 in HaCaT keratinocytes and primary epidermal cells, suggesting their dimerization in epithelial cells.

FORKHEAD BOX C1

Forkhead Box C1 (Foxc1) belongs to another subtype of the Fox family, which is an early transcription factor expressed in HFs and bulge HFSCs during development and at maturity[16,40]. In addition to the bulge HFSCs, Foxc1 is also expressed in the K6+ inner bulge layer, isthmus, IRS, and sebaceous glands. Conditionally ablated Foxc1 in HFs has an impact on the maintenance of HFSC quiescence[71], which may be related to the reduced expression level of E-cadherin in SCs. Although a new bulge can be produced, the HFs lose their ability to maintain the old bulge. Moreover, the telogen phase of Foxc1 cKO HFs is shorter than that of wild-type HFs. Applying ATAC-seq and ChIP-polymerase chain reaction analysis, Wang *et al*[72] confirmed the direct regulatory effect of Foxc1 on Nfatc1, Bmp6, and Hspb8. They indicated the existence of a collaborative regulatory network between Foxc1, Nfatc1, and BMP signals.

Moreover, in aged mice, the escape of HFSCs from the bulge to the dermis was observed, leading to HF miniaturization and even hair loss. This was related to the reduced expression levels of Foxc1 and Nfatc1. These two transcription factors collaborate to regulate some HFSC-specific cell adhesion and extracellular Mx genes[73].

MUSASHI 2

Musashi (Msi), first identified in Drosophila, is an evolutionarily conserved RNA-binding protein family[74]. Msi has two orthologs in mammals, namely, Msi1 and Msi2[75,76]. Ma *et al*[77] demonstrated that Msi2 is an important posttranscriptional regulator of HFSC quiescence by directly targeting the Shh/Gli1 signaling pathway. At P21, Msi2 is strongly expressed in the basal layer of the bulge and HG. After entering the growth phase, the expression of Msi2 is broader, including the basal and suprabasal bulge, ORS, and IRS. Thereafter, Msi2 expression gradually decreases and is concentrated again in the bulge and HG at the next telogen.

By using gain- and loss-of-function mouse models, it was demonstrated that overexpression of Msi2 significantly prolonged the telogen-to-anagen transition, hindered hair cell entry into anagen, and impaired hair cell regeneration after depilation[77]. In contrast, the lack of Msi2 accelerated hair growth. In combination with several other HFSC markers, including K15, Sox9, and Nfatc1, Msi2 induced more Nfatc1+ cells, fewer K15+ cells and Ki67+ proliferating cells, while the opposite results were observed with Msi2 knockdown. Altogether, Msi2 plays a key role in the resting state of HFSCs.

CONCLUSION

Due to their relatively superficial location, easy isolation and observation, and periodic activation in adults, epithelial HFSCs are not only considered the preferred source for adult SC research but also the ideal seed cells for tissue-engineered skin. SC markers can not only provide a basis for the labeling and identification of HFSCs and serve as a precondition for the isolation and purification of HFSCs but also provide a foundation for the molecular research on the biological characteristics and related regulatory networks of HFSCs. The HFSC markers described here have their own characteristics. Some are classic and commonly used markers, such as CD34 and K15. Others, such as Nfatc1 and Lhx2, are molecules closely related to the important life processes of HFSCs, including but not limited to resting, proliferation, and aging. There was some colocalization between these markers. The combination of multiple markers can greatly improve the labeling rate of HFSCs.

The heterogeneity of SCs in the bulge region of HFs has attracted increasing attention. Some analyses of single-cell RNA-sequencing (scRNA-seq) data also support this view in recent years[2,17,25,78,79]. Through quantitative scRNA-seq to sequence cells from the murine telogen and anagen epidermis, Some studies identified 13 distinct main groups of epidermal cells[17,79]. The bulge had one inner and one outer compartment, characterized by K6a and CD34, respectively. Upon taking a second round of unsupervised clustering, the OB keratinocytes, characterized by high expression of CD34, K15, and Lgr5, were further divided into five subpopulations (OB I-V). Most OB cells were proximal OBI (CD34^{high}, Postn^{high}, Lgr5^{high}, K24^{high}) and central OBII (CD34^{high}, Postn^{high}, Lgr5^{dim}, K24^{dim}) populations. However, the study was not able to distinguish previously reported cell populations containing certain specific markers, such as Gli1+ or Lgr5+ cells in the lower bulge. The cell populations distinguished by specific markers often contain more than one subpopulation in the study. The features and functions of these new cell subpopulations require further in-depth research. In addition, the study also compared SCs and progenitor cells, but did not find any significant distinguishable stemness characteristics. Considering the different spatial locations of these cells in the tissue, space transcriptomics may become an important complement to scRNA-seq.

Combined with fluorescence-activated cell sorting, another interesting scRNA-seq study was on K14-H2BGFP+CD34+ cells from the early/mid-anagen stage of murine skin[78]. During this stage, HFSCs undergo self-renewal. Four bulge subpopulations were identified as migration-, low-, mid-, and up-bulge parts. All four subpopulations were positive for several known HFSC markers, such as K15, Sox9, Lhx2, and Col17a1. However, some other used markers had different expression patterns, including CD34, Lgr5, Nfatc1, Foxc1, Runx1, and Postn. The mid-bulge subpopulation was mainly dormant and speculated to be a reserve pool. According to the differential gene expression analysis, upregulated genes included Zfp36, Atf3, Sfn, Btg2, Cdkn1a and Hspb1. Consistent with the murine study, scRNA-seq of human skin has been performed and revealed 23 primary cell clusters[25]. The bulge subpopulation was immunostained with CD200 and Cxcl14, and a Gene Ontology analysis revealed that the bulge subpopulation was enriched in cell adhesion and extracellular Mx molecules.

This review mainly summarized the recent progresses and functions of some classic and commonly used molecular markers in HFSCs. These molecules can be used as candidate molecules for the identification, labeling, and screening of HFSCs to a certain extent. Along with the development of sequencing technology, our understanding of HFSCs will increase gradually. There is still a long way to go to clarify the molecular heterogeneity of HFSCs in more detail. With the assistance of various new technologies, more in-depth characteristics of HFSCs will be revealed, such as transcriptional heterogeneity, spatial location information, and chromatin accessibility. These new researches will help in developing reasonable methods for treating hair loss in clinic.

FOOTNOTES

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