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REVIEW ARTICLE

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Regulation of melanocyte stem cells in the pigmentation of skin and its appendages: Biological patterning and therapeutic potentials

Weiming Qiu[1] | Cheng-Ming Chuong[2,3] | Mingxing Lei[3,4]

[1]Department of Dermatology, Wuhan General Hospital of Chinese People's Liberation Army, Wuhan, China

[2]Department of Pathology, University of Southern California, Los Angeles, California

[3]Integrative Stem Cell Center, China Medical University Hospital, China Medical University, Taichung, Taiwan

[4]Institute of New Drug Development, College of Biopharmaceutical and Food Sciences, China Medical University, Taichung, Taiwan

Correspondence
Mingxing Lei, Integrative Stem Cell Center, China Medical University Hospital, China Medical University, Taichung, Taiwan.
Email: mingxing@mail.cmu.edu.tw
Cheng-Ming Chuong, Department of Pathology, University of Southern California, Los Angeles, CA.
Email: cmchuong@usc.edu

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Abstract

Skin evolves essential appendages and indispensable types of cells that synergistically insulate the body from environmental insults. Residing in the specific regions in the skin such as epidermis, dermis and hair follicle, melanocytes perform an array of vital functions including defending the ultraviolet radiation and diversifying animal appearance. As one of the adult stem cells, melanocyte stem cells in the hair follicle bulge niche can proliferate, differentiate and keep quiescence to control and coordinate tissue homeostasis, repair and regeneration. In synchrony with hair follicle stem cells, melanocyte stem cells in the hair follicles undergo cyclic activation, degeneration and resting phases, to pigment the hairs and to preserve the stem cells. Disorder of melanocytes results in severe skin problems such as canities, vitiligo and even melanoma. Here, we compare and summarize recent discoveries about melanocyte in the skin, particularly in the hair follicle. A better understanding of the physiological and pathological regulation of melanocyte and melanocyte stem cell behaviours will help to guide the clinical applications in regenerative medicine.

KEYWORDS
hair follicle stem cells, hair greying, hair regeneration, melanocyte stem cells, pigmentation disorders

1 | INTRODUCTION

In animals, skin and its appendages such as hair and feather show diverse colours which are crucial for beauty and survival.[1] Feedback and feedforward loops between evolutional pigmentation and the adaptive requirement to the environment affect behaviour and multiplicity of animals. In birds such as flamingo and peacock, the male with colourful feathers is more easily recognized by the female to mate during their breeding season. Animals such as snake, frog and chameleon that evolve with the ability to synchronize their skin colours with the surrounding environment are enabled a camouflage benefit for self-protection and food-hunting. In human, skin colour

also shows regional specificity in different species and within an individual, which is also the evolutionary product of adaptation to different environment.[2] Distinct length, thickness and pigmentation of hairs in different body regions allow diverse appearance to emerge.[3] The melanin-based pigmentation not only endows human skin with abilities to shield the body from ultraviolet (UV) radiation, but also facilitates the discharge of harmful substances such as heavy metal out from the body. Hypopigmentation deficiency-related diseases can cause mental trauma and significantly affect one's social contact.

Melanocyte arises from the neural crest in vertebrates (Figure 1). During embryogenesis, melanoblasts differentiated from neural crest cells migrate from the neural tube through skin dermis to epidermis and hair follicles.[4] In epidermis, melanoblasts differentiate into melanocyte precursors which resides in the basal layer of the epidermis. And in hair follicles, the melanoblasts differentiate into melanocyte stem cells (McSCs) which are homed to the hair follicle bulge, the niche where hair follicle stem cells (HFSCs) reside.[5] Melanocyte precursors and McSCs differentiate into mature melanocytes in the skin epidermis and hair matrix. Each mature melanocyte extends its dendrites and targets a defined population of epidermal or hair follicular keratinocytes at a ratio about 1:36 to create a pigmentary unit.[6] The melanin is produced and transported by melanocytes to the surrounding keratinocytes, leading to the formation of pigmented hair and skin. The distribution of melanocytes shows diversity in mammal skin, yet the mechanisms remain elusive. Interestingly, melanocytes reside in both hair follicle and epidermis in human and young mice skin (eg, <2-month-old), but not in adult mouse epidermis.

Hair follicle is an attractive surrogate model to study the cellular and molecular mechanisms involved in the maintenance and collapse of integument pigmentation. McSCs share the same niche with HFSCs in the bulge and show cyclical activation (anagen), degeneration (catagen) and quiescence (telogen) in synchrony with HFSCs during cycling (Figure 2).[7] At the onset of pigmented hair regeneration, these two different lineages of adult stem cells are activated to proliferate and differentiate in a synchronized manner. At catagen, mature melanocytes undergo apoptosis along with keratinocytes in the hair matrix and outer root sheath. Upon entry into telogen, HFSCs and McSCs turn to be relatively quiescent. The coordination between McSCs and HFSCs allows them to divide synchronously to ensure the maximal efficiency in formation of a pigmented hair.[8] On the contrary, silence or loss of McSCs in hair follicles causes generation of grey hairs. However, under stress situations such as wounding, UV radiation or exposure to chemical carcinogens like TPA/DMBA, McSCs can migrate towards epidermis and differentiate into melanocytes for epidermal pigmentation.[9–11]

In this review, we focus on McSCs in hair follicles and summarize the recent progress in studying McSC niche in the hair follicle. We review the signals that are involved in McSC development, and signals that regulate the behaviours of McSCs during hair regeneration. We also discuss the potential therapeutic applications of McSCs in skin hypopigmentation diseases. These data provide a foundation for how to target hair bulge to stimulate hair and epidermal repigmentation.

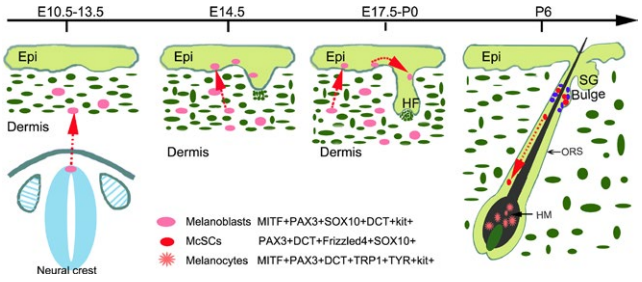

FIGURE 1 Development of melanocytes. Melanoblasts originate from neural crest and migrate through the dermis to the epidermis and finally to the hair follicles. When homing to the hair follicle bulge region, melanoblasts differentiate into McSCs, which migrate downward and differentiate into the mature melanocytes in the hair matrix. The mature melanocytes further differentiate to generate melanin for hair pigmentation. Melanoblast, McSCs and melanocyte express specific genes as shown in the figure. The red arrows indicate the developmental process of the melanocyte lineage. E, embryonic day; Epi, epidermis; HF, hair follicle; HM, hair matrix; ORS, outer root sheath; P, postnatal day; SG, sebaceous gland

2 | COMPLEX SIGNALLING NETWORKS GUIDE DEVELOPMENT AND HOMING OF MELANOCYTE TO THE HAIR FOLLICLE

Melanocyte stem cells are differentiated from neural crest during vertebrate development. Multipotent neural crest cells can

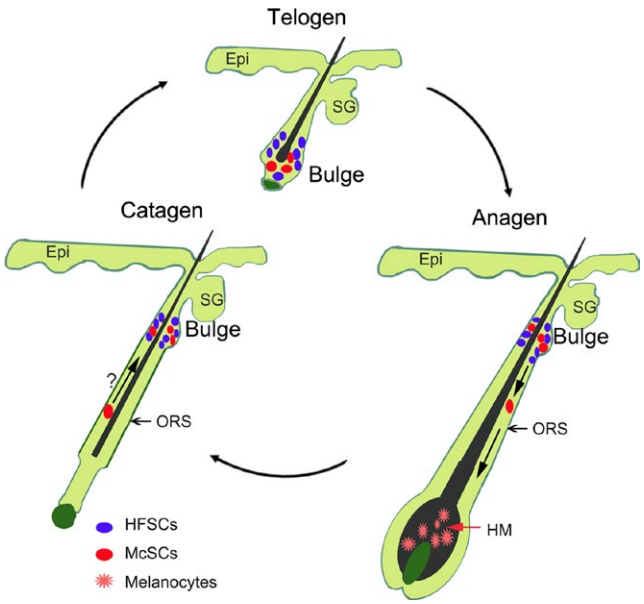

FIGURE 2 Cyclical regeneration of McSCs during hair cycling. McSCs are activated in synchrony with HFSCs in anagen. They migrate downward along the ORS and differentiate into mature melanocytes in the hair matrix. In catagen, mature melanocytes undergo apoptosis. Whether the non-apoptotic McSCs migrate back to bulge niche remain elusive. McSCs keep quiescent in telogen until the initiation of the next anagen. Epi, epidermis; HM, hair matrix; ORS, outer root sheath; SG, sebaceous gland

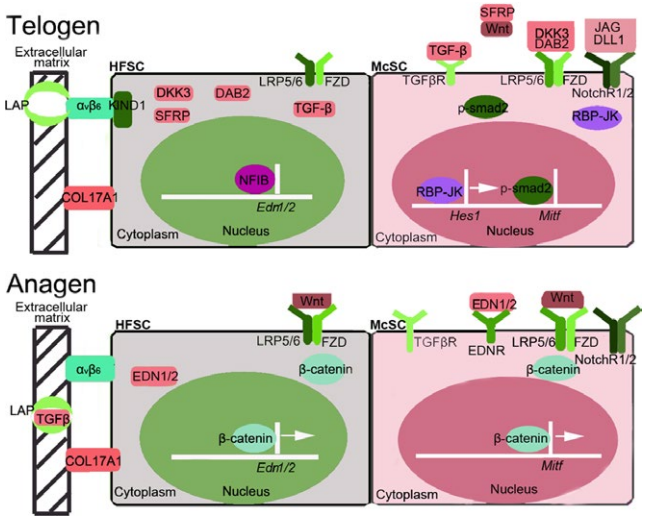

FIGURE 3 Molecular mechanisms that regulate quiescence and activation of McSCs niche in telogen and anagen, respectively. In telogen, HFSCs-derived signalling molecules, such as TGF-β, Sfrp1, DKK3, Dab2, Jagged, keep McSCs quiescent by activating the intracellular signalling pathway. In early anagen, paracrine-derived signalling molecules, such as Edn1/2 and Wnt, induce regeneration and migration of McSCs

differentiate into neuronal, glial and melanogenic lineages. Between murine embryonic day 8.5 (E8.5) and E10.5, neural crest cells which migrate between the somite and the non-neural ectoderm differentiate into melanoblasts.[12] Complex signalling networks have been unveiled to be involved in the melanoblast specification process. Microphthalmia-associated transcription factor (Mitf) functions as a master transcriptional factor for the specification and survival of melanoblasts. Loss of function by deletion or mutation of Mitf causes melanocyte absence and pigmentation defects.[13–15] Function null of MITF causes melanoblasts to transdifferentiate to glia cells.[16] As the master regulator, MITF promotes the expression of many melanogenesis-related genes, including tyrosinase, tyrosinase-related protein 1 (TRP1) and dopachrome tautomerase (DCT).[17,18] Sox10, Pax3 and Wnt3a-mediated Wnt/β-catenin signalling induce the transcription of MITF and promote differentiation of neural crest into melanoblasts,[19–23] though MITF is not expressed in the neural crest. Thus, activation of MITF can be deemed as the initiation of melanoblasts lineage specification from neural crest in mouse and zebrafish.[24–27] This process is governed by a transcriptional factor Foxd3, which is expressed in the neural tube before neural crest migration and in migrating neural and glial precursors.[28–31] Through interacting with PAX3, FOXD3 prevents binding of PAX3 to MITF promoter to repress melanogenesis in zebrafish, quail and chick neural crest cells,[29,32] suggesting that downregulation of Foxd3 is a crucial step during the early phase of melanoblast lineage specification from neural crest cells.

After E10.5, melanoblasts start to migrate ventrally towards the face, ventral abdomen and the developing limbs. In *Xenopus*, Ednrb2-positive melanoblasts migrate to their destinations where endothelin 3 (Edn3) is expressed. Aberrant Edn3/Ednrb2 signalling interrupts melanoblast migration, suggesting that Edn3/Ednrb2 signalling might determine the melanophore localization in *Xenopus*.[33] EdnrB signalling is a temporally required for neural crest development between embryonic days 10 and 12.5 in mice. EdnrB is indispensable for the migration of murine melanoblasts in the dorsolateral pathway.[34,35] Ednrb knockout mice have typical Waardenburg–Shah syndrome IV phenotypes (WS IV) showing hypopigmentation, megacolon disease and hearing loss.[36] Moreover, mutation of Ednrb is also found in human WS IV.[37] At around E13.5, melanoblasts progress upward through the developing dermis into the epidermis. The migration of melanoblasts to their destination might be regulated by migratory or chemotactic signals. Site-specific overexpression of hepatocyte growth factor HGF in epidermal keratinocytes significantly increases the number of melanoblasts and melanocytes in dermis but not in epidermis,[38] suggesting that HGF acts as a paracrine agent to promote and maintain the survival and proliferation of melanoblasts in dermal compartment. Then what is the mechanism that melanoblasts migrate from dermis towards epidermis? Overexpression of stem cell factor (SCF) in epidermal keratinocytes promotes the extension of melanocytes in epidermis and causes melanocytes to reside in oral epithelium and footpad,[39] indicating that SCF signalling plays important role in melanoblast migration towards epidermis.

Distinct mechanisms of melanoblasts localization emerge in between murine and human skin after birth. Melanoblasts gradually disappear in murine epidermis, only staying in the developing hair follicles by postnatal day 4 (P4), responsible for hair pigmentation. From E17.5 to P0, the melanoblasts in hair follicles are segregated into two populations: one population resides in the putative bulge region to become the small and round McSCs and the other one is differentiated into mature melanocytes located in the hair matrix.[7,40–43] However in human, melanoblasts-derived melanocytes are normally located in both interfollicular epidermis and hair follicles.[44] This might be another evolutionary outcome between different species to their facing environment. Humans are diurnal primate with skin bears fewer hairs but often exposes to the external stresses such as UV radiation from the sun,[45] thus maintaining melanocytes in skin epidermis to directly protect the body from environmental insults. As cave animals, murine retains hair follicles throughout their skin to keep warm. Nevertheless, bulge melanocytes can still migrate into the epidermis when the skin is injured.

It is interesting that melanoblasts are recruited to hair bulge region to become the McSCs during embryonic development. Similar to the migration of melanoblasts from skin dermis to epidermis during embryogenesis, hair follicle-derived SCF also induces melanoblasts migration towards hair follicles by binding its specific receptor c-Kit, which is expressed by the melanoblasts.[46] Elevated level of SCF is expressed in the bulge cells, suggesting that HFSCs may help the recruitment of melanoblasts to the bulge niche, and give rise to McSCs which express PAX3, DCT, Frizzled4 and SOX10 (Figure 1). Receiving signals from hair matrix keratinocytes and dermal papilla (DP) cells, McSCs migrate downward along ORS and differentiate to melanocyte precursors which generate mature melanocytes in the hair matrix. Foxn1 expressed by hair matrix keratinocytes recruits

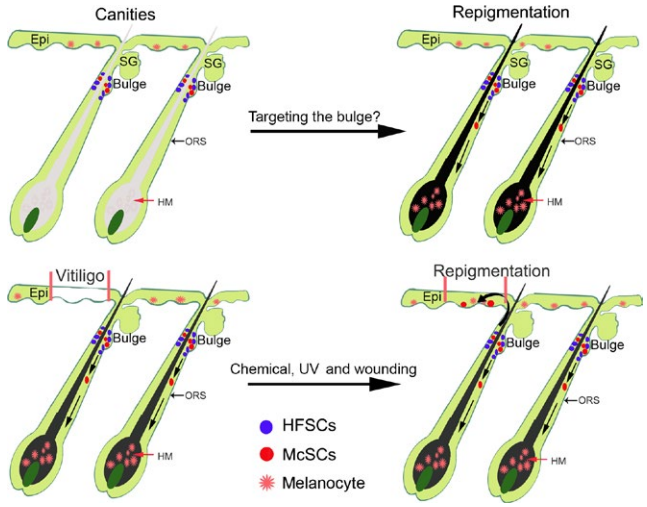

FIGURE 4 Targeting the hair follicle stem cell niche for epidermal and hair repigmentation. In canities, some of the McSCs reside in the bulge niche. Activation of McSCs by targeting the hair bulge may be an excellent choice for hair repigmentation. After TPA, UV radiation and wound healing, McSCs are recruited to the skin epidermis for epidermal repigmentation. UV radiation has been used to treat epidermal hypopigmentation diseases, such as vitiligo. Chemicals such as TPA can activate McSCs for pigmentation of regenerated hairs. During wound healing, McSCs can also migrate towards epidermis and differentiate to generate melanocytes for epidermal pigmentation. Epi, epidermis; HF, hair follicle; HM, hair matrix; ORS, outer root sheath; SG, sebaceous gland

some melanoblasts to migrate downward by regulating the expression of bFGF for hair pigmentation.[47] The signals from dermal papilla are also involved in melanoblast differentiation. Chemotactic signal secreted by the DP may control the localization and migration of melanocytes.[48] The melanoblasts in the hair matrix differentiate into mature melanocytes which can be characterized by the expression of MITF, PAX3, DCT, Trp1, TYR and Kit.[7,40–43]

3 | CYCLIC ACTIVATION OF MCSCS IN THE BULGE REGIONS OF HAIR FOLLICLES

Melanocyte stem cells undergo quiescence in synchrony with HFSCs under physiological conditions. As catagen ensues, differentiated melanocytes undergo apoptosis along with the rest of the matrix, resulting in degeneration of the lower portion of hair follicles.[49] Exquisite histone and nucleotide double-pulse-chase and lineage tracing experiments show that early HFSCs descendent which become transit-amplifying cells during anagen phase, retain stemness and home back to the bulge niche when hair growth phase stops.[50] On the other hand, known evidence shows nearly all the differentiated melanocytes undergo apoptosis during catagen phase, leaving a few McSCs at the bulge region when hair follicle enters telogen phase. However, there is also a population of MITF-positive progenitor cells located in the outer root sheath and hair bulb region of anagen hair follicles.[41,51,52] Whether there is a similar home back de-differentiation mechanism in regulating McSCs behaviour as in HFSCs remains further investigation. At least McSCs can differentiate into transient amplifying cells and some of them survive after catagen and maintain the stem cell fate.[49,53] As aforementioned, hair follicle bulge expresses an elevated level of Kit ligand, which may help recruit these cells back into the niche.[46]

Microphthalmia-associated transcription factor is a key transcription factor that promotes melanogenesis by upregulating two melanogenic enzymes, TYR and TRP1. The central role of MITF in melanocytes is well reviewed in previous studies.[54,55] MITF is expressed in the progeny of hair follicle McSCs during anagen phase.[9] Expression of MITF can be regulated by Pax3, a transcription factor which has dual functions in regulating melanogenesis. Pax3 not only promotes melanogenesis by activating the expression of MITF, but also maintains McSCs quiescence by competing with MITF through binding an enhancer responsible for the expression of dopachrome tautomerase (DCT), an intermediate in the biosynthesis of melanin. Pax3-mediated repression of melanogenesis is relieved by activated β-catenin.[43] This means that Pax3 functions as a crucial nodal point for self-maintenance and activation of McSCs.[43,56]

In human melanoma cells, MITF also interacts directly with β-catenin and redirects β-catenin transcriptional activity away from target genes regulated by Wnt/β-catenin signalling, towards MITF-specific target promoters to activate transcription.[57] Activation of Wnt/β-catenin signalling in McSC promotes their differentiation.[8] These data indicate that MITF may enhance the role of Wnt/β-catenin signalling in proliferation and differentiation of McSCs in a feedback mechanism. Activation of Wnt signalling or increased Edn1/2 in the hair bulge promotes McSCs proliferation by binding Edn receptor B, which also governs the regenerative response of both mouse and human McSCs during large wound healing.[8,58] Both Edn1 and Edn2 are important for McSCs activation. End1 is induced by Wnt signalling at early anagen.

Downstream side, MITF modulates expression of microRNAs to regulate pigmentation in melanoblasts and melanocytes. For example, MITF-dependent expression of microRNA-211 promotes pigmentation in melanoblast and melanocyte cell lines by inhibiting the expression of TGF-β receptor 2, which is involved in the maintenance of McSCs quiescence. On the contrary, downregulation of microRNA-211 significantly inhibits skin pigmentation,[59] suggesting that MITF-mediated microRNA-211 acts as a negative regulator of TGF-β signalling in tissue pigmentation.

4 | MAINTAINING QUIESCENCE OF MCSCS IN THE BULGE POTENTIATES CONTINUOUS REGENERATION OF MELANOCYTES

Elucidating the molecular mechanisms underlying the regulation of the TGF-β signalling will be of great importance for controlling

the cyclic activation and quiescence of McSCs. Since McSCs reside in hair bulge surrounded by HFSCs, adhesion molecules and direct cell-cell contact are involved in the interaction between McSCs and HFSCs. The transmembrane protein collagen XVII (COL17A1) projects beneath hemidesmosomes in epithelial basal keratinocytes into the basement membrane to mediate anchorage.[60] COL17A1 is expressed in HFSCs but not in McSCs. COL17A1 maintains HFSCs at a quiescent state.[61] Knockout of COL17A1 in HFSCs initiates the activation of McSCs via repressing the TGF-β level, indicating that COL17A1 is required for maintenance of McSCs by controlling the TGF-β expression.[62] TGF-β signalling functions as a key signalling on maintaining McSCs quiescence.[62,63] TGF- β is activated in McSCs when the hair follicle enters the resting phase. TGF-β binds TGF-β receptors in melanocytes, leading to the phosphorylation of downstream effector Smad2, which inhibits melanocyte growth and melanogenesis through downregulating PAX3 and MITF transcription.[63,64] Knockout of TGF-β receptor 2 in hair follicle melanocyte lineage blocks the Smad2 phosphorylation, resulting in a loss of quiescence state of McSCs.

Residing at the bulge niche at telogen, HFSCs secrete several inhibitors that may be responsible for maintaining quiescence of McSCs (Figure 3). Some Wnt inhibitors, such as DKK3, Sfrp1 and Dab2, are expressed by HFSCs and set into the hair bulge.[65,66] Injecting Wnt inhibitor DKK1 into skin inhibits TPA induced the proliferation and differentiation of McSCs.[52] These data indicate that Wnt inhibitors expressed by HFSCs may suppress the proliferation and differentiation and contribute to McSCs quiescence. Thus, inhibited Wnt signalling and activated TGF-β signalling are the keys to the dynamic changes of the microenvironment for maintaining quiescence of McSCs in the bulge niche.

Notch signalling plays an important role in maintaining survival and quiescence of McSCs.[67,68] Notch ligands including Jagged1, Jagged2, Delta-like1, Delta-like3 and Delta-like4 bind to Notch receptor, which induces signal transduction cascade through the induction of transcription factor RBP-JK to initiate the transcription of target genes.[69] Notch and its target gene Hes1 are highly expressed in quiescent McSCs. GSI is a specific inhibitor of Notch receptors 1 and 2. The number of McSCs is significantly decreased in the bulge of GSI-treated mice.[67,70] Deletion of either Notch receptor 1 or 2 or both leads to loss of hair pigmentation. However, it has not been genetically proven that RBP-JK is involved in Notch signalling-mediated maintenance of melanocyte homeostasis. Mice with deletion of RBP-Jk in melanocytes show grey hairs comparable to that Notch1 and Notch2 receptors-null mice.[71] The grey hair phenotype in RBP-Jk-null mice cannot be rescued by transgenic expression of Notch 1. Thus, Notch1/2-RBP-Jk signalling also acts as a crucial regulator of McSC quiescence and homeostasis.

The African striped mouse (*Rhabdomys Pumilio*) has a periodic pattern of dorsal stripes in hair colour. The transcription factor Alx3 is expressed in both melanocytes and keratinocytes at the hair bulb of the follicle, and its expression is elevated in the light stripe compared to the dark stripe.[72] Alx3 decreases melanin production by directly suppressing the expression of MITF, by indirectly inhibiting the secretion of Edn3 and by indirectly promoting the expression of ASIP. This discovery identifies Alx3 as a novel factor that modulates McSC differentiation to arrange the spatial colour variation of the hair coat.

5 | REGULATION OF MCSCS BY KERATINOCYTES

Hair follicle stem cells and McSCs share the same niche. Organized and timely cooperation between HFSCs and McSCs contributes to the successful regeneration of pigmented hairs. HFSCs coordinate the McSCs behaviour by both intrinsic and extrinsic mechanisms during hair regeneration (Figure 3). At the initiation of anagen phase, quiescent HFSCs and McSCs are both activated and coordinately repopulate the hair bulb with differentiated pigment-producing progeny.[73] The differentiated melanocytes produce and transfer melanin to the adjacent hair follicle keratinocytes. Multiple factors and signalling pathways govern this process during pigmented hair regeneration. Nuclear factor I/B (NFIB) was recently identified as a transcription factor expressed in the HFSCs to coordinate HFSCs and McSCs behaviour.[73] NFIB plays an important role in lung and brain development and is involved in epithelial cancers.[74–76] Conditional knockout of NFIB in HFSCs promotes McSCs proliferation and differentiation, indicating a role of NFIB as a regulator of McSC behaviour.[73] Inhibition of NFIB signalling in HFSCs directly stimulates expression of endothelin 2 (Edn2), which is required in HFSCs-dependent McSCs activation.

Forced β-catenin stabilization in hair follicle drives proliferation and differentiation of both HFSCs[77,78] and McSCs.[8] On the contrary, lack of β-catenin arrests proliferation of HFSCs and McSCs, indicating the importance of Wnt/β-catenin signalling pathway in regulating these two stem cell populations. Inhibition of Wnt signalling by a Wnt antagonist secreted frizzled-related protein 4 (sFRP4), which is exclusively expressed in the epithelial cells but not the melanocytes of the hair follicle, results in a decrease of melanocytes differentiation in the regenerating hair follicle.[79] However, whether McSCs release instructive signals to regulate HFSCs behaviour is an interesting question awaiting future investigation.

Kindlins, a family of evolutionarily conserved proteins, are expressed at cell-matrix adhesion sites that increase integrin affinity for the ligand to bind the integrin tails.[80] Kindlin family members include Kindlin-1, Kindlin-2 and Kindlin-3. Kindlin-1 and Kindlin-2 are expressed in epidermis and hair follicles.[81] Loss of Kindlin-1 in mouse keratinocytes results in formation of hyper thickened epidermis, development of ectopic hair follicle and increased susceptibility of skin tumor,[81] suggesting an enlarged and hyperactive stem cell compartments in mouse skin. Mechanistically, knockout of Kindlin-1 promotes cutaneous epithelial stem cells differentiation via inhibiting α(v)β(6) integrin-mediated TGF-β1 liberation and promoting integrin-independent Wnt ligand expression to activate Wnt/β-catenin signalling.[82] These indicate that Kindlin-1 functions as an essential factor to control HFSCs homeostasis by balancing TGF-β-mediated growth-inhibitory signals and Wnt-β-catenin-mediated

growth-activating signals. Thus, the altered molecular environment must influence the activities of McSCs. Indeed, lack of Kindlin-1 results in a reduced hair pigmentation.[82]

At anagen, HFSCs generate seven types of uni-lineage epithelial progenitors that are spatially arranged juxtaposed to the DP, to give rise to seven differentiating layers of the hair shaft, inner root sheath and companion layer.[83] McSCs also migrate downward and differentiate into progenitor cells in the hair bulb. Yet melanocytes only produce and transmit melanin to the hair cortex and medulla. Krox20-positive hair matrix progenitors regulate melanocyte differentiation and hair pigmentation by secreting SCF.[84] Foxn1-positive hair cortex epithelial progenitors secrete Fgf2 which instructs melanocytes to produce and transmit melanin to the epithelial cells.[47] Both Foxn1 and Krox20 are regulated by Wnt/β-catenin signalling.[84,85] This further indicates that Wnt signalling is indispensable for activation and differentiation of McSCs during initiation and progression of the hair cycle.

Asymmetric differentiation expression in the keratinocytes of the hair bulb region also influences pigment patterning of hair follicles. Transcription factor TBX3 is expressed in the outer cuticular layer and asymmetrically in hair cortex of Dun hair follicles, but only in the outer cuticular layer of non-Dun hair follicles in Dun horses. This asymmetric expression pattern of TBX3 in the growing hairs results in pigment dilution and asymmetric pattern of pigment in hairs,[86] indicating that hair cortex keratinocytes can regulate melanocyte location and hair pigmentation.

6 | REGULATION OF MCSCS BY DERMAL PAPILLA CELLS

Melanin includes black pigment (eumelanin) and yellow pigment (pheomelanin). The key point to decide the type of melanin is the Mc1r activity. Basal Mc1r activity is sufficient to maintain the production of black pigment.[87] The production of black melanin can be augmented by the Mc1r agonists such as α-MSH,[88] whereas inhibiting the Mc1r activity by DP-derived agouti promotes the production of yellow pigment.[89] Although α-MSH can derive from epithelial cells and systematically, the activation of Mc1r signalling by α-MSH is in the DP, which further regulates melanocyte pigmentation. This indicates that DP directly regulates melanocyte pigmentation. On the contrary, when agouti activity is inhibited by β-defensin, or Corin, or β-catenin in the DP, the yellow coat turns to be black.[90–92] This indicates that as a signalling centre, DP not only regulates various hair follicle behaviours,[93] but also plays an important role in regulating hair pigmentation.

Regulation of mesenchyme-derived agouti allows variation of coat colours for animals to accommodate their living environment. For example, mainland *Peromyscus polionotus subgriseus* mice residing in a field with dark soil have a colour pattern shown as an expanded dark dorsum and narrower light ventral region. To inhibit the light-coloured sand dunes of Florida's Gulf Coast, their descendants *Passeriformes Phoenicurus leucocephalus* who live at the beach have an overall lighter pigmentation and even no pigmentation on the face, flanks and tail.[94] Evolutionarily, the black colour coat formation is negatively regulated by agouti, and ectopic expression of agouti in the hair follicles of mainland *Peromyscus polionotus subgriseus* mice indeed inhibits eumelanin expression. Complementary to the expression of agouti which is expressed in the ventral mesenchyme, transcription factor Tbx15 is expressed in dorsal mesenchyme during early embryonic development, implying a positive regulatory role on black colour patterning in dorsal hair follicles.[95]

7 | REGULATION OF MCSC BY EXTRA-FOLLICLE SIGNALS

Hair follicle bulge resides in a complex macroenvironment.[96] Other tissues surrounding the hair follicle, such as neuron, arrector pili muscle, dermis, adipocytes and blood vessels, influence the stem cell behaviours. Deletion of nerve-derived Shh does not change the morphology of the regenerated hair follicles, but inhibits the proliferation of HFSCs. As a result, the expression of molecular markers of HFSCs such as K15 is significantly decreased,[97] suggesting that nerve-derived Shh is required for maintaining a functional niche for HFSCs. Nevertheless, the bulge stem cells deposit nephronectin into the underlying basement membrane to create a smooth muscle cell niche for the arrector pili muscle.[98] McSCs sharing the same bulge niche with HFSCs should be impacted by the macroenvironmental factors. For example, Gli3 has been shown to be able to restore differentiation of neural crest cells to melanoblasts during melanocyte development,[99] indicating that nerve-derived Shh-Gli signalling can affect behaviours of melanocyte lineages.

Bmp2 and Bmp4 in the dermis and subcutaneous adipose tissue inhibit transitioning of hair cycling from telogen to anagen.[100,101] Bmp2/4 also inhibits TYR activity and melanogenesis,[102] suggesting that dermis- and subcutaneous adipose tissue-derived Bmp might also serve to maintain McSCs quiescence. On the other hand, adipose regeneration is in synchrony with the activation of HFSCs. Adipocytes are necessary and sufficient to activate HFSCs,[103] and this is regulated by Platelet-derived growth factor (PDGF). PDGF promotes the proliferation of human melanoblasts and differentiation of melanocytes,[104] indicating that adipose-secreted PDGF may also regulate McSCs activation and differentiation. Still, how this relatively small amount of McSCs maintain homeostasis and regenerate in the complex macroenvironment remains further exploration.

8 | COMPLEX FEATHER PIGMENT PATTERNS RESULT FROM A COMBINATION OF MULTI-SCALE REGULATORY MECHANISMS

Avian feathers show complex colour patterns. This is the combination of multiple mechanisms. At the tissue level, there are

regional-specific feather follicles which are regulated differently by hormones or seasons.[105] Furthermore, avian pigments can derive from melanocytes (eumelanin or pheomelanin), chemical colours and structural colours.

Feather melanocytes sit in the bottom of the follicles. In each cycling, they may proliferate and emigrate or remain quiescent. Even the melanocyte progenitors emigrate into feathers, the differentiation may be suppressed by agouti, made by the peripheral pulp fibroblasts.[1] In this case, the expression of agouti is influenced by sex hormone, and feathers from the same position of male and female chickens show different pigment pattern.[1] It would be interesting to learn how sex hormones affect the agouti expression. So the white colour can be due to the absence of melanocytes by non-migration or death of melanocytes, or due to the suppression of differentiation by agouti or other inhibitors.

Chemical colours are generated by enzymes. One category is the carotenoids. For example, golden and silver pheasants (*Chrysolophus*) have striking colour differences. Genome and transcriptome analyses show carotenoids in the feathers of the golden, but not silver, pheasants.[106] Carotenoid is a lipochrome and the distribution pattern is further regulated by its synthesis, transport, deposit ad degradation. For example, golden pheasant feather follicles contain high APOA1 (for deposit) and low BCO2 (for degradation).

Structural colour is based on the arrangement of organelles (eg, melanosomes) or cell shapes (eg, barbules). Peacock tail feathers look blue or green based on iridescent and light interference.[107] Another good example is the blackness produced by the tight interwoven of barbules in paradise birds. This structural colour black is much blacker than melanin black and can over-write the presence of other colours. An interesting example of combinatorial structural and chemical colour is seen in budgerigar, which has a structural colour mechanism to make it blue (or white, when this mechanism is not present). Then, there is polyketide synthase which produces yellow pigment, or no pigment when this enzyme contains a mutation. When yellow is with white, the feather is yellow. When yellow is with blue, the feather is green.[108] Interestingly, polyketide synthase is present in chicken but not in the skin. In budgies, this enzyme is co-opted to be expressed in the axial plate epithelia.

9 | UPON INJURY, MCSCS CONTRIBUTES TO EPIDERMAL MELANOCYTE REPOPULATION

Melanocyte stem cells and HFSCs are not only responsible for pigmented hair regeneration but also provide cells resource for epidermal homeostasis and shield from harmful stress. After receiving the external stress such as UV radiation or exposure to chemical reagent, the epidermis is significantly thickened, with an increased number of melanocytes.[9,10,109,110] The epidermis in the lesion of vitiligo is also thicker than the normal epidermis.[111] These pathological phenotypes suggest that both epidermal and McSCs can initiate coordinated mechanisms to protect our body from environmental insults.

Hair follicle stem cells contribute to reepithelialization during wound healing[112–116] and even preferentially differentiate into epidermal cells when expression of collagen XVII is decreased during skin ageing.[61,117] McSCs can also be recruited from the bulge to the epidermis upon wounding or UV radiation.[9–11,52] Recent study shows that forced activation of Wnt signalling in melanocytes promotes epidermal pigmentation during wound healing.[118] Epithelial β-catenin activation induces the follicular melanocytes to epidermis through the Endothelin signalling.[8] The regenerated hair follicles during healing of large wound are unpigmented.[119] Nevertheless, the regenerated hair follicle can turn to be pigmented when Endothelin is overexpressed.[58] SCF/c-kit signalling and melanocortin 1 receptor (Mc1r) guide the McSCs directly migrate from the hair bulge to epidermis and mediate their differentiation for epidermal pigmentation.

UV and ionizing radiation-induced DNA damage triggers McSC differentiation, leading to McSC exhaustion and hair greying.[8,11,120] Wnt7a secreted from UV-radiated epidermal keratinocytes activates β-catenin signalling in McSCs, which are differentiated to melanocytes that migrate into the injured epidermis for epidermal pigmentation.[11] Continuous activation of β-catenin signalling in McSCs promotes McSCs differentiation, exhaustion and premature hair greying.[8] Although DNA damage is irreparable, eukaryotic cells have the ability of self-protection and self-repair. In respond to DNA damage, eukaryotic cells immediately initiate the activation of ataxia-telangiectasia mutated (ATM) and ATR protein kinases signalling cascades. Deletion of ATM results in ectopic McSCs differentiation and deficiency of ATR also causes premature hair greying.[120,121] Additionally, deficiency of TTD and XPD, responsible for DNA damage repair, results in premature hair greying.[122]

10 | HYPOPIGMENTATION, LOOKING INTO THE RELATION BETWEEN THE CLINICAL CASES AND THE UNDERLYING FUNDAMENTAL PRINCIPLES

Hair greying is one of the prominent phenotypic signs of human ageing. About 50% hairs change to grey in 50% people around age 50. Loss of McSCs and melanocytes is demonstrated in ageing human scalp.[123] Destruction of McSCs niche, shortening of telomere[124,125] and oxidative damage-induced melanocyte death[126] directly promote grey hair formation.

Canities is a common hypopigmentation disease showing loss of hair pigment (Figure 4). Although canities can be caused by multiple factors such as deficiency in heredity, nutrition and mental state, there is no effective therapy currently. Loss of hair follicle melanocytes, inhibition of melanogenesis and overactivation of McSCs may be the causations for canities. Intriguingly, under certain conditions, grey hair can get repigmented.[127–129] A case study shows that a patch of heavily repigmented hairs is detected within the otherwise grey hair on the vertex scalp of a women.[129] Although skin examination shows multiple melanomas occur in the scalp, there is no infiltration

of melanoma cells in the repigmented hair follicles, indicating that McSCs may still reside in hair follicles but are not activated for generating pigment. Pigmented hairs turn grey after therapy for the scalp melanoma, indicating that melanoma cells are able to produce factors that induce human McSCs to differentiate into mature melanocytes for hair pigmentation.[127] Interestingly, in canities patients, the grey hairs remain cyclic growth, indicating that the signalling pathways regulating the HFSCs behaviour function well. This in turn suggests that the synchrony of activation or quiescence between HFSCs and MSCs can be uncoupled in certain pathogenetic conditions.

Vitiligo is another common hypopigmentation disease due to heredity, physical and mental stress, innate immune inflammation and T cell–mediated melanocyte destruction. The pathology phenotype of vitiligo shows a decreased number of melanocytes in vitiligo area in skin. Several therapeutic approaches have been developed to increase the number of functional melanocytes to treat vitiligo, though few approaches effectively retain complete repigmentation of vitiligo skin with long-lasting effect (Figure 4).

In the molecular level, both external and internal factors contribute to hair greying. The expression of pMel17, an early marker of melanocyte lineage, is significantly decreased in human hair bulge of patients between age 40 and 60 and negative in those between age 70 and 80.[130] MITF is colocalized with pMel17 in amelanotic melanocytes in the outer root sheath and hair matrix. These factors are absent in most hair follicles of human between age 80 and 90.[131] Loss of the key signalling molecules (eg, Bcl2) that maintain the survival of McSCs causes the deletion of McSCs and hair greying.[131] Forced activation or increased expression of Wnt signalling in McSCs induces exhaustion of McSCs and premature hair greying;[8,132] TGF-β type 2 receptor deficiency in hair follicle bulge causes incomplete maintenance of McSC quiescence, leading to mild hair greying in mice.[63]

HGF, SCF and End3 have been revealed to promote melanoblast or melanocyte proliferation and differentiation.[133,134] SCF, HGF and End3 are effective factors for preventing hair greying. Among these factors, SCF seems to function more effectively in preventing hair greying.[134] Indeed, inhibition of SCF/c-kit signalling by c-Kit antibody induces grey hairs formation through reducing the number of hair follicle melanocytes.[135] On the other side, continuous expression of SCF in epidermal keratinocytes is capable of rescuing the number of McSCs compromised in a Bcl2-null mouse, which displays a characteristic of loss of pigmentation.[136] This suggests that these extrinsic factors play crucial roles in regulating hair pigmentation through maintaining and regulating melanocytes behaviour. Thus, targeting the McSCs by modulating these signals in hair bulge potentiates curing hair greying disorders.

11 | CONCLUSION AND PERSPECTIVE

Melanocyte stem cells are a distinctive population of stem cells colocalizing with HFSCs in the hair follicle bulge niche and coordinating with HFSCs for regeneration of pigmented hair follicles. Complex external micro- and macroenvironments combined with internal factors regulate the quiescence and activation of the McSCs under physiological hair regeneration and pathological injuries. Among them, TGF-β, Notch, Shh and NFIB function as crucial regulators for quiescence of McSCs, while Wnt and Edn1/2 act as activators for proliferation and differentiation of McSCs. Emerging achievements in McSCs studies shed light on the hope for curing various pigmentation-related skin disorders such as canities, vitiligo, nevi and melanoma. Recruiting McSCs from hair bulge to the skin epidermis for epidermal pigmentation can be deemed as the fundamental conceptual idea for future therapeutic treatment of these disorders.

Nevertheless, several outstanding questions regarding McSCs and skin pigmentation remain. For example, McSCs originate from neural crest stem cells during embryonic development.[12] A population of neural crest stem cells also resides in hair bulge.[137] Is it feasible to induce neural crest stem cells in hair bulge to transdifferentiate these cells into melanocyte lineages for repigmentation of grey hair? During hair follicle regeneration at anagen, heterogenous hair follicle transit-amplifying cells differentiate into the hair follicle layers.[83] Melanocytes located at the hair matrix region also align adjacent to the DP. Does heterogeneity of melanocyte precursors exist during McSCs regeneration and is the heterogeneity involved in pigmentation of different hair layers? During catagen, can McSCs home back to the bulge niche as what HFSCs behave? During telogen, whether the McSCs in the bulge also secrete signals to support the balance of the niche environment, and influence HFSCs behaviours? How susceptible of McSCs is to their macroenvironment? Whether the McSCs migrated out from the bulge to the epidermis during skin injury create a new niche for their maintenance? How exactly are the levels of signals modulated to mobilize the inactivated McSCs for hair repigmentation without the risk of deleting the number of McSCs in the bulge? Whether the extrafollicular dermal McSCs are recruited to migrate to hair bulge for repigmentation of grey hair? As work continues at the intersection of McSCs and HFSCs biology, understanding these and other biological questions will be yielding exciting and even unexpected valuable insights into the pathological and regenerative medicine fields.

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CONFLICT OF INTEREST

The authors have declared no conflicting interests.

AUTHOR CONTRIBUTIONS

WQ, CMC and ML conceived and wrote the manuscript.

ORCID

Mingxing Lei https://orcid.org/0000-0002-4271-2714

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