Review

The biology of melanocyte and melanocyte stem cell

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The melanocyte stem cells of the hair follicle provide an attractive system for the study of the stem cells. Successful regeneration of a functional organ relies on the organized and timely orchestration of molecular events among distinct stem/progenitor cell populations. The stem cells are regulated by communication with their specialized microenvironment known as the niche. Despite remarkable progress in understanding stem cell-intrinsic behavior, the molecular nature of the extrinsic factors provided to the stem cells by the niche microenvironment remains poorly understood. In this regard, the bulge niche of the mammalian hair follicle (HF) offers an excellent model for study. It holds two resident populations of the SCs: epidermal SCs (EpSCs) [2,3], which cyclically regenerate the HF and make the hair and melanocyte SCs (McSCs) [4], which on differentiating into mature melanocytes are responsible for hair pigmentation. The McSCs are an attractive model for studying the SCs cellular function and differentiation for several reasons: first, the McSCs work as a single-cell unit. Secondly, they can be relatively non-invasively harvested from the skin. Thirdly, they have a distinctive differentiation product in the form of melanosomal organelles and melanin. In this review, I will summarize the recent advances in the studies of melanocyte, McSCs, and their regulation by the niche environment with focus mainly on the in vivo studies in mice.

Melanocyte Development

The precursor cells of the melanocytes called melanoblasts, arise from the neural crest in vertebrates. During embryogenesis, the melanoblasts migrate away from the neuroepithelium in an extraordinary journey with cycles of migration and proliferation to eventually populate the skin and the HF [5]. In mice, neural crest cells undergo two migration pathways. Those that migrate in the space between the somites and the neural tube (ventral pathway) give rise to neuronal and glial cells, whereas those that migrate between the somite and the non-neural ectoderm (dorsolateral pathway) differentiate into melanoblasts [6]. Studies indicated that this melanogenic fate is determined by the expression of Kit (the major tyrosine kinase receptor in the melanocytes) before the migration [7]. In addition, the importance of Wnt signaling on the neural crest-derived melanoblasts was also suggested. Wnt1, Wnt3a-deficient mouse have almost no Dct+ melanoblasts [8]. Studies in fish also have suggested that Wnt signaling, through activated β-catenin, promotes a fate decision toward the melanoblasts [9] (Fig. 1).

Melanoblasts reside in an area just near the neural tube, called the migration staging area, where they receive proliferation and survival signals from Kit-ligand (KitL), the
ligand of Kit. Between E8.5 (embryonic day) and E10.5, their migration is dorsolateral and induced by the transient expression of KitL in dermatome. After E10.5, they begin to progress ventrally toward the face, ventral abdomen, and the developing limbs. They also undergo a migration in the z-plane, as they emerge upward through the developing dermis and into the epidermis at around E13.5.

Over the next few days they enter the developing HFs. At birth, melanocytes are observed in both the epidermis and the HFs, but within a few days, epidermal melanoblasts disappear, leaving only those in the HFs, which differentiate and produce melanin by around P4 (post-natal day) [10–12]. As a consequence, the skin in the adult mouse is mostly unpigmented. In contrast, human melanocytes normally located in the bottom layer of the skin's epidermis, comprise from 5% to 10% of the cells in the basal layer of the epidermis [13].

To date, several genes have been identified which are associated with the coat-color abnormalities in mice, indicating defects in melanoblast migration, proliferation, and survival [5,14,15] (see also the useful website by the International Federation of Pigment Cell Societies, http://www.ifpcs.org). Some of these genes are also associated with the neural crest and melanocyte disorders in humans. Example of these genes include the transcription factors: Mitf [16–18], Sox10 [19,20]; Receptor tyrosine kinase Kit and its ligand KitL [21–24]; G-protein coupled endothelin-3 receptor/ligand pair [25,26]; Notch signaling [27]; small Rho GTPase Rac1 [28] and its activator P-Rex1 [29]; and actin bundling protein Fascin1 [30–32] and secreted protease of the ADAM family of the metalloproteinase ADAMTS20 [33].

Signaling to Kit via KitL is known to trigger proliferation and survival via activation of the Ras and downstream MAPK/ERK signaling [24,34–36], and has been speculated to be involved in motility [11,37]. The application of the Kit function blocking antibody to the mouse embryo was able to block proper melanoblast development [4,38]. Furthermore, ectopic expression of Kitl in the basal layer of the skin promotes the dermal melanoblasts survival [39].

Endothelin-3 signaling functions are necessary for the development of the melanoblasts. Endothelin-3 is not required for melanogenic fate specification, but is needed for the migration and survival of the melanoblasts between E10.5 and E12.5 [40], and is also required for melanoblast development in the epidermis beyond E12.5 [38]. Similar to Kitl., ectopic expression of endothelin-3 in the keratinocytes driven by the keratin 5 promoter also result in increased melanoblast proliferation and pigmented skin harboring dermal melanocytes, suggest an important role of endothelin-3 in Kit independent survival of the melanocytes [41].

While signaling drive melanoblast migration at different stages is being discovered, it is still not known how melanoblast migration is regulated at the molecular level. It is suggested that the reorganization of the actin cytoskeleton and cell–cell adhesion molecules is important for cell movement [42,43]. The interesting question is how do the melanoblasts regulate their actin structures to migrate within the tissue. Recent study using live ex vivo imaging of the skin showed that the melanoblasts navigate in the epidermis by using two classes of protrusion: short stubby protrusions and long pseudopods [28,44]. Rac1 positively regulates the frequency of initiation of the long pseudopods, which promote the migration speed and directional plasticity [28]. Fascin is an actin bundling protein, which is normally enriched in filopodia and invadopodia during cell migration in vitro.
[30,31]. Interestingly, fascin expression is transiently upregulated in the melanoblast and unregulated in the melanocytes [32], suggesting that fascin is required for efficient melanoblast migration. Indeed, in fascin-deficient mice, the melanoblasts migrate slowly and form less longer pseudopods [32]. More importantly, both Rac1 and fascin are dispensable for the formation of short stubs. It will be interesting in the future to study the mechanism of the formation of the short stubs.

**Discovery and Localization of the McSCs**

Melanoblasts start entering newly developed HFs from embryonic day 14.5 (E14.5) during embryogenesis. Once in the HFs, they are segregated into two populations: differentiated melanocytes that localize at the hair matrix and the McSCs that colonize at the bulge region of the HFs. How this process is regulated is currently unknown. In human skin, the melanocytes are maintained in the interfollicular epidermis, while in adult mouse skin, the melanocytes are located exclusively in the HFs [13]. The McSCs are quiescent cells that are located in the lower part of the permanent HF known as bulge niche [4,45]. The McSCs are unpigmented and were recently found to be Dct⁺, Pax3⁺, and Sox10⁺ [20,46,47]. In the initiation of a new anagen, the active phase of the HF cycle, the McSCs proliferate, and give rise to melanocyte progenitor cells. These transient amplifying cells are proliferative progenitor cells present in the outer root sheath. The melanocyte progenitors then differentiate to yield mature melanocytes, which are located in the HF bulb and which express all of the major melanogenic enzymes (Tyrp1⁺, Dct⁺, and tyrosinase⁺) [45,47], which produce melanin and transfer the pigment in the melanosomes to the adjacent keratinocytes.

The discovery of the McSCs comes from a study, where an anti-c-kit antibody (ACK2) when injected into C57BL/6 neonates resulted in the growth of white hairs and an associated decrease in melanocyte proliferation and differentiation with only Dct⁺ melanocytes left in the lower permanent part of the HF [4]. Despite this, subsequent hair cycles produced black hairs with normal numbers and patterns of melanocytes, showing that the McSCs in the bulge were SCF/kit independent and were able to proliferate, differentiate, and fully repopulate the entire melanocyte lineage at the onset of the new HF growth cycle.

**Regulation of the McSCs by the Niche**

Single-cell transcript analysis of the gene expression in purified McSCs cells and immunofluorescence has shown that the McSCs lack many of the markers seen in the melanoblast or the differentiated melanocytes such as Kit, Tyr, Tryrp1, Mitf, Ednrb, and Mlana, suggesting that the niche may maintain the SC population thus avoiding the loss of the SC pools, as well as promoting quiescence to prevent the overgrowth of the cells [47]. A histological analysis of the McSCs indicates their direct cell—cell contact with the surrounding EpSCs [4], suggesting the involvement of the cell-to-cell interaction in the regulation of the SCs. Although, the in vitro study suggested that the keratinocytes may provide a complex of paracrine factors and adhesion molecules for the melanocytes and play a dominant role in regulating melanocyte survival, proliferation, and differentiation (reviewed in [48]). The mechanism of the regulation of the McSCs by the bulge niche in vivo has emerged recently.

**Wnt signaling**

By using transgenic mice, Rabbani et al. [49] activated or blocked canonical Wnt signaling in either the epithelial or the McSCs through the expression of a constitutively active β-catenin, or via conditional knockout of the β-catenin. Interestingly, hair graying is induced when the β-catenin activity was either increased or decreased within the McSC. The expression of the active β-catenin induced differentiation of the McSCs in the bulge, phenotypically parallel to prior studies in which the SCs were depleted. Loss of β-catenin also led to hair graying, despite a different mechanism. The McSCs were not depleted but failed to differentiate into pigmented melanocytes. More intrinsically, stabilization of the β-catenin in the EpSC caused an extrinsic effect on the McSCs, which led to an increase in the proliferation of the melanocyte pool through an endothelin-dependent signaling mechanism. This elegant study, as well as earlier studies demonstrates the importance of the Wnt signaling in the McSCs proliferation and differentiation and further suggest that the activation of the McSCs is dictated at least in part by the signals directed by the EpSCs [8,9].

**Transforming growth factor-β signaling**

In addition to the Wnt signaling, other factors such as transforming growth factor-β (TGF-β), have been shown to regulate the McSCs quiescence and maintenance of an undifferentiated state [50]. The TGF-β is a signaling molecule that can regulate a number of cellular responses, such as cell growth, differentiation, and survival [51]. Nishimura et al. [50] showed that in the HF, the TGF-β proteins are expressed as the HF regresses during catagen. Exposure to the TGF-β results in cell-cycle arrest and immaturity in the in vitro melanocytes in vitro. In vivo conditional knockout of TGFβRII in the melanocyte lineage results in hair gray phenotype due to premature differentiation of the melanocytes in the bulge and the loss of the McSCs. The key transcription factors in the melanocytes such as MITF and PAX3, as well as melanin-associated enzymes such as tyrosinase are downregulated in response to TGF-β [50,52], suggesting that TGF-β signaling is a major mechanism for the
maintenance of the McSCs. Interestingly, TGF-β2 secreted by the dermal papilla results in elevated TGF-β signaling in quiescent EpSCs and induces tissue regeneration during the anagen phase which counterbalances the bone morphogenetic protein (BMP)-mediated repression [53]. This data suggest that the TGF-β signaling is likely have a dual function in both suppressing and fine-tuning the activation of the McSCs. The function of the TGF-β signaling in the McSCs activation during the anagen phase required further study.

**Nuclear factor I/B**
Recent study identified a transcription factor called Nuclear factor I/B (NFIB) in the EpSCs as a coordinator of SC behavior between the EpSCs and the McSCs [54]. The NFIB is a transcription factor that is required for lung and brain development and is often amplified and/or found on the oncogenic chromosomal breakpoints in epithelial cancers [55–57]. The NFIB was first detected in the epidermis at E14.5, concomitant with the upregulation of the established skin progenitors and later expressed in the EpSCs [58]. Intrinsically, the conditional knockout of the NFIB EpSCs promotes the McSCs proliferation and differentiation and production of melanin in the hair germ. The NFIB directly targets and suppresses the expression of endothelin-2, where the expression is aberrantly elevated in the NFIB-deficient HF SCs. Ectopically induced endothelin-2 mimics the NFIB-deficient phenotypes in the wild-type mice [54]. This study demonstrates how the EpSCs can prevent the McSCs differentiation by suppressing the expression of the extrinsic factors important for McSCs activation.

**Notch signaling**
The notch signaling pathway is a highly conserved cell signaling system in most multicellular organisms. Mammals possess four different notch receptors, which interact with specific ligands (Jagged 1 and 2, Delta-like 1, 3, 4) through the cell–cell interactions. Once activated, the Notch intracellular domain (NID) is cleaved by the gamma secretase [27]. The NID can disassociate from the membrane and translocate into the nucleus, where it interacts with the transcription factor called RBP-J in mice and activates the Notch downstream genes. Targeted deletion of the RBP-J results in a mixture of the pigmented and the unpigmented hairs due to the reduction of the melanoblasts and the follicular melanocytes by apoptosis during HF morphogenesis [59]. In the second HF cycle, the melanocytes completely disappeared from the HFs, thus rendering almost all of the hairs unpigmented. An examination of the skin in these murine models demonstrated that the Dct<sup>+</sup> cells in the bulge had been depleted, suggesting a permanent effect on the McSC population [59]. Similarly, the phenotype of the melanocyte conditional knockout of Notch1/2 is similar to that of mice with a melanocyte-targeted deletion of the RBP-J gene [60]. Deletion of one receptor in the melanocyte population led to a partial pigmentation defect, suggesting that Notch1 and Notch2 can partially compensate for each other. Together, these studies raise many interesting questions about how the Notch signaling pathway is regulated in the bulge niche, and what are the ligand-expressing cells that mediate the signal. One could imagine that the EpSCs will possibly play an important role in the Notch pathway thus regulates the self-renewal of the McSCs.

**Col17a1**
The EpSCs in the bulge niche also regulate themselves and the McSCs through the production of the extracellular matrix protein. The EpSCs highly express the hemidesmosomal transmembrane protein called Col17a1 (BP180/BPAG2) [61]. This protein attaches to the underlying basement membrane, and the deficiency of this protein leads to a reduction of the cellular anchorage, hair graying and loss, and follicular atrophy, [62–64]. Mice lacking Col17a1 show premature hair graying before bulk hair loss [61,65]. Col17a1 is critical to maintain the SC niche architecture within the bulge for normal maintenance of both the EpSCs and the McSCs [61]. This work provides an insight into the architecture of the follicular SC niche, and may provide the foundation for creating artificial niches for growing SCs ex vivo. Interestingly, it is shown that while the adjacent keratinocytes form mature hemidesmosomes in the bulge area, the MsSCs, however, lack hemidesmosome formation in the basement membrane zone [61]. Cell adhesion is important for asymmetric cell division and SC differentiation and self-renewal [66]. This raises an important question about how the MsSCs maintain their polarity during asymmetric cell division and what is the function of the adhesion molecule in the MsSCs? Conditional knockout of the adhesion molecules enriched in the MsSCs would be useful in answering this question.

**Wound and UVB irradiation-induced McSC activation**
The McSCs in the bulge can not only migrate to the hair bulb to be differentiated into mature melanocytes during the anagen phase, it can also move up to the epidermis following skin injury or Ultraviolet B (UVB) exposure. Interestingly, the McSCs migrate to the epidermis in a melanocortin 1 receptor (Mc1r)-dependent manner and differentiate into functional epidermal melanocytes in response to wound and UVB irradiation [67]. It was also demonstrated that under UVB exposure, the epidermal keratinocytes secrete Wnt7a to trigger the McSCs differentiation into the melanoblast through the activation of the Wnt signaling pathway. The expression of the Kit ligand in the epidermis induces the migration of the melanoblasts to the epidermis and differentiation into functional epidermal melanocytes, providing a pigmented protective barrier against ultraviolet irradiation over the damaged skin [68]. It is very likely that
these two signaling pathways are cross-talked and function together during this process. Overall, these findings will help in developing therapeutic technologies for vitiligo and other pigmentation disorders.

Conclusion and Perspectives

A picture of how the McSCs function and are maintained is starting to emerge. They are maintained and regulated in a distinct location of the lower permanent portion of the HF as bulge, and can be regulated by the EpSCs, the extracellular matrix proteins, and the extrinsic secreted factors from the EpSCs or the dermal papilla. The high volume of SC research shed a light on the hope for the SCs and their role in regenerative medicine [69]. The McSCs will provide new clues to understand the pathological conditions caused by the defects of the SC system including various pigmentation disorders, such as vitiligo, nevi, and melanoma. Melanoma is one of the most deadly forms of cancer, which causes a thousand deaths every year in western countries [70]. Future studies will reveal the exact origin of human melanoma and the correlation with the McSCs in the HFs. Understanding this biological system will be valuable to human medicine beyond its explanation of aging, aging-associated degenerative diseases, and cancer development.

References


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