A Dominant Role of the Hair Follicle Stem Cell Niche in Regulating Melanocyte Stemness

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Melanocyte and hair follicle bulge stem cells share a common niche. In this issue of *Cell Stem Cell*, Nishimura et al. (2010) show that hair follicle stem cells, by expressing high levels of TGF-β2, regulate the quiescence, the stemness, and the long-term renewal of melanocyte stem cells.

Melanocytes are neural crest-derived cells that colonize different tissues during development and are responsible for the pigmentation of skin epidermis and for the color of hairs and eyes (Lin and Fisher, 2007). The melanocytes and their pigments protect the genomic information of the different cell types located in the skin epidermis by absorbing the highly mutagenic UV irradiation of the sun that constantly assaults animals' bodies. The color of scales, feathers, and hair is also mediated by the integration of melanocyte pigments into these skin appendages. The ingenuity and the beauty of the various color assortments found across the animal kingdom help animals hide from predators and can influence their reproductive behavior. The maintenance of the melanocyte population is dependent on a population of melanocyte stem cells (MSCs) that are present in the bulge region of the hair follicle (HF). In this issue, Nishimura et al. (2010) reveal that TGF-β produced in this specialized niche regulates the function of MSCs in the HF.

The HF has the remarkable ability to alternate cycles of growth and degeneration throughout the life of an animal (Blanpain and Fuchs, 2009). After reaching a certain size, the HF stops proliferating and rapidly degenerates by massive programmed cell death in a stage called catagen. During this process, all cells of the lower HF, including the mature melanocytes and rapidly cycling transit-amplifying (TA) matrix cells, die by apoptosis. Multipotent HF stem cells (SCs) and the MSCs, which share a common niche located within the permanent portion of the HF called the bulge, however, escape this destructive stage. During the next stage of regeneration called anagen, HF bulge SCs and MSCs proliferate, migrate downwards, and give rise to TA matrix cells and TA melanocytes that differentiate to produce the colored hair shaft (Figure 1A; Blanpain and Fuchs, 2009; Nishimura et al., 2002).

Hair graying in both mice and humans is due to a progressive loss of MSC (Nishimura et al., 2005), and this process can be reproduced in mutant mice. For example, *Bcl2* null mice present a complete loss of MSC after HF morphogenesis resulting from failure to promote survival of the MSC when they re-enter their quiescent stage. Mice with a hypomorphic mutation in the *Mitf* gene, called $Mitf^{Vit}$, exhibit progressive hair graying secondary to the loss of bulge MSC that differentiate prematurely. Premature differentiation of bulge MSCs is also observed during physiological hair graying in mice and humans (Nishimura et al., 2005) and after DNA damage (Inomata et al., 2009).

A key question in SC biology is what are the intrinsic and extrinsic factors that regulate the fate and behavior of SCs within their niche. TGF-β signaling regulates diverse cellular functions such as growth arrest, apoptosis, migration, and differentiation. TGF-β2 is expressed at a high level and is active in HF bulge SCs, as demonstrated by the large fraction of quiescent HF bulge SCs that express nuclear phospho-Smad2 (Tumbar et al., 2004). Because MSCs and HF bulge SCs are in close contact within the same cellular niche, Nishimura and colleagues investigated whether TGF-β2 secretion by bulge SCs may regulate the function of MSCs (Nishimura et al., 2010). MSCs also express high levels of phospho-Smad2 in their nucleus when they re-enter the quiescent stage. Treatment of primary melanocytes, which contain a mixture of MSCs and more differentiated cells, with TGF-β increases the quiescence of melanocytes in vitro, and in the absence of mitogenic or survival factors, melanocytes treated with TGF-β underwent apoptosis. However, the presence of survival factors such as Kit ligand prevented apoptosis of melanocytes in response to TGF-β signaling. Moreover, TGF-β treatment of primary melanocytes induced the downregulation of Mitf, a transcription factor essential for melanocyte differentiation, and tyrosinase, a Mitf target gene and a rate limiting enzyme in melanin biosynthesis, suggesting that TGF-β maintains MSCs in an immature state by repressing key genes associated with melanocyte terminal differentiation. Interestingly, it has been recently demonstrated that UV irradiation represses the expression of TGF-β by keratinocytes, which in turn promotes melanocyte differentiation through the loss of *Pax3* repression mediated by TGF-β signaling (Yang et al., 2008). Altogether, these data are consistent with a role of TGF-β in promoting the quiescence of MSCs, as well as controlling their survival and inhibiting their differentiation (Figure 1B).

To address the functional relevance of these observations in vivo, the authors conditionally ablated the *tgbr2* gene, an essential coreceptor of the TGF-β signaling, in MSCs and their progeny ($Tgfr2^{fl/fl}/Dct^{CRE}$ mice) (Nishimura et al., 2010). These mice develop a mild but progressive hair graying phenotype, which correlates with the appearance of ectopically differentiated pigmented melanocytes with a dendritic morphology within the bulge SC niche (Figure 1B), a situation reminiscent of the MSCs observed in prematurely hair graying $Mitf^{Vit}$ mice

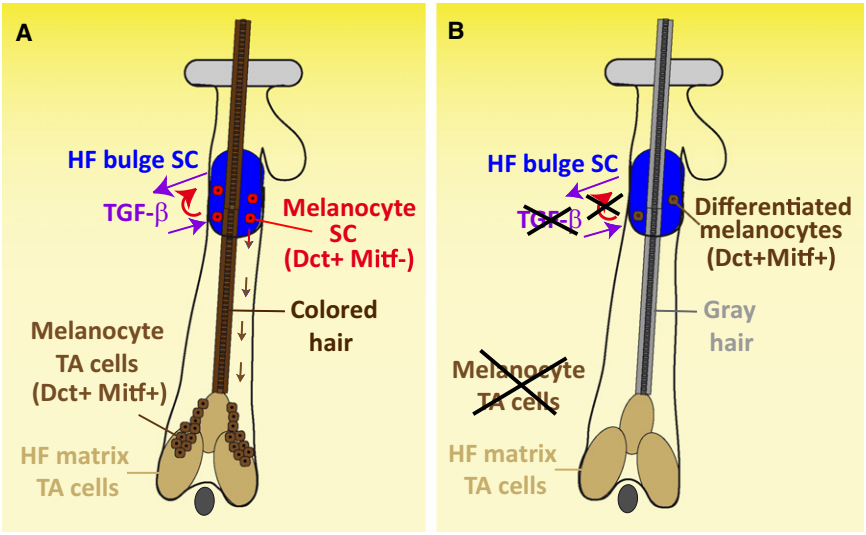

Figure 1. Loss of TGF-β Signaling Induces Premature Differentiation of Melanocyte Stem Cells within Their Niche

(A) During HF regeneration, HF bulge SCs (blue) and MSCs (red) give rise to TA cells (brown) that migrate downwards in the hair matrix, proliferate actively, and differentiate to form the new colored hair. The secretion of TGF-β by HF bulge SCs regulates the quiescence and the stemness of MSCs.

(B) TGF-β signaling may control the quiescence and immaturity of MSCs by directly regulating the expression of genes associated with cell cycle arrest and melanocyte terminal differentiation such as *Mitf*. In the absence of TGF-β signaling, MSCs undergo a premature differentiation within their niche, leading to MSC depletion and accelerated hair graying.

(Nishimura et al., 2005) or after DNA damage (Inomata et al., 2009). A complete depletion of MSCs from the bulge of unpigmented (gray) HFs, the matrix of which is devoid of melanocytes, is observed in $Tgfr2^{fl/fl}/Dct^{CRE}$ mice, suggesting that the progressive depletion of MSCs in the absence of TGF-β signaling precedes and is responsible for the hair graying phenotype (Figure 1B).

Given that MSCs in *Bcl2*-deficient mice exhibit massive apoptosis when the cells re-enter the quiescence stage (Nishimura et al., 2005) and also express high levels of nuclear phospho-Smad2 at this same point (Nishimura et al., 2010), the authors investigated whether TGF-β signaling might play a role in the SC depletion observed in *Bcl2* null mice. Administration of blocking TGF-β antibodies to *Bcl2* null mice inhibits apoptosis and MSC depletion at the time when MSCs re-enter their quiescent stage. These data suggested that TGF-β signaling promotes MSC apoptosis in the context of limited prosurvival factors, such as in the absence of *Bcl2*.

The incomplete and progressive hair graying phenotype observed when *Tgfbr2* is deleted in melanocytes, and thus the persistence of MSCs in some HF SC niches suggests that additional factors could regulate the maintenance of MSCs in the absence of this receptor. Consistent with this notion, the ablation of *Tgfbr2* in the epidermis did not lead to the permanent activation and/or depletion of HF bulge SCs (Guasch et al., 2007), whereas epidermal deletion of *Smad4*, a common downstream effector of TGF-β signaling, leads to the progressive loss of HF bulge SCs and alopecia (Yang et al., 2009). Future studies will be important to determine whether TGF-β directly regulates the quiescence of MSCs within their natural niche, whether Mitf and/or Pax3 are directly regulated by TGF-β to maintain the undifferentiated state of MSCs, what other factors present in the bulge SC niche regulate the quiescence and undifferentiated state of both HF and melanocyte bulge SCs, and whether TGF-β promotes the maintenance of adult SCs in other tissues. The absence of *tgfr2* in the epidermis leads to the development of squamous cell carcinoma in mice (Guasch et al., 2007), so it would be interesting to determine whether the premature differentiation of MSCs that is observed in the absence of TGF-β signaling (Nishimura et al., 2010) or after ionizing radiation (Inomata et al., 2009) acts as a tumor suppressor mechanism to eliminate overactive or damaged SCs.

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