

niche-stem cell communication (Schofield, 1978). These efforts will inform circuit-based approaches to modulate adult hippocampal neurogenesis and optimize memory processing in brain disorders characterized by cognitive and mood impairments.

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Location, Location, Location: Spatio-Temporal Cues That Define the Cell of Origin in Melanoma

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It is unclear whether melanoma initiates from mature melanocytes or stem cell precursors. In this issue of *Cell Stem Cell*, Moon et al. (2017) and Köhler et al. (2017) use *in vivo* lineage tracing to demonstrate that these two possibilities may occur downstream of the same pro-tumorigenic lesions, depending on environmental factors or the anatomical location.

Adult stem cells have been reported as the cell of origin in various cancer types (White and Lowry, 2015), but whether this is the case in cutaneous melanomas, the most aggressive form of skin cancer, is unknown. In this issue of *Cell Stem Cell*, two studies by Moon et al. (2017) and Köhler et al. (2017) report new lineage-tracing mouse models that provide insight into spatio-temporal cues defining melanoma initiation *in vivo*.

The need for functional analyses of melanocytic cells in their physiological niches has emerged from previous animal models. For example, live imaging in zebrafish has revealed melanomas resulting from de-differentiation to an early neural crest precursor, a feature conditioned by microenvironmental factors (Kaufman et al., 2016). A phenotypic switch involving loss of lineage specifiers has also been observed in genetically en-

gineered mice that exploit promoters of melanocytic genes, such as tyrosinase (Tyr) or dopachrome tautomerase (Dct), to recapitulate the disease's characteristic genetic alterations in oncogenes or tumor suppressors (i.e., Braf or Pten) (Pérez-Guijarro et al., 2017). While this de-differentiation reflects epithelial-to-mesenchymal transitions in human melanoma cells (Caramel et al., 2013), the precise nature of the cells of origin is unclear. Moreover, an acknowledged limitation of these mouse models is that the induction protocols to activate Braf and delete Pten (typically at neonatal stages) result in dermal lesions instead of the interfollicular epidermal-dermal presentation seen in most human cutaneous melanomas (Pérez-Guijarro et al., 2017). Another method, neonatal UVB irradiation, has also been reported to mobilize melanocytes to drive epidermal mela-

noma with a marked inflammatory component (Zaidi et al., 2011). However, whether mature melanocytes and stem cells play differential roles in adult mice is still not well understood.

Moon et al. (2017) exploit tamoxifen-inducible Tyr (Tyr::CreERT2) and doxycycline-regulated Dct (Dct-rtta) mouse strains to drive fluorescent reporters (*LSL-tdTomato* or *TreH2B-GFP*, respectively) for *in vivo* tracing of the melanocytic lineage. These animals were further used to generate *Tyr::CreERT2; Braf^{AV600E}* derivatives in the absence or presence of Pten (to monitor nevi or melanoma, respectively). Different protocols for depilation, or hair removal, and topical administration of tamoxifen were tested to assess the transformation potential of hair-follicle-associated melanoma cancer stem cells (MCSCs). With this approach, *Braf^{AV600E}; Pten^{-/-}* were found to induce dermal



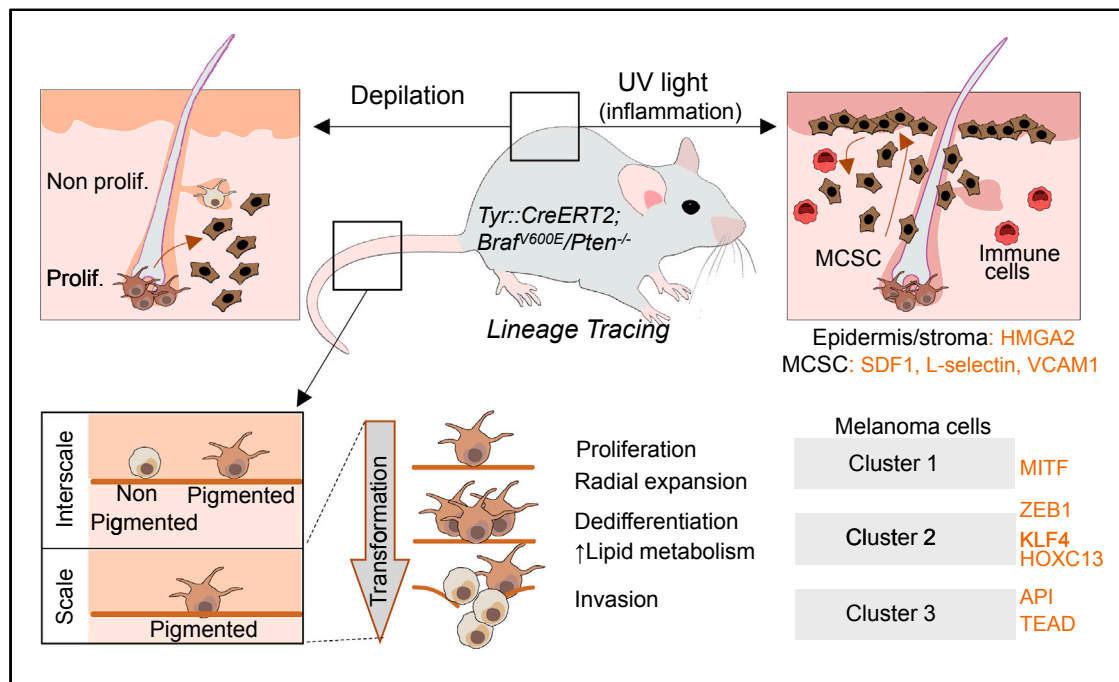


Figure 1. New Fluorescent Reporters for *In Vivo* Tracing of the Melanocytic Lineage Provide New Insights on the Cell of Origin of Melanoma
Differential outcome of two characteristic melanoma-associated genetic alterations (activation of oncogenic $Braf^{V600E}$ and $Pten$ loss) depends on the anatomical location (i.e., back versus tail skin) as reported by Köhler et al., or microenvironmental conditions related to UV irradiation as described by Moon et al. MCSC, melanoma cancer stem cell.

melanomas as previously described (Dan-kort et al., 2009), but only if MCSCs were in an activated stage. Interestingly, a single acute dose of UVB (180 mJ/cm²) had a dual effect: (1) favoring oncogenic transformation of MCSCs at any stage of the hair cycle, and (2) changing the histopathology of the melanocytic lesions (from dermal to epidermal growth; see Figure 1). Intriguingly, these UV-induced epidermal melanomas did not appear to de-differentiate as described in zebrafish or other mouse models (Caramel et al., 2013; Kaufman et al., 2016). RNA sequencing of td-Tomato-labeled MCSCs ultimately uncovered a series of pro-inflammatory signals driven by UVB. The relevance of UVB-driven inflammation in $Braf^{V600E}; Pten^{-/-}$ melanomas was demonstrated using TPA as an alternative inducer, and dexamethasone or sunscreens as blockers. Of note, additional roles of UVB in MCSC migration and melanoma development were found to be linked to the overexpression of the non-histone chromosomal high mobility group A2 protein (Hmga2) in the epidermis and the stroma. The specific mode of action of Hmga2 on MCSCs has yet to be defined, but it may be associated to various

proinflammatory cytokines (Figure 1, upper right panel).

Köhler et al. (2017) also exploit Dct-H2B-GFP and derivatives of Tyr::CreERT2 for lineage tracing of the melanocytic compartment. In addition to *LSL-tdTomato* and *LSL-YFP* reporters, these authors include *LSL-“Confetti”* strains for short and long-term analyses of the cell of origin and cell fate in $Braf^{V600E}; Pten^{-/-}$ -induced melanomas. Combining depilation with different time points of tamoxifen administration in the back skin, these authors independently demonstrate that MCSCs in the bulge were refractory to $Braf^{V600E}$ -driven melanomagenesis. Curiously, they visualized an additional population of mature and pigmented melanocytic cells in the hair follicle matrix that could be driven to transformation if appropriately activated after depilation. These activated mature melanocytes generated multiclonal hyperproliferative lesions that extended through the dermis.

Köhler and collaborators then tested other possible sources of mature melanocytic cells competent for transformation beyond the hair follicle. The tail was selected for its particular distribution of

melanocytic cells, wherein the interscale regions contain non-pigmented and pigmented melanocytes, while the scale areas are enriched in pigmented and dendritic melanocytes. Histological analyses combined with time-lapse *in vivo* microscopy revealed that tamoxifen induced a similar $Braf$ activation and $Pten$ loss in all these melanocytic cells in the adult mice (inferred by positive signals of the fluorescent lineage tracing used). However, only pigmented melanocytes in the scale areas were found to hyperproliferate, expanding in a radial phase through the epidermis. Interestingly, these activated melanocytes were then found to lose pigmentation markers (differentiate) as they became invasive (Figure 1, bottom panels). Single-cell RNA sequencing analyses of normal melanocytes and melanoma cells at early and late time points after development (i.e. before and after differentiation) revealed three main gene signatures. These were enriched in factors associated with cell proliferation (Cluster 1), dedifferentiation and lipid metabolism (Cluster 2), and cell invasion (Cluster 3), with distinct transcription factors in each category (Figure 1, bottom right panels). The downregulation of the

transcription factor MITF and the acquisition of ZEB1 are reminiscent of EMT-like phenotypic changes described in other mouse models and reflect analyses of transcriptomic profiles previously reported in human melanoma cells (Caramel et al., 2013).

Together, these two papers provide *in vivo* evidence of how microenvironmental cues shape the transformation potential of pro-oncogenic signals. In particular, the refractory nature of the MCSCs in the bulge, and the melanomas generated from adult mature melanocytes in the tail, suggest that stemness may not be an obligate requirement for malignant transformation in this disease. Perspectives for future studies are exciting. Considering the plasticity of the neural stem cell precursors that give rise to melanoblasts and mature melanocytes during development (Mort et al., 2015), it appears to be necessary to perform comprehensive kinetic and functional analyses of transcriptomic/proteomic profiles in melanocytic cells at different anatomical locations and in

response to intrinsic and extrinsic stimuli (including, but not limited to, oncogenes, UV radiation, or pro-inflammatory signals). In this context, it should be noted that the quest is still open for the identification of human melanocytic stem cells and the characterization of their role in dormant versus proliferative melanocytic lesions (Merlino et al., 2016). In any case, results in these two papers raise an important note of caution on the need for standardization of experimental procedures in the *Tyr::CreERT2* mice. Users should be aware of the markedly differential impact that timing and site of depilation and/or tamoxifen administration may have on the malignant potential of (epi)genetic alterations. After all, location matters.

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Vitamin C: C-ing a New Way to Fight Leukemia

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Metabolic cues and (epi-)genetic factors are emerging regulators of hematopoietic stem cell (HSC) potency. Two new studies in *Nature* and *Cell*, from Agathocleous et al. (2017) and Cimmino et al. (2017), respectively, show that vitamin C regulates HSC function and suppresses leukemogenesis by modulating Tet2 activity.

DNA methylation is an epigenetic modification that plays a critical role in hematopoiesis, controlling proper hematopoietic stem cell (HSC) self-renewal, and lineage differentiation (Jeong and Goodell, 2014). Dysregulation of DNA methylation leads to aberrant stem cell function and cellular transformation. Tet proteins have been identified as key players of DNA demethylation by acting as Fe²⁺ and α -ketoglutarate-dependent dioxygenases (Tahiliani et al., 2009). These enzymes catalyze the

oxidation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), which leads to DNA demethylation mediated by either replication-dependent dilution or base excision repair (BER). TET2 recurrently undergoes loss-of-function mutations in a wide range of myeloid and lymphoid malignancies (Jan et al., 2012). These lesions are early events in leukemogenesis and are associated with DNA hypermethylation, tumor progression, and poor patient outcome. In homeostasis,

the stability and activity of Tet proteins are regulated at multiple levels. Acting as a cofactor, vitamin C (also known as ascorbate) promotes the activity of Tet enzymes (Blaschke et al., 2013). Now, two new studies published in *Nature* and *Cell* provide mechanistic insights on how vitamin C regulates HSC frequencies and leukemogenesis by augmentation and restoration of Tet2 function, respectively (Figure 1) (Agathocleous et al., 2017; Cimmino et al., 2017).

