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## Superficial Spreading-Like Melanoma in *Arf<sup>-/-</sup>::Tyr-Nras<sup>Q61K</sup>::K14-Kitl* Mice: Keratinocyte Kit Ligand Expression Sufficient to “Translocate” Melanomas from Dermis to Epidermis

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### TO THE EDITOR

Being amenable to genetic modification, mice are critical *in vivo* tools to test the role of gene mutations in malignant melanoma (MM) development. They are the animal model of choice for studying MM growth and treatments either as autochthonous tumors or as xenografts. However, adult mice do not have epidermal melanocytes (MCs), and primary murine MMs are usually dermal, reminiscent of human malignant blue nevi (see, e.g., Dhomen *et al.*, 2009), animal type, or nodular MM. In contrast, most human MMs appear to originate in the epidermis, although they can progress to a vertical growth phase and invade the dermis. Although most murine MMs are dermal, lesions from the hepatocyte growth factor model often exhibit epidermal “pagetoid” spread on the albino (FVB) but not C57BL6 background (Noonan *et al.*, 2001; Florell *et al.*, 2007). Focal pagetoid spread is also seen in *Pten<sup>F/F</sup>::Tyr-CreERT2::LSL-Braf<sup>CA/wt</sup>* mice (Dankort *et al.*, 2009).

One factor that differentiates mouse from human skin is the expression of KIT receptor ligand (KITL), highly expressed in the human but not murine epidermis (Kunisada *et al.*, 1998; Longley and Carter, 1999). It is expressed in 76% of human MMs, not only in adjacent keratinocytes, but also sometimes in the tumor cells (Giehl *et al.*, 2007). Mice with engineered keratinocyte *Kitl* expression (*K14-Kitl*) have epidermal MCs throughout life

### CONFLICT OF INTEREST

HPS is a shareholder and consultant for Molemap, Australia. The other authors state no conflict of interest.

### SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/jid>

(Kunisada *et al.*, 1998). Remarkably, they do not develop MMs even after chronic UVR exposures (Yamazaki *et al.*, 2004). When crossed onto a DNA repair defective (*Xpa*-null) background, they developed lentigo-like lesions (Yamazaki *et al.*, 2005) after a harsh chronic UVR regimen. Although the physiological relevance of this study is questionable, as many animals died from severe sunburn, epidermal MMs did develop. Recently, *K14-Kitl* mice were crossed with animals carrying activation of the metabotropic glutamate receptor-1 (Abdel-Daim *et al.*, 2010), but only dermal MMs were reported.

We previously reported the development of dermal MMs in *Arf<sup>-/-</sup>::Tyr-Nras<sup>Q61K</sup>* mice (Ferguson *et al.*, 2010). These mice develop MMs with early age of onset (average of 114 days) after neonatal UVR. We hypothesized that the same mutations (in *Arf* and *Nras*) should lead to the development of epidermal MMs in mice with epidermal MCs. *Arf<sup>-/-</sup>::Tyr-Nras<sup>Q61K</sup>::K14-Kitl* mice were exposed to a single neonatal UVB treatment and followed for the development of MMs. Experiments were undertaken with institute animal ethics approval. As with *K14-Kitl* mice, we observed MCs along the basal layer of the compound mutants (Figure 1a). The hyperpigmented skin was bleached, and histopathological analysis revealed nests of MCs in the epidermis and scattered MCs in the dermis (Figure 1b). We confirmed the epidermal localization of MCs by staining with anti-S100 or anti-Tyrp1 antibodies and anti-keratin 14 to stain keratinocytes (Figure 1c). The presence of the increased numbers of MCs singly and in small nests in all levels of the epidermis, some of which stained for Ki-67 (Figure 1d), is suggestive of early epidermal MM. As MCs do not normally remain in the adult murine epidermis, we wanted to determine how long they survive there when supported by constitutive *Kitl* expression. We performed label retention experiments beginning with injection of *K14-Kitl* neonates with BrdU. When skin was examined 8 weeks later, many epidermal MCs still carried label (Supplementary Figure S1a and b online), indicating that they are slow cycling and can remain for long periods in the epidermis. This is consistent with the MC nests emanating from expansion of these epidermal MCs rather than invasion of dermal MCs.

By eye, it was not possible to discern the MC nests from “normal” skin because of its hyperpigmented nature, but slightly elevated plaques were also observed (Figure 1e–k), developing at an average age of onset of 100 days. Although this is earlier than *Arf<sup>-/-</sup>::Tyr-Nras<sup>Q61K</sup>* lesions (Supplementary Figure S2 online), the difference may be because of strain differences between the cohorts. Critically, whereas *Arf<sup>-/-</sup>::Tyr-Nras<sup>Q61K</sup>* mice developed deep dermal lesions clearly separated from the epidermis by layers of collagen (Figure 2a), the plaques developing in *Arf<sup>-/-</sup>::Tyr-Nras<sup>Q61K</sup>::K14-Kitl* animals exhibited an epidermal pagetoid growth pattern with epidermal hyperplasia (Figure 1g–k), as well as spread within the adjacent dermis (Figure 2b). The deeper highly pigmented dermal portion of the plaques contains mostly melanophages interspersed with scattered MCs (Figure 1h). As atypical MCs are present within all levels of the epidermis, and to some extent beneath the epidermis in the papillary dermis, there are clear similarities between these murine lesions and human superficial spreading MMs (Figure 2c).

We also examined skin from other anatomical locations (Supplementary Figure S3 a–i online). In the ears and tail, we saw slightly atypical MCs along the entire dermoepidermal junction and to some degree in the dermis. The effect of keratinocyte *Kitl* expression was especially noticeable in the footpads (Supplementary Figure S3 g–i online). In *Arf<sup>-/-</sup>::Tyr-Nras<sup>Q61K</sup>* mice, the footpads are white with no MCs present (data not shown). However, *Arf<sup>-/-</sup>::Tyr-Nras<sup>Q61K</sup>::K14-Kitl* mice have black footpads, and histopathology shows MCs singly, and as nests, throughout the epidermis. We did not observe visible plaques on any anatomical location other than the dorsal skin.

A common criticism of mice as MM models is the dermal localization of lesions. Using a dermal murine MM model, we show that overexpression of just one cytokine in keratinocytes—Kitl—essentially translocates MMs into the epidermis, where they exhibit nesting and pagetoid spread. Hence, it is likely that many aspects of transformation of murine MCs are similar to those occurring during human MM progression. It is noteworthy that there are no good mutation signatures that differentiate superficial spreading and nodular MMs. At least in our experimental model, the same mutations (a complement of mutations commonly present in human melanomas that together with neonatal UVR are sufficient for MC transformation) can induce both MM subtypes. The growth pattern/localization of the lesions is totally controlled by a keratinocyte cytokine. The development of better methods of specifically targeting mutations to epidermal MCs in *K14-Kitl* mice should engage the full power of the mouse as a preclinical model to study the mechanisms of epidermal MM growth. Such models, along with the one we report in this study, will facilitate improved *in vivo* modeling of gene–environment interactions in MM carcinogenesis in adult mice.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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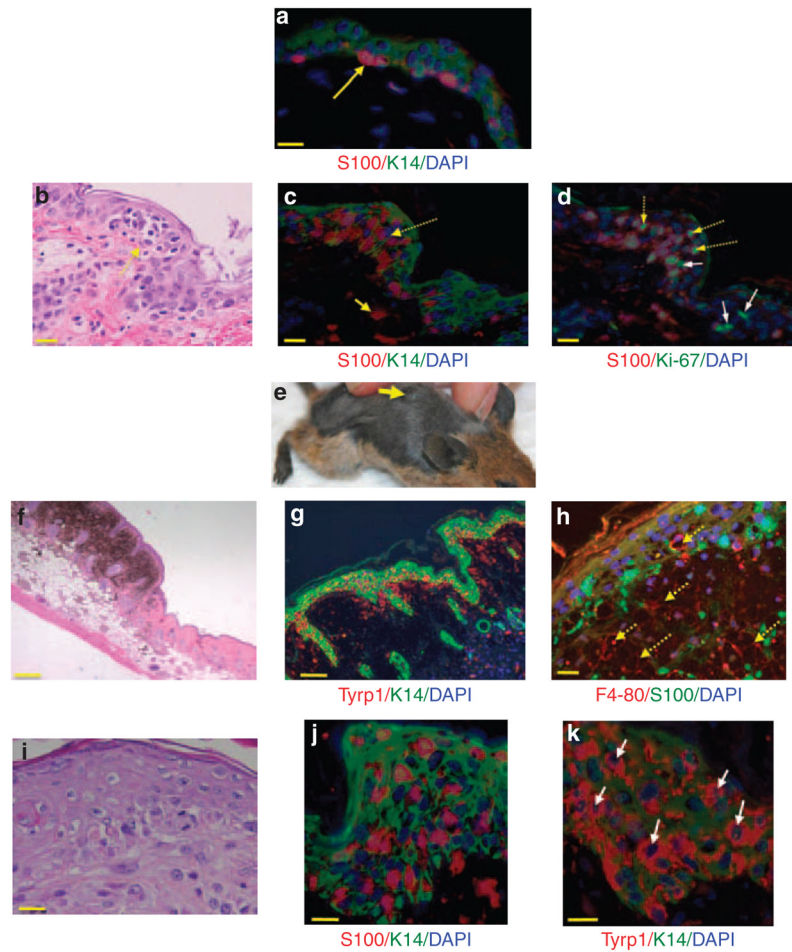
## Abbreviations

MC	melanocyte
MM	malignant melanoma

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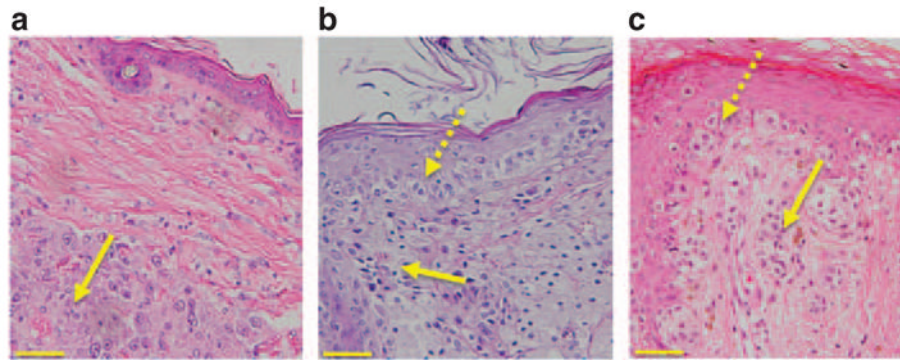
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**Figure 1. Histopathology of superficial spreading melanoma equivalents in  $Arf^{-/-}::Tyr-Nras::K14-Kitl$  mice**

(a) Double-label immunofluorescence (IF) staining for S100 and K14 on “normal”  $Arf^{-/-}::Tyr-Nras::K14-Kitl$  mouse skin (methods fully described in Supplementary Material online). Briefly, sections of formalin-fixed and paraffin-embedded skin were stained with anti-S100 (Dako, Eli, UK) primary antibody, followed by an AlexaFluor 555-labeled donkey anti-rabbit secondary antibody (Jackson ImmunoResearch Laboratories, West Grove, PA). After several washes, we stained keratinocytes using anti-K14 (Dako) followed with AlexaFluor 488-labeled donkey anti-rabbit (Jackson ImmunoResearch Laboratories). The yellow arrow points to red S100 staining MCs sitting at the dermoepidermal junction. Scale bar = 20  $\mu$ m. (b) Hematoxylin and eosin (H&E) staining of bleached formalin-fixed, paraffin-embedded sections of  $Arf^{-/-}::Tyr-Nras::K14-Kitl$  mouse skin. Bleaching is described in Supplementary Methods online. Note cluster (nest) of atypical MC cells in the epidermis, indicated by a dotted yellow arrow. Scale bar = 20  $\mu$ m. (c) IF staining of a similar portion of epidermis with a dense cluster of S100-positive epidermal MCs (indicated by dotted arrows). Solid yellow arrow points to a dermal MC. Scale bar = 20  $\mu$ m. (d) Epidermal MC cluster stained for S100 and Ki-67. Dotted yellow arrow shows MCs in the suprabasal epidermis positive for Ki-67. Solid white arrows denote keratinocytes (S100-negative cells) staining for Ki-67. Scale bar = 20  $\mu$ m. (e)  $Arf^{-/-}::Tyr-Nras::K14-Kitl$  mouse with a slightly elevated pigmented plaque on its back. Note that because of the heavy pigmentation of the whole skin, the melanoma is not easily discernible in this photograph. Mouse background: FVB, two generations down C57BL6. (f) H&E-stained section showing

raised plaque. Scale bar = 200  $\mu\text{M}$ . **(g)** IF staining of a *Arf*<sup>-/-</sup>::*Tyr-Nras*::*K14-Kitl* plaque for Tyrp1 (red) and K14 (green). Scale bar = 100  $\mu\text{M}$ . **(h)** Double-layer IF image of a pigmented plaque staining for F4/80 (red) and S100 (green). Dotted yellow arrows denote macrophage lineage cells (melanophages) in a “cobblestone” pattern. The pigmented part of the tumor consists of many melanophages interspersed with some melanocytes/melanoma cells. Scale bar = 20  $\mu\text{M}$ . **(i)** Higher-power H&E image of MC nesting in the thickened epidermis within a plaque. Scale bar = 20  $\mu\text{M}$ . **(j)** Higher-power image of IF staining of S100-positive epidermal MCs within an epidermal melanoma. K14-positive keratinocytes are green. Scale bar = 20  $\mu\text{M}$ . **(k)** Double-label IF staining for Tyrp1 using the PEP1 antibody, a gift from Dr Vince Hearing. K14-positive keratinocytes are green. Whereas S100 IF staining somewhat obscures the 4,6-diamidino-2-phenylindole (DAPI)-stained nuclei, Tyrp1 staining allows better visualization of MC nuclei of varying sizes and shapes (indicated by white arrows). Scale bar = 20  $\mu\text{M}$ .



**Figure 2. Comparative histopathology of murine and human epidermal melanomas**  
**(a)** Hematoxylin and eosin (H&E)-stained section showing a deep dermal malignant melanoma (MM) in an *Arf*<sup>-/-</sup>::*Tyr-Nras* mouse. The yellow arrow denotes a nodular melanoma composed of sheets of pleomorphic melanocytes (MCs). This nodular lesion is clearly separated from the epidermis by layers of collagen. **(b)** MM from an *Arf*<sup>-/-</sup>::*Tyr-Nras*::*K14-Kitl* mouse. Dotted yellow arrow denotes atypical MCs in the epidermis, and filled yellow arrow atypical MCs in the dermis. There is an increased number of MCs, varying in size and shape, not only at the dermoepidermal junction but also at all levels of the epidermis. **(c)** Human superficial spreading MM. Dotted yellow arrow denotes atypical MCs in the epidermis, and filled yellow arrow atypical MCs in the dermis. The morphological similarities between Figure 2b and c are striking. Scale bars = 100 μM.