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Expression of oncogenic BRAF^{V600E} in melanocytes induces Schwannian differentiation *in vivo*

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Dear Editor

Although melanocytes represent one of several terminally differentiated neural crest-derived lineages, they exhibit a surprisingly high degree of phenotypic plasticity (Adameyko et al., 2009, Real et al., 2006). Interestingly, the cell fate switching in melanocytes can be observed *in vivo* under pathologic conditions including nevi, as nevus cells phenotypically resemble Schwann cells in multiple ways (Aso et al., 1988). However, it is unclear whether the phenotypic switch occurs coincidental with nevus formation or whether it functionally contributes to the tumor suppressive effect of nevogenesis.

BRAF^{V600E} is the most prevalent mutation in melanoma (Girotti et al., 2014). Our laboratory generated a transgenic mouse in which *BRAF*^{V600E} is expressed in melanocytes (Goel et al., 2009). The *BRAF*^{V600E} mice exhibited skin hyperpigmentation, melanocytic hyperplasia and developed melanomas at a low frequency; moreover, metastatic melanomas can be induced in combination with *Cdkn2a*^{+/-}, *Tp53*^{+/-}, *Arf*^{+/-} or UV irradiation (Luo et al., 2013).

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Two founder lines of the transgenic mice with high and low *BRAF*^{V600E} expression, denoted 470 and 476, respectively, were backcrossed to C57Bl/6 mice. The resultant progeny retained the phenotypes observed in the mixed background, with profound hair graying being prominent (Figure 1A). Histology confirmed accumulation of pigmented cells in cutaneous regions and deep dermis, consistent with elevated pigment contents (Figure S1A-B). The premature hair graying phenotype, which was observed as early as postnatal day 7 (P7, Figure S1C), was a result of white and black hair mixture (Figure S1D). Hair pigmentation is a direct contribution of hair follicle melanocytes. Using tyrosinase-related protein-2 as a lineage tracker, we found *BRAF*^{V600E} melanocytes displayed a developmental defect in proper localization, leaving the majority of hair follicles devoid of melanocytes, and thereby melanin, in aged skins (Figure S2A-B).

Although *BRAF*^{V600E} melanocytes undergo hyperproliferation (Figure 1B), spontaneous melanomas are rare. Oncogene-induced senescence undoubtedly serves as the primary barrier to malignant transformation (Dhomen et al., 2009); however, not all *BRAF*^{V600E} melanocytes displayed a senescent phenotype (Figure 1C, arrowhead). Instead, ectopic melanocytes in *BRAF*^{V600E} skins emerged as cutaneous masses, which were initially detected in P13 subcutis and became evident near hair follicle bulk at P22 (Figure 1D, arrowhead). These masses progressed over time to form intermediately to highly pigmented tumors in both superficial dermis and subcutis (Figure 1D, circle). Morphologically, the masses contained spindle and epithelioid cells forming oval or polygonal nests and fascicles with smooth and defined boundaries, reminiscent of Schwannian differentiation. Histopathologically, the masses resembled hyperpigmentation overlying (plexiform) neurofibroma, a benign nerve sheath tumor derived from Schwann cells occurring frequently with *neurofibromin 1* (*Nf1*) inactivation.

Despite *Nf1* transcript was unaffected, chronic activation of *BRAF*^{V600E} significantly triggered several Schwannian markers in melanocytes, suggesting oncogenic *BRAF*^{V600E} reprograms melanocytes toward the Schwannian lineage (Figure 1E-F). This Schwannian phenotype was indeed a consequence of the *BRAF*^{V600E} *in vivo*, as all the masses stained positive for *BRAF*^{V600E} protein (Figure 1G). This finding was not an artifact of insertional effects in the 470 *BRAF*^{V600E} line, as similar tumors were present in the 476 line in the context of *Arf* or *Cdkn2a* loss (Figure 1H), and Schwannian-like differentiation was also reported in other melanoma models in which the *BRAF*^{V600E} is expressed from the endogenous locus (Marsh Durban et al., 2013, Damsky et al., 2015). Intriguingly, the Schwannian masses seldom progressed to malignancy, remaining benign for the life-span of the animals, suggesting the trans-lineage differentiation between melanocytes and Schwann cells is tumor suppressive, supplementing senescence to inhibit melanoma formation in response to *BRAF*^{V600E}. Indeed, features characteristic of Schwann cells can be detected in human nevi, while invasive melanoma cells never adopt a Schwannian phenotype (Aso et al., 1988, Reed et al., 1999).

It is likely that melanocytes driven into the Schwannian differentiation “trap” by *BRAF*^{V600E} must be diverted back to the melanocytic pathway before they can undergo malignant transformation. AKT is likely to play a key role in this process, as AKT activation has been reported to retard Schwann cell differentiation (Sherman et al., 2012), whereas

AKT activation positively correlates with melanoma progression. Interestingly, we found that active AKT was absent in the BRAF^{V600E} precancerous lesions containing Schwannian masses but abundant in melanomas (Figure 2A-B). We also observed that during progression of BRAF^{V600E} melanocytes to melanoma, expression of Schwannian markers was drastically reduced (Figure 2C), indicating suppression of the Schwannian phenotype occurs concomitant with transformation. Furthermore, when we treated BRAF^{V600E} mouse melanoma cells with AKT inhibitor, Schwannian markers were significantly elevated (Figure 2D), confirming AKT suppression enhanced Schwannian differentiation of melanoma cells.

Taken together, oncogenic BRAF^{V600E} in melanocytes, in addition to instigate senescence, rewires the schwannian differentiation program, leading to benign nerve sheath tumors. As such schwannian tumors can remain benign for the life-span of the animals, they represent a dead end for oncogenic BRAF^{V600E} thereby functioning as a novel melanoma suppressive mechanism. An individual BRAF^{V600E} cell, in which AKT is hyperactivated, will downregulate the Schwannian program, and presumably reactivate the melanocytic pathway to arise as a tumor focus, ultimately leading to melanoma initiation (Figure 2E). This hypothesis not only provides another layer of functionality to AKT in melanomagenesis, namely, lineage switching from the tumor-suppressive Schwannian pathway to the tumor-prone melanocytic lineage, but also emphasizes the high degree of phenotypic plasticity of melanocytes. Thus, deciphering the mechanisms that regulate the plasticity and maintenance of neural crest-derived differentiated cells is likely to be an important step towards understanding mechanisms of melanoma progression.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Schwannian marker glial fibrillary acidic protein (Gfap) in primary melanocytes from WT and BRAF^{V600E} mice. Data represent mean $\pm$ SD. n=3, *p<0.05, **p<0.01, student t-test. (G) IHC staining of human BRAF^{V600E} (VE) in skins from age-matched WT and BRAF^{V600E} mice. (H) H&E staining of skins from BRAF^{V600E} mice (476 line) with or without deletion of Arf or Cdkn2a. Bars represent 100 μ m if not otherwise indicated.

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