Advanced stage melanomas are refractory to most available therapies including recently approved targeted therapies. This is also true for most biological (IFN-α or IL-2) treatments and therapy involving adoptive transfer of melanoma-reactive T cells (de Souza et al., 2012). A number of mechanisms have been proposed to explain the lack of T-cell functional activity in vivo. Some of these mechanisms include emergence of antigen-loss tumor variants, induction of immune inhibitory molecules such as CTLA4 or PD1 and induction of T-reg (regulatory) cells or myeloid-derived suppressor cells (MDSC) or presence of immune-suppressive cytokines such as TGF-β or IL-10 (Grivennikov et al., 2010). A number of environmental factors can further contribute to genetic instability and induce epigenetic changes of progressing heterogeneous tumors. Presence of inflammatory factors at the tumor site can cause selection of preexisting tumor cell variants resulting in preferential growth of antigen-negative tumor variants or tumor cells that have lost MHC-class I molecules (Khong and Restifo, 2002). Immune editing or alterations of tumor antigens due to immune pressure can also be one of the reasons for lack of T-cell-mediated tumor cell death in vivo (Singh and Paterson, 2007).

In clinical trials, adoptive cell therapies (ACT) with melanoma-reactive tumor-infiltrating lymphocytes (TILs) or polyclonal T cells engineered to express chimeric antigen receptor (CAR) with re-directed specificity to melanoma have shown great promise with dramatic regression of bulky tumors (Robbins et al., 2011). However, in most patients, initial regression of tumor cells was followed by appearance of new lesions with therapy resistant melanoma cells (Robbins et al., 2011). Until now, the exact nature of ACT-related tumor resistance and the cause of emergence of antigen-negative melanoma cells were largely unknown.

In the current edition of Nature, Landsberg et al. describe a novel mechanism of T-cell-induced loss of melanocytic antigens as a possible cause of tumor cell resistance to ACT. To demonstrate therapy resistance, the authors have used a previously published genetically engineered hepatocyte growth factor (Hgf)-CdK4R24F mouse melanoma model and anti-gp100-specific CTLs obtained from a TcR transgenic pmel-1 (gp100) mouse model. TNF-α, a pro-inflammatory cytokine predominantly secreted by activated T cells caused down-modulation of gp100 antigen expression on the mouse melanoma cells resulting in loss of recognition by anti-gp100-specific CTLs used in ACT. TNF-α also down-modulated pigmented-related gene expression and tyrosinase-related protein (TRP)-2 on melanoma cells. Interestingly, the pro-inflammatory environment of TNF-α secretion was sufficient to down-modulate the expression of non-melanocytic antigens. On the contrary, there was an increased expression of nerve growth factor receptor (NGFR) on melanoma cells. Down-modulation of gp100 expression was reversible when the activity of TNF-α was neutralized or the resistant tumor cells were re-transplanted into a normal mouse lacking an inflammatory environment. The mechanism of non-genetic reversible antigen down-modulation is novel and has not been described before. This is in contrast to other genetic heterogeneity and immunoselection-related mechanisms described earlier. Understanding inflammation-induced plasticity and the non-genetic heterogeneity of melanoma cells are critical for the development of new therapeutic strategies for melanoma.

The authors then corroborated their mouse studies using human melanoma cell lines where they report similar down-modulation of gp100 and MART-1/Melan-A expression in the presence of TNF-α. Similar to mouse studies, human melanoma cell lines also showed up-regulation of NGFR expression in the presence of TNF-α. This was confirmed in melanoma patients’ tumor tissue sections with abundant infiltrating immune cells further supporting the clinical relevance of the above findings.

The results of Landsberg et al. have important clinical implications if the phenotypic plasticity of melanoma cells in an inflammatory microenvironment is validated in a larger cohort of patients with melanoma after ACT. Down-modulation of gp100 expression and concurrent up-regulation of NGFR expression of melanoma cells in an inflammatory environment raises the possibility of using ACT with a cocktail of CAR-engineered T cells with dual or multiple specificities to target more than one melanoma antigen to ensure complete regression of tumor lesions. For this, a personalized approach of analyzing melanoma antigen expression on tumor cells before and after ACT therapy will be essential for the selection of CAR-engineered T cells with defined tumor antigen specificity. The proposed approach may enhance the long-term disease-free survival of patients with metastatic melanoma.

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**Decayed melanoma proliferation and cell survival: turn down your SOX10**

Stacie K. Loftus
e-mail: sloftus@mail.nih.gov

Many transcription factors involved in early developmental lineage specifica-
tion, cell proliferation, and survival are also found at key regulatory nodes controlling similar functions in adult differentiated cell populations. SOX10 and its downstream target MITF, key lineage-defining transcription factors found in melanocytes, are such examples. Both were initially identified by their fundamental roles in melanocyte development. Mutation of either gene causes a reduction in melanocyte cell numbers during development, resulting in the hypopigmentation phenotype of Waardenburg syndromes type 4C (OMIM# 613266) and type 2A (OMIM #193510), respectively. In differentiated melanocytes, subtle variations in MITF levels are known to affect melanocyte proliferation and survival; however, the role of SOX10 in the regulation of tumor cell proliferation and survival is only now being appreciated.

Shakhova et al. recently evaluated the role of SOX10 in melanin cell proliferation and tumor formation in a mouse model for the developmental melanocytic abnormalities known as giant congenital nevi (Tyr:: Nras<sup>G61K</sup>l<sup>nrk4a</sup>−/−). Histological analysis of giant congenital nevi from both human patients and Tyr:: Nras<sup>G61K</sup>l<sup>nrk4a</sup>−/− mouse skin found ectopic pigmented cells located in der-
mal regions surrounding hair follicles. Patients with congenital nevi often have NRAS mutations rather than BRAF

mutations, and the presence of giant congenital nevi is also correlated with an increased risk of developing mela-
noma during childhood and adolescence (Krengel et al., 2006). Similarly, Tyr:: Nras<sup>G61K</sup>l<sup>nrk4a</sup>−/− mice exhibit hyperpig-
mentation of the ears, paws, snout, and back skin, and 90% of these mice show melanocytic tumors by 6 months of age. In isolating KiT<sup>–</sup> flow-sorted cells from the Tyr:: Nras<sup>G61K</sup>l<sup>nrk4a</sup>−/− model, Shakhova et al. observed that Sox10 expression levels are increased in comparison with normal control melanocytes. Given these findings, they asked whether lowering Sox10 levels within the context of the Tyr:: Nras<sup>G61K</sup>l<sup>nrk4a</sup>−/− animal model would attenuate both congenital nevus formation and, at later ages, melanoma tumor formation. In crossing to Sox10<sup>−/−</sup> mice, in which one copy of the Sox10 coding sequence is replaced with LacZ coding sequence, Tyr:: Nras<sup>G61K</sup>l<sup>nrk4a</sup>−/− Sox10<sup>−/−</sup> animals that exhibited a visible reduction in pigment on back skin, snout and paws were generated. Furthermore, haploin-
sufficiency for Sox10 in these mice also dramatically reduced tumor formation when assessed at 6 months and compared with Tyr:: Nras<sup>G61K</sup>l<sup>nrk4a</sup>−/− animals. Histological analysis demonstrated a reduction in the ectopic dermal hyperproliferation surrounding hair follicles. Cell proliferation, as measured by DCT- and Ki67-positive cells in the hair follicle, was also rescued to near normal levels by reduction of Sox10. A second genetic cross utilized Sox10<sup>−/−</sup> TYR-CreERT2 mice, to condi-
tionally knockout one copy of Sox10 in 2-month-old Tyr:: Nras<sup>G61K</sup>l<sup>nrk4a</sup>−/− animals. When evaluated at 12 months, even though reduction of Sox10 occurred subsequent to establishment of the melanocyte lineage, rescue was again observed, as measured by visible

reduction in back, snout, and paw skin pigment and lessening of the ectopic hyperproliferation of DCT+ dermal cells. These results highlight that a threshold for Sox10 expression must be reached for the proliferation, maintenance, and survival of melanocytes in the Tyr:: Nras<sup>G61K</sup>l<sup>nrk4a</sup>−/− model.

As Sox10 expression levels were found to be critical for melanocyte sur-
vival in the murine Tyr:: Nras<sup>G61K</sup> cells in vivo, Shakhova et al. next reduced Sox10 levels in human melanoma cell lines using RNAi. This led to a dimin-
ished colony-forming ability of these cells both in vitro and in vivo, when injected subcutaneously in nude mice. Knockdown also increased the propor-
tion of cells in the G1 phase of the cell cycle and increased numbers of Annex-
in-V staining cells. When Sox10 levels are reduced and microarray analysis performed at 48 and 96 hr, expression of known direct transcriptional targets, such as TYR, DCT and MITF, was decreased (GEO accession, GSE37059). These same shSox10 cells also had a significant decrease in expression levels for cyclins and CDKs, consistent with the G1 arrest observed, while a cohort of genes representing an apoptotic response were increased as compared to controls.

In addition to the observed effects on genes regulating proliferation and sur-
vival, knockdown of Sox10 in mela-
noma cell lines also resulted in a decrease in the number of neural crest stem cell marker CD271+ (p75NTR) cells. This is interesting given that both Sox10 and CD271+ primary tumor expression has been correlated with an increase in tumor metastatic potential (Civerni et al., 2011). CD271+ expres-
sion has also been proposed as a mar-
ker for ‘melanoma initiating cells’ (Boiko et al., 2010; Civerni et al., 2011). Sox10 thus becomes one regulatory