**BRAF-targeted therapy alters the functions of intratumoral CD4⁺ T cells to inhibit melanoma progression**

Ping-Chih Ho¹, Susan M Kaech¹,², *  

¹Department of Immunobiology; Yale University School of Medicine; New Haven, CT USA; ²Howard Hughes Medical Institute; Chevy Chase, MD USA

**Keywords:** BRAF, melanoma, T cell, CD40L, IFNγ

**Abbreviations:** DAMP, damage-associated molecular pattern; MDSC, myeloid-derived suppressor cell; TAM, tumor-associated macrophage; TIDC, tumor-infiltrating dendritic cell; Treg, regulatory T cell

The establishment of an immunosuppressive tumor microenvironment is a hallmark feature driving cancer cell evasion of immunosurveillance. In a murine melanoma model, we recently demonstrated that decreased intratumoral CD4⁺ T-cell expression of CD40L and interferon γ (IFNγ) is critical to maintain this immunosuppressive microenvironment. Altered effector functions of tumor-associated CD4⁺ T cells is essential for B-Raf⁶⁰⁰E inhibitor-mediated restoration of antitumor immunity.

Oncogenic mutations in the gene encoding BRAF kinase are driver mutations found in roughly half of human melanoma patients. Remarkably a single lesion, the V600E mutation (BRAF⁶⁰⁰E), is the predominant mutation found in these patients. Oncogenic BRAF⁶⁰⁰E constitutively activates MEK-MAPK signaling cascade to promote proliferation and survival as well as protect transformed melanocytes from apoptosis.¹ Selective BRAF⁶⁰⁰E inhibitors, vemurafenib and dabrafenib, are effective at halting disease progression or even inducing tumor regression in a high frequency of melanoma patients harboring BRAF⁶⁰⁰E mutation. However, melanomas resistant to BRAF⁶⁰⁰E inhibitor inevitably arise after a few months of treatment, after which there are no further therapeutic options. The lack of treatment for this aggressive form of melanoma demands a better understanding of tumor progression and regression to prevent resistance and identify novel therapies.³

The tumor microenvironment consists of tumor cells, stromal cells and a variety of immune cells. Clinically, the increase of tumor-infiltrating T lymphocytes has been shown to correlate with prolonged survival of patients with colon cancer as well as other types of cancers.³ Increasing the frequency of intratumoral T cells by transferring tumor-specific T cells can also diminish tumor progression in small portion of patients. These findings reveal that immune cells may be able to eliminate tumor cells by recognizing tumor antigens or moieties. However, recent studies have suggested that tumor-infiltrating immune cells may provide both pro-tumorigenic and anti-tumorigenic effects in the tumor microenvironment. The tumor microenvironment is generally immunosuppressive and the accumulation of different immunomodulatory cells, including regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), within the tumor microenvironment can compromise antitumor immunity.¹ Immunotherapies that target checkpoints of immune responses (including PD-1 and CTLA-4 blockade) or the immunomodulatory cells themselves have been shown partially restore antitumor immunity.³ Host immunity has also been suggested to inhibit melanoma progression in melanoma-bearing mice receiving B-Raf⁶⁰⁰E inhibitor treatment, but the underlying immune responses are not well defined.⁶ Therefore, understanding how cancer cells promote an immunosuppressive microenvironment in tumors, and, whether the underlying mechanisms can be targeted to activate antitumor responses, are critical, clinically relevant aspects of tumor immunology. The knowledge attained from targeted therapies and chemotherapies may facilitate the development of new treatment regimens that harness the power of the immune system in combating malignant disease.

In our recent study, we first determined the kinetics of both adaptive and innate immune responses during tumor growth using a genetically modified melanoma mouse model (referred as Braf/Pten mice).⁷ In this mouse model, melanomas were induced by the oncogenic B-Raf⁶⁰⁰E mutation and simultaneous depletion of Pten in melanocytes.⁸ Since Braf/Pten mice harbor a common driver mutation and exhibit similar pathophysiological features as those observed in melanoma patients, we took the advantage of this..
We found immune responses could be restored after progression and, if so, whether these responses are diminished during tumor characterization whether specific immune in an immunocompetent setting. We first model to examine the immune responses underappreciated. To our surprise, our evidenced that B-RafV600E inhibitors may alter certain activities of melanoma cells that normally allow them to suppress T helper cell type 1 (T\(_{H1}\)) effector and helper functions.

In summary, these findings inspire conceptual advances on the roles of immune signaling in BRAFV600E inhibitor-mediated antitumor responses. Most importantly, treating Braf/Pten mice with agonistic anti-CD40 mAb is a proof-of-concept that re-introduction of CD40L:CD40 signal could impede tumor growth, suggesting that diminished CD40L expression on intratumoral CD4^+ T cells may be involved in immune escape. Of note, we showed that the therapeutic benefit of agonistic anti-CD40 mAb is T cell-independent. Therefore, an important future direction would be exploring whether combining agonistic anti-CD40 mAb with immunotherapies targeting inhibitory checkpoints can elicit stronger antitumor response in both BRAFV600E inhibitor-sensitive and –resistant melanomas. Additionally, determining whether the CD40L expression level after BRAFV600E inhibitor therapy correlates with the antitumor responses and the emergence of resistant melanomas will be of great interest for clinical applications.

**Disclosure of Potential Conflicts of Interest**

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