IN THE SPOTLIGHT

Immunotherapy and Oncogenic Pathways: The PTEN Connection

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Summary: Peng and colleagues describe the effects of PTEN inactivation on antitumor immunity and response to immune checkpoint blockade in melanoma. These results shed light on the intricate interplay between oncogenic pathways and antitumor immune response. *Cancer Discov; 6(2); 128–9. ©2016 AACR.*

See related article by Peng et al., p. 202 (6).

Immune checkpoint inhibitor therapy (ICT) has improved clinical outcomes in a number of advanced malignancies, such as melanoma and non–small cell lung cancer (1–3). As of 2015, anti–PD-1 therapy has been approved by the FDA for melanoma, renal cell carcinoma, and non–small cell carcinoma, with approvals for other tumor types being actively pursued. A common theme seen across a number of tumor types is that the benefit from anti–PD-1 therapy is seen primarily in tumors that possess an adaptive immune escape phenotype. The microenvironment of these tumors is frequently characterized by the presence of immune cells and activation of the PD-1 axis (4). It is becoming increasingly clear that this adaptive immune escape phenotype is, in part, determined by features of the tumors' genetic landscape and is associated with high mutation burden (5). The genetic "drivers" in this case are likely to be specific epitopes formed by mutations, or neoantigens, recognized by T cells as foreign. There is currently intense effort aimed to identify these neoantigens.

The promise of immune checkpoint blockade as a cancer treatment has so far hinged on the ability to achieve durable tumor responses in some tumors. However, only a minority of tumors respond. Interestingly, not all tumors with inflamed tumor microenvironments respond to ICT and some tumors without inflamed microenviroments do respond. Clearly, our understanding of immune checkpoint blockade is incomplete. Several unanswered questions are particularly important to unravel. First, how is the immunosuppressive environment in noninflamed tumors established despite the presence of immunogenic tumor antigens? Second, what factors drive the establishment of "innate" immune escape? And third, what is the influence of known oncogenic pathways, such as the PI3K pathway, on antitumor immunity and response to immunotherapy?

In their study, Peng and colleagues present data that help address these important questions. The investigators present evidence that inactivation of *PTEN* in melanoma promotes immune resistance by the tumor (6). First, Peng and colleagues silenced *PTEN* in melanoma cell lines and evaluated antitumor responses to T cell–mediated immunotherapy. In *in vitro* experiments, knockdown of PTEN decreased the ability of T cells to kill tumor cells expressing the melanoma tumor antigen gp100. Then, using the ACT murine model, they also showed that silencing of PTEN reduced the ability of adoptively transferred T cells to kill melanoma tumors *in vivo* when compared to tumors expressing PTEN.

To determine the clinical relevance of their findings, the investigators analyzed PTEN expression in tumor samples from patients with melanoma. When they examined PTEN expression using immunohistochemical analysis of a cohort of 39 patients with metastatic melanoma treated with anti–PD-1 antibodies (pembrolizumab or nivolumab), they observed that patients with tumors that expressed PTEN generally achieved greater reduction of tumor size than patients with tumors that did not express PTEN ($P = 0.029$). Interestingly, when they attempted to grow tumor-infiltrating lymphocytes (TIL) from the tumors, they found that more melanomas that did not yield TIL growth had lost PTEN (26%) than what was observed in tumors that yielded TIL growth (11%; $P = 0.04$). Moreover, an examination of a cohort of 135 resected stage IIIB/C melanoma regional metastases found that melanomas with *PTEN* loss have significantly less $CD8^+$ T-cell tumor infiltration compared with tumors with PTEN expression (6, 7).

Peng and colleagues examined the mechanisms that may link *PTEN* loss to lack of immune activity in melanoma tumors. They found that PTEN status did not correlate with PD-L1 levels but did correlate with levels of CCL2 and VEGF. IHC showed that VEGF levels were increased in regions with PTEN loss. The authors interpreted this finding to indicate that loss of PTEN promotes resistance to immune infiltration through the production of inhibitory cytokines. However, analysis of gene expression of 609 inflammation-related genes showed a broader decrease in expression in tumors with PTEN loss. Moreover, a microarray analysis showed that cells with and without PTEN silencing did not reproduce the results of the 609-gene analysis. Therefore, the immunomodulatory mechanisms underlying PTEN loss are still unclear and may or may not be a direct effect on cytokine regulation.

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The hypothesis that PTEN may have more broad effects on immunity is supported by the effects of PTEN status on autophagy observed by the authors. They altered expression of genes required for activation of autophagy in patient-derived melanoma cell lines and exposed them to autologous TILs. Enforced expression of autophagy-related genes increased the susceptibility of tumor cells to apoptosis induced by their autologous TILs, whereas silencing caused resistance. The authors interpret their data as showing that PTEN loss protects tumor cells from T-cell killing through an autophagy-dependent mechanism.

Loss of *PTEN* results in hyperactivity of the PI3K pathway. Interestingly, the PI3Kβ isoform can regulate AKT activity in tumors with PTEN loss but is not needed for the activation of the TCR signaling pathway. The authors, therefore, examined whether a PI3Kβ-selective small-molecule inhibitor (GSK2636771) could synergize with immunotherapy treatments. Using *in vitro* models consisting of melanoma cell lines engineered to express gp100 and murine H2-D^d, they tested the cell-killing ability of PMEL-1 T cells (murine T cells that target gp100) in the presence or absence of the PI3Kβ inhibitor. They found that the inhibitor improved the T cell–induced tumor killing of the melanoma lines. Moreover, using a genetically engineered murine model that spontaneously developed *BRAF*-mutant, PTEN-null melanomas, the investigators showed that treatment with PI3Kβ inhibitor and anti–PD-1 or anti–CTLA-4 antibody significantly improved tumor growth inhibition compared to treatment with the antibodies alone. Importantly, these data indicate that PI3Kβ inhibition may improve the efficacy of immune checkpoint inhibitor therapy.

The data presented by the authors are very interesting and important. The varying effects (or lack thereof) of different oncogenic pathways on antitumor immune activity is a critical issue to resolve as the field moves forward. If the data described by Peng and colleagues can be confirmed in further studies, this finding will have a strong impact on future clinical trial design. However, so far, *PTEN* mutation has not been observed to be significantly enriched in genomic studies of tumors from nonresponding patients with melanoma or lung cancer treated with anti–PD-1 or anti–CTLA-4 therapy (5, 8, 9). However, protein levels have not been thoroughly investigated in these cohorts and it is possible that epigenetic silencing is the dominant cause of *PTEN* inactivation in the melanomas reported by Peng and colleagues. These issues need to be resolved by examination of larger numbers of samples from patients treated with immunotherapy.

Dissecting the links between oncogenic processes and tumor immunity is a critical effort. The significance of observed links between activation of other oncogenic pathways linked to immunomodulation, such as RAS–RAF–MEK and β-catenin, and response to immune checkpoint blockade is currently not entirely worked out. RAS–RAF–MEK activation is reported to promote immunosuppression in melanoma (10); yet, in lung cancer, it is the *RAS*-mutated cohort that preferentially responds to pembrolizumab treatment. Similarly, although β-catenin has been linked to the actions of immune checkpoint therapy in melanoma, it likely has little to do with determining response in colon cancer, where the vast majority of tumors (both microsatellite unstable and stable) have activated WNT/β-catenin activity and β-catenin activity is not predictive. Therefore, determinants of response may be tumor-type specific and dependent on oncogenic context. Much more work needs to be done to clarify these relationships.

In summary, Peng and colleagues present some very interesting observations on the effects of PTEN status and PI3K pathway activity on immunotherapy efficacy. Future studies will be needed to answer some of the questions raised by these data. Nevertheless, the results by Peng and colleagues go a long way toward shedding light on the intricate interplay between oncogenic pathways and antitumor immunity.

Disclosure of Potential Conflicts of Interest

N.A. Rizvi and T.A. Chan are co-founders of Gritstone Oncology.

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