IN THE SPOTLIGHT

Debugging the Black Box

James C. Yang

Summary: A better understanding of the mechanisms by which tumor rejection succeeds and fails is needed to improve immunotherapies. Here, Anagnostou and colleagues find that mutations predicted to be the most immunogenic are preferentially lost when cancer progresses through checkpoint blockade. *Cancer Discov; 7(3); 250–1. ©2017 AACR.*

See related article by Anagnostou et al., p. 264 (4).

Tumor immunologists have spent decades pursuing the goal of inducing the intact immune system to reject a malignant autologous tissue as it does a transplanted allogeneic organ. Historically, the rare anecdotes supporting the former stood in stark contrast to the inevitability of the latter. Enormous progress has been made in the past two decades. A molecular understanding of tumor-associated antigens, MHC, and antigen processing has led to the ability to identify, induce, or indeed construct meaningful immune responses against human cancers. Most recently, a better understanding of the inhibitory tumor microenvironment and the advent of checkpoint inhibitors to release the T-cell response have produced clinical benefit against a number of cancers.

Currently, our understanding of tumor rejection requires that an adequate antitumor T-cell repertoire be present, the tumor exhibit sufficient tumor-associated antigens, which are properly processed and presented, and that immunosuppressive factors in the tumor microenvironment be neutralized. It has recently become clear that mutated "neoantigens" resulting from tumor-specific mutations are also an important (perhaps *the* important) target of the endogenous antitumor T-cell response (1–3). This is both good and bad news. It is good because all tumors have mutations, but it is bad because some do not have enough and mutations are typically patient specific, stymieing the development of shared therapeutic reagents.

The checkpoint inhibitors, such as anti-CTLA4 and anti–PD-1/PD-L1, have shown consistent clinical activity against only a limited array of tumors characterized by high mutational frequencies. In addition, they have a low rate of complete responses, even against susceptible cancers. In this issue of *Cancer Discovery*, Anagnostou and colleagues (4) sought to understand the reasons why tumors escape checkpoint antibody therapy. They serially sampled tumors from 4 patients with non–small cell lung cancer who initially responded to anti–PD-1 antibody with or without anti-CTLA4 antibody, but then relapsed, using whole-exome sequencing (WES) of pre- and post-relapse specimens. After WES, potential MHC class I–restricted mutation-associated neoantigens (MANA) were identified *in silico* using MHC-binding prediction algorithms. In 3 of these patients, candidate MANA peptides were synthesized and used to stimulate peripheral blood, using quantitative T-cell receptor (TCR) clonotype analysis as the readout of a proliferative immune response. In summary, the findings were that mutations were both lost and gained in the relapsed tumors. Of particular note was the loss of 6 to 18 tumor-associated (predicted) mutated antigens per patient and the fact that these lost MANAs had higher predicted affinities for an autologous MHC allele than MANAs either retained or gained in the relapsed tumor. This finding was reinforced by limited data showing that the lost MANAs were also associated with more proliferative TCR responses on peptide stimulation of peripheral blood lymphocytes, suggesting they could represent bona fide antigens being "immunoedited" (5). A better understanding of the homogeneity of neoantigen expression and the functional significance of each tumor-associated mutation might be needed to assess the contribution from an immune attack on a specific target. Other shortcomings of this study were that the specificity of the responsive TCR clonotypes responding to candidate MANA peptides was not validated, and there were no data on class II–restricted T-cell epitopes. Isolated data make a compelling story that such class II–restricted T cells can also be sufficient to achieve tumor regression in some patients (6). Yet overall, the findings of this study are not surprising, but contrast with another small study (7) showing that relapsing tumors were characterized by the loss of β2-microglobulin (with consequent loss of all MHC class I expression) and biallelic defects in JAK1/JAK2 that disrupt IFNγ receptor signaling. Similar losses were not documented in this study, nor was loss of PD-L1 found.

It is clear that such small studies cannot yield a comprehensive picture of the true "landscape" of immunotherapy resistance, and, in fact, these studies were limited to therapy with checkpoint blockade. Evidence from adoptive T-cell therapy using tumor-infiltrating lymphocytes (TIL) in patients with melanoma show that these TILs contain extensive reactivity against mutated neoantigens (1) and can achieve high rates of durable complete clinical response (8). The additional observation that TIL adoptive transfer can achieve responses in patients who have progressed through checkpoint blockade (8) also points to an insufficient T-cell repertoire as one cause of unresponsiveness to checkpoint blockade.

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So as we look at what was once a "black box" concealing the process of tumor rejection, we now have a much better understanding of what is necessary. The importance of the PD-1–inhibitory pathway in the tumor microenvironment is now clear, but even for susceptible tumor types, this is most beneficial when there is tumor expression of the PD-L1 ligand. Also central to the process is the need for a robust antitumor T-cell repertoire, likely driven by reactivity to properly processed and presented tumor-associated mutated neoantigens. Such a repertoire appears to be consistently present in only a limited number of tumor types, notably melanoma (induced by UV irradiation), lung cancer (from tobacco carcinogens), and tumors with microsatellite instability (9). Patients with other common tumors that account for most deaths from cancer in the United States (colorectal, breast, prostate, and pancreatic cancers) tend to have fewer mutations and thus limited T-cell repertoires. One promising approach for these patients is to apply a much more aggressive selection process to TIL adoptive therapy, driven by a personalized analysis of neoantigen recognition (10).

The other area for potential progress is to better understand and perhaps augment the T-cell effector functions that cause tumor rejection. Here, we encounter one of the last dark corners of the tumor regression black box. The "end game" by which T cells eliminate tumors remains obscure. The relative contributions to tumor destruction of cytolysis, cytokines, and stromal/vascular collapse are murky. There are conflicting data on the efficacy of nonlytic tumor-reactive T cells and the impact of knocking out specific host stromal elements or tumor cytokine receptors. Many studies point to a critical role for IFNγ, but what that role is remains unclear. Studies such as this one, looking at bugs in the program leading to tumor rejection, may reveal factors we are already aware of, but they also hold the promise of shedding new light on the final common pathway(s) of tumor destruction that may be critical to improving all forms of immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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