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Tumor antigenicity revealed

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Abstract

The extent to which mutated peptide antigens encoded by somatic mutations contribute to tumor antigenicity in human cancer patients has been unclear. Two recent studies using tumor exome sequencing to predict candidate mutated antigens provide insight into this question.

In recent years, strategies that harness endogenous T cells for the immunotherapy of cancer have shown promising clinical effects, mediating durable cancer regression and survival in a proportion of patients (1, 2). In adoptive cell therapy, autologous tumor-infiltrating T lymphocytes (TILs) isolated from a resected tumor are expanded to great numbers *ex vivo*, and reinfused into the patient following lymphodepletion conditioning (3). In another strategy, antibodies specific for the T cell co-inhibitory receptors CTLA-4 or PD-1 are administered to patients, thereby eliciting or re-activating endogenous T cell responses directed at tumor-expressed antigens (4). Given these compelling clinical results, it is critical to determine why some patients respond to immunotherapy and others do not.

One potential factor involves the “antigenicity” of a tumor, that is, the extent to which tumor cells display HLA-restricted antigens that can be selectively or specifically recognized by T cells. In this regard, immunologists have long been intrigued by the hypothesis that the mutational processes associated with tumorigenesis may induce the presentation of mutated peptide antigens (“neo-antigens” or “neo-epitopes”) that can be recognized by T cells. Conceptually, mutation may induce neo-epitopes in two ways. First, the mutation may fall within an epitope that is normally presented on the cell surface via HLA molecules, thereby altering the epitope and permitting specific T cell recognition. Second, the mutation may change the way a given peptide is processed in antigen presentation pathways, such as proteolytic cleavage or HLA binding, thereby inducing the presentation of a new peptide that would not normally be presented. Evidence of tumor-expressed, HLA-restricted neo-antigens stems primarily from sporadic cases in which patient-derived T cell lines reactive to autologous tumor cells were found to recognize a mutated neo-epitope (5). Most of these neo-epitopes contain single amino-acid changes induced by point mutation, although neo-epitopes resulting from translocation junction points have also been reported (5). Despite the identification of a wide array of mutated neo-antigens, it has been challenging to determine whether T cells reactive to such epitopes contribute substantially to the repertoire of endogenous T cells within tumor lesions, or whether they represent rare outliers.

What is the probability that a somatic mutation will result in the presentation of a mutated neo-epitope via HLA class I molecules on the tumor cell surface, and prime T cell responses reactive to this neo-epitope? In order for such a peptide to be antigenic, it must meet several

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criteria. First, the mutation must fall within a protein-coding region of the genome, and lie within a protein that is expressed by the tumor cell. Moreover, a short peptide epitope containing the mutated amino acid must be cleaved, processed, and loaded into HLA molecules with sufficient stability to permit presentation on the cell surface. Once on the surface, there must be T cells that are capable of recognizing the neo-epitope, distinguishing it from self antigens that may have similar antigenic conformations. The availability of T cells capable of reacting to such neo-epitopes may be restricted by deletional tolerance mechanisms in the thymus and periphery, which are thought to drive the elimination of T cells exhibiting strong reactivity to many non-mutated self antigens. Finally, since the initiation of tumor-reactive T cell responses is thought to be dependent on peptide “cross-presentation” by specialized antigen presenting cells (APCs) (6), the neo-antigen must also be released from tumor cells and cross-presented by APCs at sufficient ligand density to prime T cell responses. At each step of the antigen processing and presentation pathway, neo-epitopes face fierce competition with peptides derived from thousands of other non-mutated self proteins expressed by tumor cells. Given these odds, the extent to which mutated peptide epitopes contribute to the antigenicity of most tumors has been unclear.

Two recent studies have provided compelling insights into this issue. In one study, Robbins et al. analyzed samples from three melanoma patients that had undergone substantial tumor regression following adoptive cell therapy via autologous TIL transfer (7). The authors performed tumor exome sequencing to identify nonsynonymous mutations within protein coding regions, and used bioinformatics approaches to identify candidate HLA class I-restricted neo-epitopes encoded by mutated genes. For each patient, it was found that TIL cultures contained T cells reactive to 2–3 predicted neo-epitopes. Importantly, a measurable proportion of TIL cultures (ranging from 1–6%) produced IFN- γ upon stimulation with these neo-antigens. In addition, neo-antigen-specific T cells could be detected in the peripheral blood at least one month following TIL reinfusion therapy, demonstrating persistence of these cells *in vivo*. In a second study, van Rooij et al. analyzed samples from a melanoma patient that had undergone partial tumor regression following treatment with anti-CTLA-4 antibody therapy (8), in order to determine whether cancer regression was associated with neo-antigen-specific T cell responses. The authors performed tumor exome sequencing, and used bioinformatics approaches to identify candidate peptide epitopes encoded by nonsynonymous mutations in tumor-expressed genes. They then assayed TIL cultures for the presence of T cells specific for candidate neo-antigens using a multiplexed peptide/HLA multimer-based approach that enables the direct enumeration of T cells of many specificities in a single analysis (9). Using this approach, T cells reactive to two predicted neo-epitopes were detected in TIL cultures. Of note, T cells reactive to one of these epitopes comprised ~3% of TIL cultures, and were found to be expanded in the peripheral blood following treatment with anti-CTLA-4 antibody, suggesting a role for this specificity in tumor regression. Taken together, these studies provide an important proof-of-principle that endogenous T cells reactive to tumor-expressed neo-antigens are present within expanded TIL cultures, and may play a critical role in cancer regression. Although neo-antigen-specific T cells accounted for only a small proportion of TIL specificities, it is important to note that the approaches used may underestimate the frequency of neo-antigen-specific T cells, as all three HLA loci were not assayed in these studies. Moreover, it is possible that some *bona fide* neo-antigens were not uncovered using the *in silico* prediction methods employed.

Further work will be required to determine whether tumor mutational frequency correlates with response to immunotherapy, in both melanoma and other types of cancer, and whether neo-epitope-specific T cells are more effective than unfractionated TILs at mediating cancer regression. If so, it would suggest that neo-epitope-specific T cells may be the primary drivers of cancer regression in patients that respond to immunotherapy. More importantly, it

would suggest the viability of a personalized medicine strategy in which patients are pre-screened by tumor exome sequencing, in order to identify those individuals exhibiting a high tumor mutation frequency who are most likely to respond to immune-based therapies. In addition, it suggests that peptide/HLA multimer-based enrichment of neo-antigen-specific T cells could be used to enhance therapies based on autologous TIL transfer.

On the flip side, such findings would also suggest that T cell-based immunotherapies may be effective for those cancers associated with high mutation frequencies, such as melanoma and lung cancer, but may be less effective for the many types of cancer exhibiting low mutation frequencies (10). Moreover, tumor mutational heterogeneity provides an additional layer of complexity; if heterogeneity is common, then the elicitation of T cell responses directed at mutated neo-antigens may induce partial tumor regression, but select for the outgrowth of tumor cells bearing a different, less antigenic mutational profile. In all, the studies of Robbins et al. and van Rooij et al. provide important insights into our understanding of tumor antigenicity. Information gained from such studies will be crucial for maximizing treatment response rate and efficacy while minimizing unnecessary and costly treatments associated with adverse side effects.

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