Tracking down tumor-specific T cells

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Abstract

Two papers published in this edition of Cancer Cell (Zheng et al., 2022; Veatch et al., 2022) provide an elegant illustration of how single cell sequencing can be used to define a molecular phenotype which identifies tumour-specific T cells.

The isolation and identification of human T cells which recognize tumor associated antigens and tumor neoantigens were landmark advances, providing concrete evidence of a host adaptive immune response against cancer. The subsequent dramatic successes of CTLA-4 and PD-1/PD-L1 directed checkpoint immunotherapy closed the circle by showing that such responses could be protective. Defining the precise phenotype and function of the tumor-specific T cell populations has since emerged as a central goal of tumor immunology, with important translational implications for both patient prognosis and therapy in the context of early and metastatic cancer settings.

The pro-inflammatory and angiogenic microenvironment generated by tumors attracts an influx of a variety of haemopoietic immune cells, including memory, effector and regulatory T cells. As at any inflammatory site, most cells may be bystanders merely passing through the tissue, and these populations may mask T cells that are interacting in an antigen-specific way with the tumor. The key to unravelling this complexity, central to the strategy of the two papers in this issue (Zheng et al., 2021, Veatch et al., 2021), and to other similar publications (Oliveira et al., 2021; Caushi et al. 2021; Eberhardt et al., 2021; Lowery et al Science 2022) is the αβ T cell receptor (TCR). The TCR uniquely determines T cell antigen specificity and provides a link between tumor antigen and cell phenotype, which is usually defined by a combination of protein and RNA expression profiling. The immense diversity of the TCR sequence, emerging from unique, imprecise somatic recombination mechanisms during T cell development, means that each TCR is very unlikely to be produced more than once in an individual, providing a unique molecular barcode for the identification of T cells and their clonal progeny. Once the target antigen for a particular TCR has been determined, the TCR sequence can be used to identify the clonal progeny of that T cell within a complex population. The overall strategy is illustrated in Fig 1. Functional experiments (essentially different types of in vitro T cell antigen-specific recall assays) are used to identify T cells responding to predicted tumor antigens. The DNA sequence of the receptors expressed by responding T cells is determined. Once the sequence is determined, the antigen specificity can be validated and analyzed in detail by engineering TCR expression as a transgene in unlimited numbers of other polyclonal or transformed T cell lines. Finally, the TCR sequences are used to map the location of specific T cells within the complex phenotypic landscape of ex vivo intra-tumoral lymphocytes revealed by single cell RNA sequencing.

The two studies reported in this issue focus on very different tumour types in terms of tumor mutational burden (TMB) and response to checkpoint blockade. Zheng et al. focus on gastro-intestinal metastatic disease, specifically patients with biliary duct and pancreatic cancers. These cancers have low TMB and are notoriously resistant to checkpoint immunotherapy. In contrast, Veatch et al. focus on metastatic melanoma, a cancer with high TMB and a good response to checkpoint blockade. Despite these differences, the fundamental conclusions of both studies are remarkably similar. In both settings, T cells which were unambiguously identified as recognizing tumor antigens by an impressive series of functional T cell antigen-specific recall assays, mapped to a relatively homogenous sub-compartment of the intra-tumoral T cell phenotypic landscape. T cells responding to chronic CMV or EBV viral antigens mapped to a quite distinct region of this landscape. The dominant defining marker for both $CD4^+$ and $CD8^+$ tumor-specific T cells is high expression of PD-1, confirming many previous publications, and both studies further identify these cells as "exhausted". Both studies highlight the expression of the chemokine CXCL13, together with a small number of additional markers, as a characteristic feature of the tumor-specific T cells. Interestingly, CXCL13 expression is also an independent pan-cancer prognostic of a good response to checkpoint immunotherapy (Litchfield Reading Puttick et al., 2021).

Given the different immune profiles of the cancers in these two studies, the convergence of their findings is striking, and suggests that the identification of a common phenotype which can distinguish tumor-specific from bystander T cells may be achievable. A key follow-up question is whether this phenotype, or a similar one, can be detected in peripheral blood as well as the tumor itself. The mechanism for the peripheral recirculation of tumor-reactive exhausted T cells remains unclear, and may represent either dysfunctional priming of T cells in the draining lymph nodes or tumor-resident T cells undergoing retrograde migration via the draining lymph nodes. But PD-1 expression alone has been shown to enrich for neoantigen specific T cells in the circulation of patients with melanoma (Gros et al, 2016). If more refined phenotypes further enrich for circulating tumor-specific T cells, the results would be of great practical importance for the development of personalized adoptive T cell therapy.

These studies also highlight important limitations of the current single cell technologies. Firstly, the methods are highly labor intensive and expensive. This constrains most studies to small patient and cell numbers, which inevitably limits statistical power, interpretability and generalizability. Secondly, the first step in the pipeline, prediction and experimental identification of cancer antigen targets, remains inefficient and wasteful. Even in the in-depth studies discussed here, only a proportion of individuals, and only a small proportion of T cells could be reliably assigned to specific epitopes. Whether this reflects technical limitations or a real gap in our understanding of what constitutes tumor-derived antigens is still unclear. Perhaps artificial intelligence algorithms for matching T cell receptors sequences to cognate antigen will improve this workflow in the future. Alternatively, tumor-specific T cells responding to tumor cell lines or lysates can be identified in an antigen-agnostic fashion (Caushi et al. 2021). Thirdly, the gap between function and phenotype remains. Although these studies, and indeed most of the literature, talk about "exhausted", "effector", and other functional subsets, there is little experimental data to define how these different types of T cells are inter-related, what part they play in providing protection, or how they may be manipulated by immunotherapy. Furthermore, single-cell profiling provides a static snapshot of a highly dynamic process. The flux of antigen specific T cells through a differentiation pathway, and the potential to reverse this pathway may be as important as the number of cells with a specific phenotype in determining the outcome of the host-tumor interaction (Ghorani & Reading et al., 2020). Similarly, although CXCL13 expressing T cells emerge as an important source of antigen-specific T cells, the mechanistic role of CXCL13 remains speculative. In the context of the well-established role of

CXCL13 in germinal center formation, the increasing awareness that ectopic lymphoid structures, B cells and tumor-specific antibody can combine with T cells in mediating the anti-tumor immune response may provide an important clue (Cui et al, 2021; Meylan et al. , 2022)

In conclusion, Zheng et al. and Veatch et al. provide convincing data for the existence of a specific phenotype, or related sets of phenotypes, that discriminate tumor-specific from bystander T cells in the tumor microenvironment. The practical importance of these findings will depend on how broadly they can be applied to different types of cancers, and on whether similar signatures can be identified for circulating T cells, as well as the more inaccessible tumor infiltrating lymphocytes. New approaches will be needed which can extend these important descriptive studies to the dynamic functional differentiation processes which underly protective tumor immunity, and which can be modulated for the therapeutic benefit of patients with cancer.

Figure Legend:

Fig 1. The pipeline for the functional annotation of tumor antigen-specific T cells. Tumor infiltrating T cells are first classified as specific for tumor neoantigens, tumor-associated antigens or bystander T cells by intensive screening using antigen-specific functional recall assays. Once isolated, the T cell receptors from these cells can be sequenced, using barcoding to identify different T cell clones. Single-cell transcriptomic data can be used to link the functional and transcriptomic features of tumor antigen-specific T cells.

Declaration of interests

The authors declare no competing interests.

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Add references to Zhang et al. and Veatch et al.

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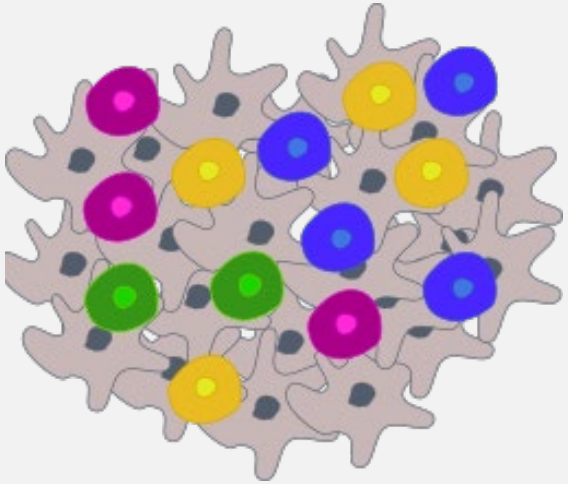
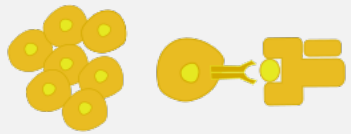
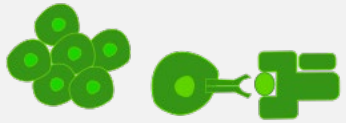
Tumour infiltrating lymphocytes



Antigen-specific functional recall assays



Neoantigen or tumour associated antigen specific



Bystander (non tumour antigen specific)

T cell receptor sequencing

Single cell transcriptomics