Spotlight

TIL classified to memory state are correlated with response to immune checkpoint blockade

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Tumor reactivity mediated by tumor infiltrating lymphocytes (TILs) is one of the hallmarks of the clinical effect of immune checkpoint blockade (ICB). Jaiswal et al. used a functional genomics approach to better characterize TIL phenotypes predictive for response to ICB.[1]

The observation that the presence of tumor infiltrating lymphocytes (TILs) is associated with improved prognosis has been well established.[2] In addition, ICB treatment or adoptive cell therapy with *ex vivo* liberated and expanded TILs can provide significant clinical effects in a substantial portion of metastatic melanoma patients.[3] However, a comprehensive characterization of the tumor-reactive T cells among the TILs is currently lacking, despite the availability of high-dimensional technologies.

In the recent issue of *Cancer Cell*, Jaiswal et al. describe a functional genomics approach to interrogate multiple publicly available single cell and bulk RNA sequencing datasets. From melanoma and other cancers datasets, they were able to classify TILs into subgroups comprising T cells enriched for memory/resident memory genetic programs and T cells with early activated, non-exhausted gene signatures.[1] Furthermore, they demonstrate that the presence of resident memory TILs was correlated with response to anti-PD-1 ICB therapy and that this coincided with an IFN-gamma response and dendritic cell maturation. In contrast, the presence of TILs with an early activation phenotype was identified to correlate with non-response, most likely because these T cells have not undergone sufficient activation and are stunted in their differentiation process.

During viral infection, naive T cells become activated and develop into effector-, memory-, and tissue-resident T cells. However, upon chronic or improper antigen stimulation, i.e., in situations of chronic viral infections or cancer, formation of adequate T cell memory fails and T cells become exhausted or dysfunctional.[4] It has become clear that exhaustion or dysfunction is not a fixed TIL differentiation state, but consists of a continuum from early dysfunction to terminal dysfunction.[5] TCF-1-expressing early dysfunctional T cells have retained self-renewal capacity and some effector T cell functionalities, whereas terminally exhausted T cells lacking TCF-1 expression exhibit high level expression of inhibitory receptors and have lost the capacity to proliferate or secrete effector cytokines upon antigen encounter.[6] Instead, these cells have acquired the capacity to express and secrete CXCL13, a chemokine required for the development of tertiary lymphoid structures (TLS), often found in cancers. Importantly, the presence of these TLS was recently shown to correlate with response to ICB,[7] hinting toward an important role for terminally exhausted cells in mediating therapeutic response.[8] Many studies applying combined single cell RNA sequencing and T cell receptor clonotype analysis of TILs, allowing coupling of cell states to TCR clonotypes, strongly suggested that (1) there is little overlap in TCR usage between TILs with clearly distinct cell states and (2) that tumor-reactive TCRs appear to be enriched in the TIL fraction residing in a dysfunctional cell state.[9] Following transcriptional profiling and multidimensional flow cytometry, tumor reactivity was attributed to TILs that exhibit high expression of inhibitory receptors, e.g., PD-1, in addition to CD69 and CD103, reminiscent of tissue-resident memory T cells.[10] Jaiswal and colleagues demonstrate that tissue-resident T cells (T$_{RM}$) develop following viral infection, display a genetic profile with high expression of inhibitory receptors (PD-1, TIGIT, Lag-3, Tim-3), develop T cell activation transcripts (GzmK, ifng), and express tissue retention markers (CD69, CD103). This genetic T$_{RM}$ profile bears high similarity with the genetic profiles of dysfunctional TILs. Indeed, gene expression profiles from a significant proportion of human melanoma TILs overlap with a T$_{RM}$ consensus signature; nonetheless, TILs diplay transcriptional profiles that show overlap with different T cell states could not be categorized easily. However, using comparative enrichment score analysis, the melanoma TIL transcriptional cell states consistently ranked higher for memory and resident memory T cell signatures over activation and exhaustion signatures, irrespective of the origin; i.e., from a primary or metastatic lesion. Using specific software, allowing the ordering of single cells in pseudotime and placing them in a developmental trajectory, the authors aim to better understand how melanoma TILs differentiate upon transition through specific cell states. By comparing TILs from ICB non-responders and responders, only two developmental trajectory TIL clusters separated responders from non-responders. In cluster 1, associated with non-response, differential gene expression analysis indicated upregulation of genes associated with G1/S cell cycle blockade and oxidative phosphorylation, but downregulation of the AP-1 pathway. Whereas in cluster 3, associated with ICB response, genes involved in G2/M cell cycle transition and the AP-1 pathway were upregulated and genes involved in oxidative phosphorylation were downregulated. When comparing this to results derived from pre-clinical models, thus far used to

classify TIL to activation and dysfunctional/exhausted states, cluster 1 genes overlapped with T cell activation, but not dysfunction (early or late) and cluster 3 genes with long-term memory and resident memory.

How should these data be interpreted given the abundance of available literature on intratumoral T cell states? There is a general consensus that T cells residing in tumors can be classified as naive cells, cytotoxic cells, and dysfunctional T cells. Importantly, based on TCR sequencing, the overlap of clonotypes between these subpopulations is small, therefore supporting that T cells with cytotoxic and dysfunctional differentiation cell states are distinctively formed. Dysfunctional T cells are functionally impaired and can be distinguished from classical T cells in that these cells secrete CXCL13, but not typical effector cytokines (e.g., IL-2, IFN-γ, TNF-α). Therefore, this very late dysfunctional cell state bares resemblance to developmental trajectory one, with downregulation of AP-1 and transcription factors required for T cell activation, and upregulation of cell cycle control checkpoints and genes involved in G1/S phase, halting cell proliferation and function. In contrast, early dysfunctional cell states show more overlap with trajectory cluster 3, with upregulation of AP-1 genes and genes involved in G2/M transition and downregulation of oxidative phosphorylation, switching metabolism to an active glycolysis dependent one. Within the field, the debate on which T cells within the TME are responsible for the clinical effects observed following ICB continues. Currently, it is unclear whether late dysfunctional or terminally exhausted T cells can switch back to effector cells or that this switch is only

allowed for TILs not yet having reached this permanent state. T cells with characteristics of long-term or resident memory as demonstrated in this paper are, from an immunological perspective, the preferred T cell states to combat and control infection. The fact that these cell states are also correlated with ICB response and that gene signatures from resident memory and late exhaustion show overlap and are highly associated with IFNγ response and dendritic cell maturation indicate that we have taken another step toward the full characterization of the ultimate effectors of tumor clearance following ICB therapy.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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