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Zooming on T cells in cancer

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Immunotherapies have had tremendous progress in treating numerous cancer types since the late twentieth century (Waldman et al., 2020). Accompanied with this, there has been an unprecedented wave of understanding the dynamic changes and the functional mechanisms of immune cells that are tumor-infiltrating and systematically circulating in cancer patients (Waldman et al., 2020). T cells are unquestionably the most focused and well-studied cell population among all immune cell types, in which $CD8^+$ T cells play a central role in killing cancer cells (Philip and Schietinger, 2022). When continuously exposed to tumor antigens or in chronic infection, cytotoxic T cells enter a dysfunctional state called "exhaustion" (Wherry, 2011). With the regulation by key transcription factors (TFs) including T-bet, EOMES, NR4A1, TCF1 and TOX, tumor-infiltrating T cells exhibit a significant degree of heterogeneity, from progenitor exhausted T cells (Tex) to terminal Tex (Beltra et al., 2020; Liu et al., 2019; Paley et al., 2012; Utzschneider et al., 2016). Progenitor Tex have been reported to show memory- or stem-like features, and remained responsive to immune checkpoint blockade (ICB) treatment. Terminal Tex, however, exhibited fixed epigenetic landscape and limited response to ICB (Pauken et al., 2016). Thus, preventing the exhaustion process or rejuvenating the Tex, especially the progenitor Tex, have been the main goals in cancer immunotherapies (Jiang et al., 2015).

In recent years, scientists have taken efforts in defining T cell populations in human tumor microenvironment by new technologies including single-cell sequencing. Although more and more valuable data from one or several cancer types have been published worldwide (Guo et al., 2018; Tirosh et al., 2016; Zhang et al., 2018; Zheng et al., 2017), it remains to be understood whether there exists a pan-cancer T cell landscape, considering the different experimental procedures and bioinformatic analyses among those studies. In this case, uniform normalization and analyses of the datasets are urgently required for matching and comparing T cell functional subpopulations in different cancer types.

A paper with the title of "Pan-cancer single-cell landscape of tumor-infiltrating T cells" was published in *Science*, which examined the transcriptome landscape of 397,810 T cells in 21 cancer types and from 316 cancer patients (Zheng et al., 2021). This became by far the largest T cell study in pan-cancer patients at single-cell level. By combining and comparing the transcriptional information of those T cells, the authors identified the common and unique features across different cancer types, analyzed the state transition among functional cell sub-populations, and provided a new approach for pan-cancer patient stratification based on T cell composition.

In this study, the investigators analyzed multiple cancer-associated T cell populations and found their functional states varied dramatically in different cancer types at the transcriptome level. Across cancer types, they identified Tex as the major population of tumor-infiltrating $CD8^+$ T cells, and found that they exhibited highly heterogenous features. Notably, they predicted the potential developmental paths among cell populations with different functions by multiple methodologies, including diffusion map, UMAP, monocle3 and RNA velocity, and found both of the prevalent paths and

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rare paths existing in pan-cancer patients. While the prevalent paths to $CD8^+$ terminal Tex from both effector memory T cells and tissue-resident memory T cells were observed across cancer types, the state transition between $CD8^+$ terminal Tex and NK-like T cells, Type 17 $CD8^+$ T cells (Tc17) or regulatory $CD8^+$ T cells ($CD8^+$ Tregs) may only exist in specific cancer types. By in-depth analyses of TFs that showed high levels of expression in Tex, the investigators noticed that *TOX*, *TOX2*, *RBPJ*, *ZBED2* and *PRDM1* were significantly highly expressed in most of the cancer types. TFs not known to be associated with T cell exhaustion were also identified, including *SOX4* and *FOXP3*. By combining the pathway information of those TFs, they inferred that besides the influence of chronic tumor antigen stimulation, the transition of effector memory and/or tissue-resident memory T cells to exhaustion state may also be affected by interferons and TGF-β in the tumor microenvironment through the activation of exhaustion-associated TFs like TOX.

The potential developmental trajectories of Tregs were also inferred by bioinformatic analyses based on the expression patterns, and the results indicated a transition from the $TNFRSF9^-$ resting Tregs to $TNFRSF9^+$ activated Tregs. Tregs also showed crucial roles in negative regulation of tumor-killing cells in the tumor microenvironment, thus precisely eliminating or inhibiting the tumor-related Tregs has always been one of the most desired treatments in cancer immunotherapies (Zou, 2006). However, the origins of tumor-activated Tregs have long been controversial. Studies have shown both the activation of natural Tregs (nTregs) that developed in the thymus and conversion of induced Tregs (iTregs) that may be derived from a population of conventional $CD4^+$ T cells (Zhou and Levitsky, 2007; Zhou et al., 2006). Since the single-cell data obtained combined information of transcriptome features and T cell receptors (TCRs), it is convenient to track the Treg developmental processes by tracing the TCR clonality of tumor Tregs with other Tregs and conventional $CD4^+$ T cells. Previously, the same group analyzed the TCR sequences of tumor Tregs in liver cancer, lung cancer and colorectal cancer (Guo et al., 2018; Zhang et al., 2018; Zheng et al., 2017), and found that TCRs of most tumor-associated Tregs were exclusive to their own population, indicating that the tumor Tregs may undergo a separate developmental process from the non-Treg cells. With a bioinformatic algorithm developed by the same group called "STARTRAC" (Zhang et al., 2018), they also noticed obvious TCR sharing between $TNFRSF9^+$ activated Tregs and $TNFRSF9^-$ resting Tregs in the vast majority of different cancer types of the pan-cancer study, further indicating a universal activation of nTregs. On the contrary, the induction of activated Tregs from the conventional $CD4^+$ T cells could only be detected in a few cancer types.

For decades, the stratification of cancer patients was mainly based on the tissue origins and the histological structure of tumor cells, until the molecular and cell features that correlated with prognosis were gradually included, such as cancer-related mutations (Lawrence et al., 2013) and immune cell abundance (Galon and Bruni, 2019). Since the cancer types are highly diverse and the tumor microenvironment is heterogenous, more systematic stratification methods are required for pan-cancer patients. In a previous study done by these investigators, patients with non-small-cell lung cancer were subtyped by the composition of different T cell subgroups (Guo et al., 2018), and the authors of the current work found that patients harboring distinct patterns of tumor-infiltrating T cells exhibited significantly different prognosis. They further put such similar idea into stratifying the pan-cancer patients, and indeed found that patients with different cancer types can be divided into two immune types, including one with high frequency of terminal Tex but low frequency of tissue-resident memory T cells ($Tex^{hi}Trm^{lo}$), and the other with the opposite compositions ($Tex^{lo}Trm^{hi}$). They noticed that patients of the $Tex^{lo}Trm^{hi}$ group showed better overall survival than those of the $Tex^{hi}Trm^{lo}$ group across cancer types or in multiple individual cancer types, thus providing a potential novel classification system for pan-cancer patients. Additionally, they reanalyzed the data from the patients treated with anti-PD1 therapies, and found that responsive patients had a lower frequency of terminal Tex and a higher frequency of naïve or tissue-resident memory T cells, thus further emphasized the relationship between T cell composition and clinical outcome.

In summary, the in-depth single-cell analyses done in the current study has precisely depicted a T cell atlas in pan-cancer patients, which provided valuable data source as well as methodology for future studies of the cancer immunology field. In their study, the authors globally characterized the functional states at the transcriptome level and potential transition trajectories among different cell subgroups, and revealed novel potential mechanisms of T cell exhaustion. Since such relationships were largely inferred via bioinformatic analyses, biological validation is necessarily required to identify the true functional regulatory genes and cellular developmental pathways. Notably, this study and others showed that the tumor-infiltrating exhausted T cells, or at least a large fraction of the exhausted T cells, showed high expression levels of genes related with effector functions but low expression levels of genes related with proliferation or stemness (Guo et al., 2018; Tirosh et al., 2016). We therefore believe that T cell exhaustion is associated with effector cells devoid of long-term persistence, due to lack of progenitor cells that supply the pool of tumor-killing effector cells following expansion and differentiation. Thus, "exhaustion" should be related to effector T cell populations that lack self-renewal and only crash in the coming stage. The authors

traced the common and unique origins of tumor-activated Tregs across cancer types. Although linking identical TCRs in different T cell populations seemed to be a reliable way to identify the transitional relationships between cell clusters, the conclusions could be biased by limited sample size. Therefore, the tumor-activated responses of Tregs should be further studied using proper biological models, which allow us to track this dynamic process. The authors also presented a novel stratification strategy for pan-cancer patients, and indicated the relationships between T cell heterogeneity and prognosis. Notably, they analyzed certain cell fractions that correlated with the clinical responses of ICB-treated melanoma patients. Such correlation analyses should also be extended to other cancer types to identify more common and reliable biomarkers, which requires larger datasets for ICB-treated patients in the future. Nonetheless, the current work is a major first step trying to link T cells associated with different cancer types, and future work along this line will offer insights into their regulatory mechanisms in the tumor microenvironment and provide basis for next-generation previsions immunotherapies.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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