645 SINGLE-CELL TRANSCRIPTIONAL AND CLONAL CHARACTERIZATION OF CD4+ T CELLS ACROSS TISSUES IN LONG-TERM MELANOMA SURVIVORS

http://dx.doi.org/10.1136/jitc-2021-SITC2021.645

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Background Melanoma patients who develop immunotherapy-related adverse events often have durable responses to treatment. We previously identified that long-term melanoma survivors presenting with the autoimmune adverse event, vitiligo, developed long lived CD8+ resident memory T cell (TRM) responses in skin and tumor with circulating memory T cell (TCIRC) clonal counterparts in blood.[1] Despite the focus on CD8+ T cells in prior studies, CD4+ T cell features remain largely in the background.

Methods Using the same patient cohort, we performed parallel single-cell RNA sequencing (scRNAseq) and single-cell TCR sequencing (scTCRseq) on CD4+ T cells sorted from matched skin, tumor, and blood using the 10X Genomics platform. The UMI counts-based gene expression matrix was processed using the R package Seurat (v.3.0).

Results Eleven distinct CD4+ T cell clusters were identified. The FOXP3 expressing regulatory T cell (Treg) cluster was comprised of cells from skin, tumor, and blood, and could be further sub-clustered into 3 distinct populations with one having transcripts associated with Treg activation. Of the T helper-like clusters, we identified subsets with transcripts associated with cytotoxicity (GZMA, GNLY, CX3CR1; TCYTO), exhaustion (PDCD1, HAVCR2, TOX; TEX), and three clusters that were excluded from blood with clear resident memory characteristics (high CD69, low KLF2, S1PR1). These three clusters were differentiated by expression of IL2 (TRM-IL2); ID2 and CD40LG (TRM-ACTIVATED) and CD28 (TRM-CD28). Paired scTCRseq revealed a high level of clonal overlap between the TCYTO and the TRM-ACTIVATED clusters, with RNA velocity analysis supporting a potential differentiation trajectory from TRM-ACTIVATED to TCYTO. Integrating our previously published CD8+ TRM and TCIRC profiles, we identified a core TRM signature and core TCIRC signature from both the CD4+ and CD8+ TRM and TCIRC cells, respectively, in melanoma patients. The core TRM signature predicted better overall survival of advanced melanoma patients in TCGA, while the core TCIRC signature did not.

Conclusions This study supports the crucial anti-tumor role of TRM in cancer patients and extends this important observation to CD4+ T cells.

REFERENCE

1. Han JC, Zhao YD, Shirai K, Molodtsov A, Kolling FW, Fisher JL, Zhang PS, Yan SF, Searles TG, Bader JM, Gui J, Cheng C, Ernstoff MS, Turk MJ, Angeles CV. Resident and circulating memory T cells persist for years in melanoma patients with durable responses to immunotherapy. *Nature Cancer* 2021;**2**(3).

Ethics Approval IRB-approved written informed consent was obtained from patients with advanced melanoma, to perform skin and tumor biopsies, draw blood, and to access historical banked tissue and blood samples for analysis. All human studies were performed in accordance with ethical regulation, and pre-approved by the Committee for the Protection of Human Subjects at Dartmouth-Hitchcock Medical Center IRB (#00029821).