Single-Cell RNA-Seq Reveals Insights into the Effects of Immune Checkpoint Inhibitors

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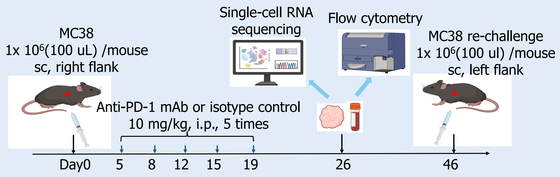
Introduction

Immune checkpoint inhibitors (ICI) have demonstrated superior clinical efficacy, with proven prolongation of patient survival in a variety of cancers. The mechanism of ICI action on the immune system and memory function needs to be continually explored. Single-cell RNA sequencing (scRNA-seq) provides a superpower tool in oncology R&D. In this study, we conducted a single-cell RNA-seq analysis following treatment with an α-PD-1 antibody in a murine colon adenocarcinoma model.

Methods



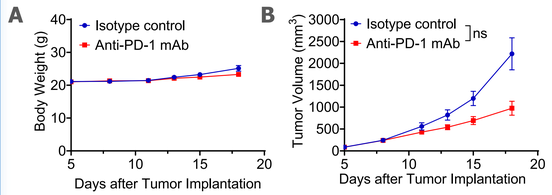
- Mice were inoculated with MC38 tumor cells and then treated with ICI. After tumor regression mice were re-challenged with the same tumor cells.
- *Ex vivo* tumor-infiltrating lymphocytes and PBMC were isolated and analyzed by scRNA-seq and flow cytometry.

Conclusion

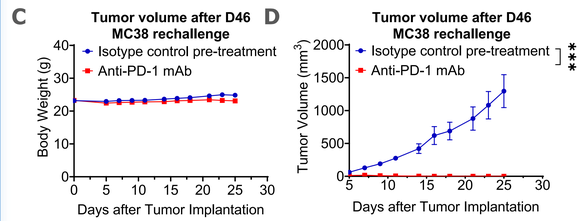
- The immune checkpoint blockade shaped a durable anti-tumor response via memory T-cell infiltration at the tumor and peripheral levels.
- Anti-PD1 therapy potentiated the tumor-killing capability of tumor-infiltrating T cells.
- Simultaneously, the treatment facilitated reprogramming of T-cell subsets in PBMCs.

1

Anti-PD-1 mAb treatment inhibited tumor growth and induced long-term anti-tumor effect



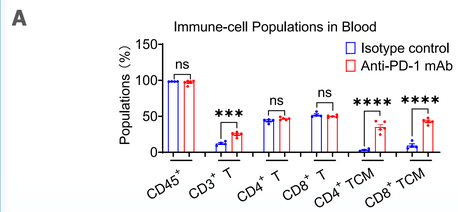
Anti-tumor activity of anti-PD-1 mAb. (A) Body weight measurements did not show any significant treatment-related changes, suggesting no severe side effects in mice. (B) Anti-PD-1 mAb at 10mg/kg inhibited tumor growth by 58 %, compared to the isotype control group.



Anti-PD-1 mAb treatment resulted in resistance to tumor re-challenge. (C) Body weight measurements showed no significant treatment-related changes. (D) Animals exhibiting complete tumor regression following anti-PD-1 mAb treatment demonstrated robust resistance to tumor re-exposure, compared with those in the isotype control-pretreated group.

2

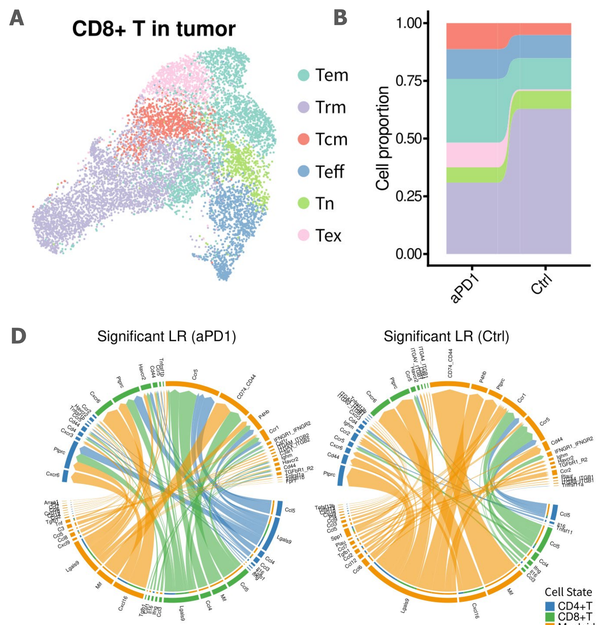
Anti-PD-1 mAb enhanced long-term memory phenotype of peripheral CD4⁺ and CD8⁺T cells



Effects of anti-PD-1 on memory T cell formation. (A) Anti-PD-1 mAb administration significantly increased percentages of long-lived CD8⁺ CD44⁺CD62L⁺ and CD4⁺ CD44⁺CD62L⁺ central memory T cells (TCM) in peripheral blood.

3

Single-Cell Analysis Reveals the Impact of Immune Checkpoint Blockade on the Tumor Microenvironment



Anti-PD 1 therapy remodels the T cell compartment and augments myeloid–T cell crosstalk in the tumor microenvironment.
(A) UMAP (Uniform Manifold Approximation and Projection) plot illustrating all subtypes of CD8⁺ T cells in a mouse tumor.
(B) Bar plot displaying cell proportion of CD8⁺ T cell subtypes in anti-PD1-treated (aPD1) and control (Ctrl) groups.
(C) Bar plot of significantly enriched GO terms in each group.
(D) Chord plot showing the significantly enriched interaction network between myeloid cells and T cells in aPD1 group (left panel) and Ctrl group (right panel). LR, Ligand-Receptor
(E) Bubble plot showing enriched communication ligand-receptor (LR) pairs from myeloid to T cells (left) and from T to myeloid cells (right).
(F) Differentiation trajectories of CD8⁺ (left) and CD4⁺ T cells (right panel).

Tn – naïve T cells; Teff – effector T cells; Tcm – central memory T cells; Tem – effector memory T cells; Trm – tissue-resident memory T cells; LR – ligand receptor