1012 DIFFERENTIATION PATHWAYS OF EXHAUSTED CD8+ T CELLS IN HUMAN BREAST TUMORS

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Background The majority of patients with either estrogen receptor positive (ER+) or triple negative (TN) breast cancer (BC) tumors demonstrate poor response to immune checkpoint blockade (ICB) therapies. Understanding what features of tumor biology and immune composition drive the generation of tumor specific T cells is critical towards improved immunotherapies for BC patients. The presence of tumor infiltrating exhausted CD8+ T cells (Tex) have been well-described to positively associate with response to ICB in a variety of cancer types. Here we profile ICB-naïve human breast tumors and paired tumor draining lymph nodes (TDLNs) to assess the differentiation pathway of Tex.

Methods Fresh surgical tissue specimens were obtained from consented patients. Single cell suspensions were analyzed by flow cytometry for immune phenotyping. Enriched T cells were subjected for single cell whole transcriptome RNA sequencing with paired T cell receptor sequencing and cell surface protein expression.

Results Tex were identified in both ER+ and TNBC tumors by co-expression of PD-1 and CD39. Tex demonstrated a transcriptionally distinct gene expression signature, with noted upregulation of a variety of genes related to exhaustion, checkpoint molecules, and T cell activation. Within the same tumors we identified additional CD8+ T cell subsets, including proliferating, resident memory, effector memory, and progenitor exhausted cells. Notably, we were able to identify sister clonotypes of Tex in other T cell subsets both within the same tumor and within matched TDLNs. Our analysis shows that Tex are composed of an oligoclonal T cell population that highly overlaps with proliferating CD8+ T cells found in the same tumor. However, Tex clonotypes were also observed in other T cell clusters suggesting there is heterogeneous differentiation of tumor specific T cells that occurs within the tumor.

Conclusions This work demonstrates that highly activated, Tex can be found in human breast tumors. We show that differentiation of tumor specific T cells occurs at multiple stages both within the TDLN and downstream tumor tissues. These findings provide insight on potential targets for immunotherapeutic interventions to optimally amplify tumor specific T cells in breast cancer patients.

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Ethics Approval Fresh tissues were obtained from patients who gave institutional review board-approved (IRB-approved) written informed consent prior to inclusion in the study (City of Hope IRB 05091, IRB 07047, and IRB 14346).

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