1027 SCTCR/RNA-SEQ IDENTIFIY TWO EXHAUSTED T CELL SUBSETS IN NEOANTIGEN-SPECIFIC CD8+ T CELLS

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Background Accurate evaluation of neoantigen (neoAg)-specific T cell status is crucial for effective immunotherapies. In a previous study, we identified three neoAgs in two murine gastric cancer cell subclones: YTN2, which regresses spontaneously in a $CD8^+$ T cell-dependent manner, and YTN16, which exhibits progressive growth in B6 mice.[1] The objective of this study was to explore the phenotypes of neoAg-specific $CD8^+$ T cells within these tumors.

Methods We performed single-cell RNA sequencing (scRNA-Seq) and T cell receptor sequencing (scTCR-Seq) on $CD8^+$ T cells infiltrating YTN2 and YTN16 tumors. Subsequently, we cloned five and four TCRs from YTN2 and YTN16, respectively, based on scTCR-Seq data. The cloned TCRs were retrovirally transduced into TG40 cells, and their reactivity to tumor cells and the three neoAgs (mCdt1, mScarb2 and mZfp106) was assessed. Furthermore, the phenotypes of neoAg-specific T cells within the tumors were evaluated using scRNA-Seq data.

Results All TCR-transduced TG40 cells exhibited reactivity to their corresponding tumor cells. Among the five TCRs derived from YTN2, three did not show reactivity to the known neoAgs, while the remaining two TCRs recognized mZfp106. Two TCRs from YTN16 recognized mZfp106, and the other two TCRs exhibited reactivity to mScarb2. Based on the scRNA-Seq data, the majority of neoAg-specific T cells in YTN2 exhibited a proliferative phenotype, whereas those in YTN16 were identified as exhausted T cells (Tex). Further reclustering of neoAg-specific Tex cells revealed two additional clusters: highly glycolytic ($Tex^{Glycolysis}$) and Tox^+ (Tex^{Tox+}) cells. The proportion of $Tex^{Glycolysis}$ and Tex^{Tox+} in YTN16 was comparable. T cells with low and high avidity TCRs were enriched in $Tex^{Glycolysis}$ and Tex^{Tox+} cells, respectively. Pseudotime analysis suggested a trajectory from $Tex^{Glycolysis}$ to Tex^{Tox+} cells. Bulk RNA-Seq analysis of tumor tissues indicated higher glycolysis levels in YTN16 compared to YTN2. From these data, we hypothesized that neoAg-specific $Tex^{Glycolysis}$ cells still retained proliferative capacity and anti-tumor activity, but high glucose consumption in YTN16 tumor microenvironment made these cells non-proliferative. To reduce glucose consumption by YTN16 tumors, we generated YTN16 lacking the Aldob gene, which is involved in glycolysis and highly expressed in YTN16 cells, using the CRISPR/Cas9 system. Aldob KO YTN16 tumors regressed in a $CD8^+$ T cell-dependent manner and exhibited higher infiltration of $CD8^+$ and $CD4^+$ T cells compared to wild-type tumors.

Conclusions NeoAg-specific exhausted $CD8^+$ T cells include a substantial proportion of a highly glycolytic subset. Tumor glycolysis-targeted therapy may reinvigorate these T cells and suppress tumor growth.

REFERENCE

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