THE SIGNAL STRENGTH OF SIGNAL 2 AND 3 DURING T CELL PRIMING AFFECT THE FUNCTIONAL FATE OF AN ANTI-TUMOR T CELL RESPONSE

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Background Fate decision in CD8+ T cells refers to the differentiation that occurs when a naïve T cell enters into effector, memory, exhausted, or other states. Multiple models of fate decision have been proposed, but the exact determinants of T cell fate are unknown. Evidence from chronic infection and cancer models support the notion that signals during T cell priming are especially important in determining both T cell fate and the functional quality of the T cell response. Priming interactions are highly complex, requiring the integration of multiple signals, each of which can vary in strength. Thus, it is unsurprising that we currently do not comprehend the combinatorial specificities and molecular underpinnings of priming interactions. Understanding the factors that drive different T cell fates could be therapeutically harnessed to reduce exhaustion phenotypes and increase effector functions and memory potential of CD8+ T cells in cancer.

Methods We used therapeutic interventions as a tool to alter signals received by a T cell during priming, and characterized the impact on T cell fate. Using a BRAFV600EPTEN-/- melanoma cell line that expresses the model antigen SIYRYYGL (SIY), we implanted tumors into mice and provided therapy during priming. We surveyed the effects of checkpoint blockade therapy (aPD1 and aCTLA4), costimulatory agonists (aCD40 and a41BB), and Type-I Interferon (IFNa and IFNb) on tumor-reactive CD8+ T cells during priming via flow cytometry and single-cell RNA sequencing, and the functional impact of these altered priming signals.

Results All treatments resulted in an increase of effector-like (TCF1+TIM3+) tumor-reactive T cells. However, each intervention induced differential expression of transcription factors. Single-cell RNA sequencing of tumor-reactive CD8+ T cells from the tumor-draining lymph node revealed distinct clustering of T cells based on the therapeutic intervention administered during priming. IFNb-treated T cells had especially high expression of genes associated with cytotoxic capacity, consistent with the activation of an effector-fate program, while aPD1-treated T cells were transcriptionally similar to untreated controls, implying no fate change was induced. Tumor outgrowth, phenotyping at later timepoints, and functional studies indicated that CD8+ T cells primed with therapeutic intervention adopt different cell fates.

Conclusions Priming signals are crucial determinants of T cell fate. Changing the signals received by a CD8+ T cell during priming results in an altered phenotypic and transcriptional profile early during priming. These early transcriptional changes appear to generate different CD8+ T cell fates, providing an opportunity to improve anti-tumor immunity particularly in a neoadjuvant setting.

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