

**CD8 T CELL ACTIVATION IN CANCER IS COMPRISED OF TWO DISTINCT PHASES**

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**Background** CD8 T cells are a crucial part of the immune response to tumors, with CD8 infiltration predicting disease progression in numerous cancer types. Recently two subsets of CD8 T cells that respond to tumors have been described, a stem-like (TCF1+) CD8 T cell that can give rise to a more cytotoxic terminally differentiated (TD) (TCF1-Tim3+) CD8 T cell. In this study we aimed to understand the origin of stem-like TCF1+ CD8 T cells within tumors.

**Methods** Human patient TDLN and tumor samples from kidney and prostate cancer were processed after resection and used for flow cytometry, RNA-seq, TCR-seq and whole genome DNA methylation analysis. We also used a prostate cancer mouse model that expresses the LCMV GP protein (TRAMP1-LCMV-GP) to track tumor-specific CD8 T cells in both TDLNs and tumors.

**Results** We studied human prostate and kidney cancer tumor-draining lymph nodes (TDLN) and found that CD8 T cells are activated but fail to acquire an effector phenotype within the TDLN. Instead, they share functional, transcriptional, and epigenetic traits with stem-like cells in the tumor. We also found that activated CD8 T cells from TDLNs shared TCR overlap with both CD8 subsets within tumors. This suggests that these activated cells are a precursor to the stem-like CD8 T cells in tumors. To further test this hypothesis, we used our TRAMP1-LCMV-GP tumor model to study tumor-specific CD8 T cell activation. We found that CD8 T cells are activated in TDLNs but fail to acquire an effector program. These cells then establish the stem-like CD8s within tumor where they require additional co-stimulation from antigen presenting cells to differentiate into TCF1- TD CD8 T cells. This is strikingly different from canonical CD8 T cell activation to acute viruses, where the effector program is acquired immediately. We also showed that human stem-like CD8 T cells require co-stimulation and TCR stimulation to divide and differentiate into terminally differentiated CD8s in-vitro, and DCs from autologous tumors can also induce this differentiation.

**Conclusions** Overall this work shows a model of CD8 T cell activation in response to tumors that has two distinct phases. The first occurs in the TDLN where CD8 T cells are initially activated, the second occurs in the tumor where CD8 T cells acquire an effector function after additional co-stimulation. This model of T cell differentiation adds to our understanding of basic CD8 T cell biology and has important implications to improve our current immunotherapies.

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