920 ETS-1 REGULATES THE ACTIVATION OF HUMAN CD8 T-CELLS IN RESPONSE TO IMMUNE CHECKPOINT INHIBITORS

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Background It is estimated approximately ~12% of checkpoint blockade recipients respond to treatment. We have previously identified NKG7 as a therapeutic target that is upregulated in CD8 T cells of responders and now ETS-1(Ets Proto-oncogene 1) as an upstream modifier of its expression. With a focus on mRNA therapeutics, we have identified ETS-1 as a modulation candidate capable of increasing response to immunotherapy. ETS-1 in hematopoietic stem cell development has been extensively researched, yet its role in mature CD8 T cell activation and persistence is still lacking. By combining RNAseq, Western blot, and cytotoxicity assay data from human CD8 T cells derived from healthy donors we identified the transcription factor ETS-1 plays an important role in a checkpoint-dependent (PD-1/PD-L1) adaptive immune response.

Methods Utilizing in silico analysis and transcription factor binding motif prediction software (PROMO) we identified ETS-1 binding motif upstream of NKG7. From here we nucleofected ETS-1 siRNA in human primary CD8 T cells to observe the consequence on NKG7 protein expression via Western blot. From here we validated that ETS1 functions as a negative regulator for expression of NKG7 and Granzyme B mRNA via RT-qPCR and Western blot in resting CD8 T cells. Next, we submitted ETS-1 knockdown samples for bulk RNA-seq and differential gene expression (DEG) analysis (3 control/ 3 siETS1). (adj p-value <0.05 and $|\text{Log2 FC}| > 0.5$). Lastly, for a functional readout, we utilized our calcein-release cytotoxicity assay. We cocultured control and siETS-1 CD8 T cells with/without Pembrolizumab (anti-PD-1) with calcein-labelled target cells (MCF7) quantifying cytotoxic tumor killing.

Results Knockdown (KD) of ETS-1 in resting human primary CD8 T cells resulted in higher expression of NKG7 in 6 of 7 RT-qPCR samples and 3 of 3 Western blot assays (p value 0.11). DEG analysis of CD8 T cells revealed 123 genes differentially expressed in response to KD of ETS-1. GO pathway enrichment analysis showed significant association of ETS-1 KD with T cell activation. In line with this, ETS1 KD in CD8 T cells resulted in higher cytotoxicity for 9 of 10 healthy donors (median control 28.2%/ siETS1 36.7% p value = .0184). Lastly, ETS-1 KD led to increase of T-cell cytotoxicity for 2 of 3 donors in response to PD-1/PD-L1 checkpoint blockade in vitro.

Conclusions We demonstrate the selective knockdown of ETS-1 in primary CD8 T cells not only increases cytotoxicity but also displays a potential avenue for increasing the response rate of immunotherapy via gene expression augmentation.

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