Abstracts

ADGRG1 CELL SURFACE EXPRESSION ENRICHES FOR NEOANTIGEN-REACTIVE CD4 LYMPHOCYTES FROM HUMAN TUMORS

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Background Treatment with naturally occurring antitumor T cells, or cells genetically modified to express their receptors (TCRs), has shown efficacy in patients with advanced malignancies.1-3 Previous studies have demonstrated success in defining the unique transcriptomic states of antitumor CD8+ and CD4+ tumor infiltrating lymphocytes (TIL) in human cancer.4-6 We recently described a CD4+ TIL transcriptomic signature (CD4+ NeoTCR) for predicting neoantigen-reactive CD4+ TCRs from metastatic tumors.7

As opposed to transcriptomic analysis, cell surface marker-based isolation of antitumor TIL would allow growth and expansion of cells for downstream applications. Previous work demonstrated that reactivity to tumor cell lines, oncoviral proteins, and patient-specific neoantigens are reliably enriched when selecting for CD8+ lymphocytes expressing specific surface markers.8-11 Although enrichment for neoantigen reactivity amongst CD4+ TIL has been demonstrated with such markers as PD-1CD39 (4.5% median reactive cells within population) and PD-1/ICOS co-expression (10%), an optimal signature for CD4+ TIL has yet to be defined.12 13 Thus, additional markers warrant investigation.

Methods Tumor digest of metastatic lesions from three patients with stage IV colon adenocarcinoma underwent single cell transcriptomic and CITE (cellular indexing of transcripts and epitopes)-seq. The CD4+ NeoTCR transcriptomic signature was used to identify likely neoantigen-reactive CD4+ clusters within samples.7 CITE-seq was used to examine the expression of 130 cell surface proteins to determine differentially expressed proteins that correspond to the CD4+ NeoTCR transcriptomic state. Several surface proteins expressed across CD4+ NeoTCR clusters of interest were selected as candidates and their performance isolating neoantigen-reactive CD4 + TCRs was validated using FACs followed by scTCR-seq.

Results CITE-seq analysis showed several candidate cell surface protein markers that were found across CD4+ NeoTCR clusters including PD-1, ADGRG1, CD86, and CD57. FACs-sorting of CD4+ cells expressing candidate markers, followed by scTCR-seq, showed ADGRG1 was the most successful single marker in isolating cells found in the CD4+ NeoTCR transcriptomic states within two patients (34.21–68.57%). From our library of 34 experimentally defined neoantigen-reactive CD4+ TCRs, ADGRG1 enrichment of neoantigen-reactive CD4+ TIL was 2.9-fold greater compared to bulk CD4+ cells (21.21% reactivity vs. 7.22%). The next best markers were CD86 with a 2.0-fold (14.71%), and PD-1 with a 1.73-fold (12.5%) improved capture of neoantigen-reactive CD4+ TIL.

Conclusions ADGRG1, as a single cell surface marker, showed the highest efficiency in isolating neoantigen-reactive CD4+ TCRs from metastatic solid tumors and could be an important marker in enriching for neoantigen-reactive CD4+ TIL. Future experiments will further evaluate these markers of interest in prospective patient samples.

REFERENCES