Targeting CEACAM5 as a tumor-associated surface antigen in neuroendocrine prostate cancer

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Background: Neuroendocrine prostate cancer (NEPC) is a highly aggressive variant found in up to 20% of lethal metastatic castration-resistant prostate cancers. Patients diagnosed with NEPC face a grim prognosis as no standard and effective treatments exist for NEPC. In most cases, NEPC arises from prostate adenocarcinoma through a process of transdifferentiation as a mechanism of treatment resistance. NEPC is a divergent cancer differentiation state marked by substantial DNA methylation, gene expression, and phenotypic changes. Cell surface phenotypes often reflect distinct differentiation states of cells in normal development and cancer. Defining unique cell surface markers would allow for the development of targeted antibody-based therapies for NEPC.

Methods: RNA-Seq gene expression analysis and cell surface proteomics were performed on a panel of human prostate cancer cell lines representing NEPC and prostate adenocarcinoma. Rank-rank hypergeometric overlap analysis was performed on differentially expressed cell surface genes and cell surface proteins to identify high-confidence NEPC cell surface candidates. CEACAM5 expression was confirmed by immunoblot, immunohistochemistry, and flow cytometry of NEPC cell lines. Further characterization of CEACAM5 expression was performed by immunohistochemistry on LuCaP patient-derived xenografts, small cell NEPC tissues archived at UCLA, and a prostate adenocarcinoma tissue microarray.

Chimeric antigen receptors (CARs) were cloned using single chain variable fragments specific for CEACAM5. CAR-transduced human T cells were co-cultured with CEACAM5+ (NCI-H660) and CEACAM5- (MSKCC EF1) NEPC cell lines. T cell activation and cytotoxicity were determined by measurement of interferon-γ release by ELISA and NEPC cell enumeration by time lapse microscopy.

Results: CEACAM5 was nominated as a NEPC cell surface antigen by rank-rank hypergeometric overlap analysis of the prostate cancer cell lines. RNA-Seq gene expression data (from Beltran et al., Nature Medicine, 2016) showed outlier levels of CEACAM5 gene expression in 66.7% (10/15) of NEPC samples. CEACAM5 protein expression was confirmed in the NCI-H660 NEPC cell line by immunoblot and flow cytometry. Further, 100% (4/4) of NEPC LuCaP xenografts and 61.1% (11/18) of small cell NEPC samples archived at UCLA demonstrated intermediate to strong staining by CEACAM5 immunohistochemistry. In contrast, CEACAM5 was expressed in 0% (0/22) of prostate adenocarcinoma LuCaP xenografts and in 0% (0/33) of prostate adenocarcinoma samples including two metastatic tissues.

Multiple second- and third-generation CAR clones were engineered that demonstrate antigen-specific T cell activation and cytotoxicity when co-cultured with NEPC cell lines. In vivo evaluation of these CEACAM5 CAR-T cells in mouse xenograft models of NEPC is ongoing.

Conclusions: CEACAM5 is a tumor-associated surface antigen that is expressed in over 60% of small cell NEPCs. Our studies provide a strong rationale for the clinical evaluation of anti-CEACAM5 antibody-drug conjugate therapy and the continued development of CEACAM5 CAR-T cell immunotherapy in NEPC.

Conflict of Interest Statement: No conflict of interest declared.