T cell receptor engineering targeting FOXM1 for the treatment of lung cancer

Isabel Perez-Sandi, Minying Zhang, Hui Nie, Peixin Jiang, Cassian Yee, Gregory Lizee, John V. Heymach, Alexandre Reuben

All authors from The University of Texas MD Anderson Cancer Center

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Abstract

Background: Despite its success, checkpoint blockade immunotherapy has proven challenging in selected lung cancer patient populations. This is in part due to the extensive intratumor heterogeneity at play and infiltration of bystander T cells which recognize non-tumor antigens. Recent clinical trials have demonstrated some efficacy for adoptive cell therapy using bulk unenriched tumor-infiltrating lymphocytes, but success remained limited. Accordingly, novel tumor antigens are needed to further improve upon this success of cellular immunotherapy in lung cancer. Forkhead Box M1 (FOXM1) is a transcription factor expressed in 90% of lung cancers and lacks expression in brain tissue, making it an appealing target for T cell receptor (TCR) engineering. Interestingly, up-regulation of FOXM1 is associated with drug resistance to tyrosine-kinase inhibitors (TKIs), highlighting another potential therapeutic application for this target. Here, we assessed the immunogenicity of FOXM1 and its potential as a cellular therapy target in non-small cell lung cancer.

Methods: Antigen-specific T cells were isolated and then expanded by peptide stimulation of HLA-matched healthy donor PBMCs. Antigen-specific T cells were then isolated by tetramer sorting and underwent single-cell TCR sequencing to identify full length alpha and beta chains of the TCR. TCRs were retrovirally-engineered into healthy donor PBMCs and function was assessed via chromium-51 release (cytotoxicity), ELISpot (IFN-γ secretion) and ELISA (MIP-1β secretion).

Results: An epitope of FOXM1 (*YLVPIQFPV*) was immunogenic when presented on HLA-A*02:01 (42% of United States population). This epitope was confirmed to be naturally-processed and presented using H1975 cells. Assessment of cytotoxicity revealed that 51% of H1975 cells were lysed by TCR-engineered PBMCs, compared to only 10% for H1975 parental cells devoid of FOXM1 expression ($p<0.0001$). Cytokine assessment via ELISpot demonstrated a significant increase in IFN-γ spots ($p<0.05$) and MIP-1β secretion by ELISA ($p<0.05$).

Conclusion: Our findings confirm the immunogenicity of FOXM1 when presented on the most prevalent HLA allele in the United States and support the feasibility of TCR-engineered targeting FOXM1 for the treatment of lung cancer.

FOXM1 expression profile

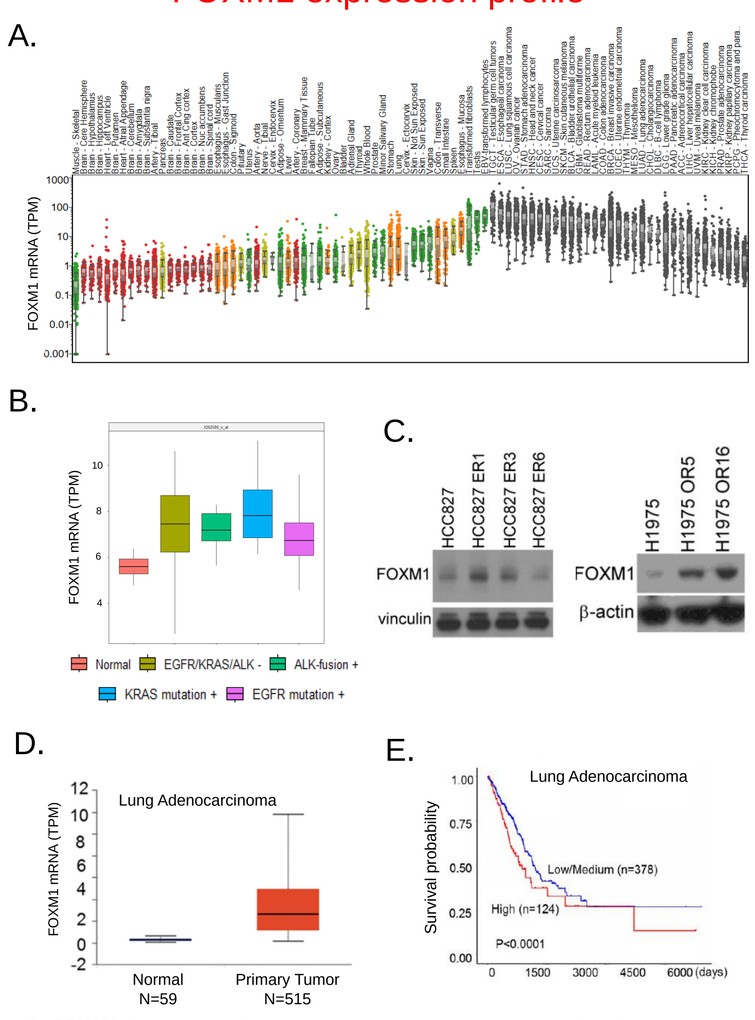

Fig. 1. FOXM1 is expressed in many cancer types, associated with resistance and shortened survival. A) *FOXM1 expression across healthy tissues and cancers.* ***B)*** *FOXM1 expression in EGFR/KRAS/ALK-mutant lung adenocarcinoma [Okayama et al., Cancer Res (2012)].* ***C)*** *FOXM1 in EGFR TKI resistant & parental cell lines [Nilsson et al., Sci Transl Med (2020)]* ***D)*** *Overexpression of FOXM1 LUAD vs adjacent normal lungs.* ***E)*** *Survival according to FOXM1 expression [Liao et al., Cell Comm.& Sig (2018)].*

HLA allele prevalence

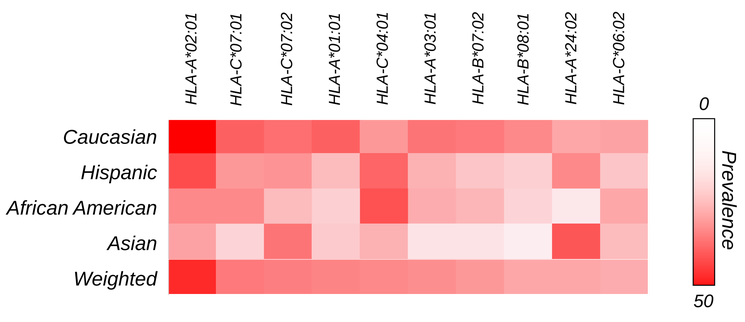

Fig. 2. Frequencies of the ten most common HLA alleles in the United States. *HLA-A*02:01 is most prevalent HLA among US demographics, and therefore was chosen for FOXM1-specific T cell generation [Adapted from Pearlman et al., Nature Cancer (2020)].*

T cell generation workflow

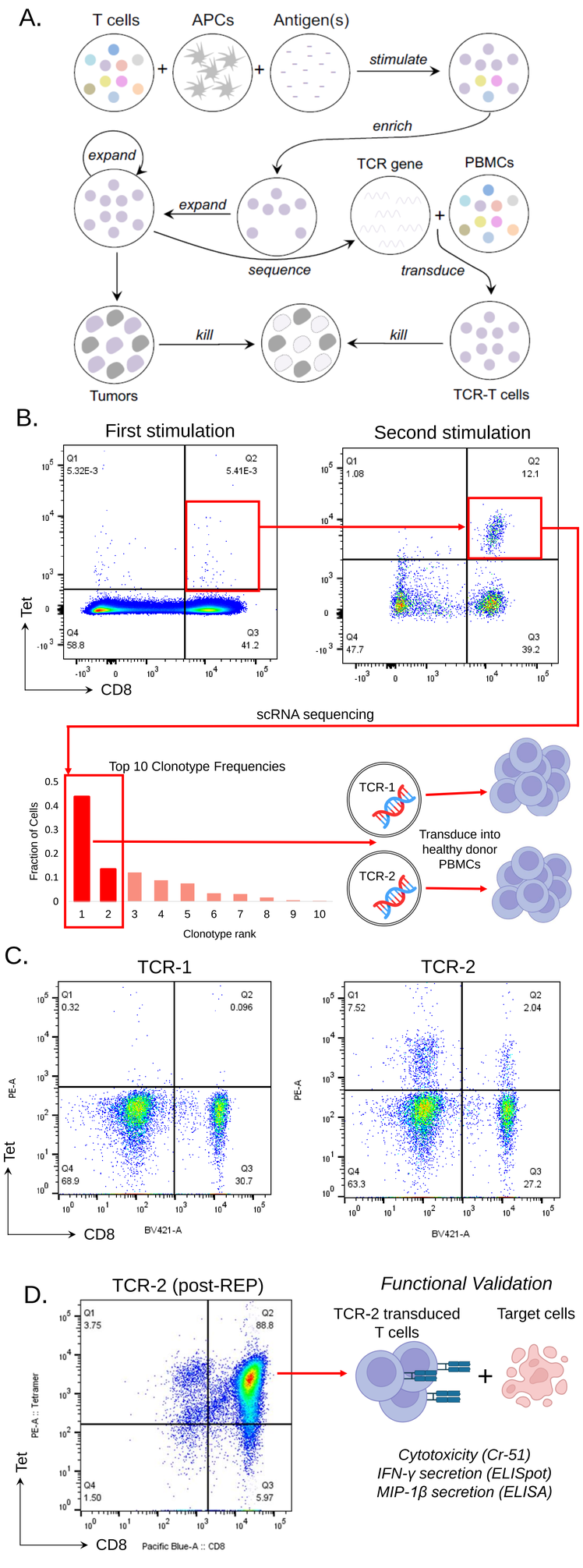

Fig. 3. FOXM1 epitopes are immunogenic and can be targeted by T cell engineering. A) *Overview of workflow for antigen-specific T cell generation and validation.* ***B)*** *First sorting of healthy donor PBMCs stimulated against FOXM1 HLA-A*02:01 peptides followed by scRNA sequencing to identify the FOXM1 TCR clonotypes most highly expressed in the sorted population. The top 2 were subsequently transduced into healthy donor PMBCs.* ***C)*** *CD8/tetramer staining for TCR-1 and TCR-2 transductions.* ***D)*** *TCR-2 was increasingly expressed and expanded through Rapid Expansion Protocol (REP) for subsequent functional validation. [cell icons sourced from BioRender.com]*

FOXM1 T cell reactivity

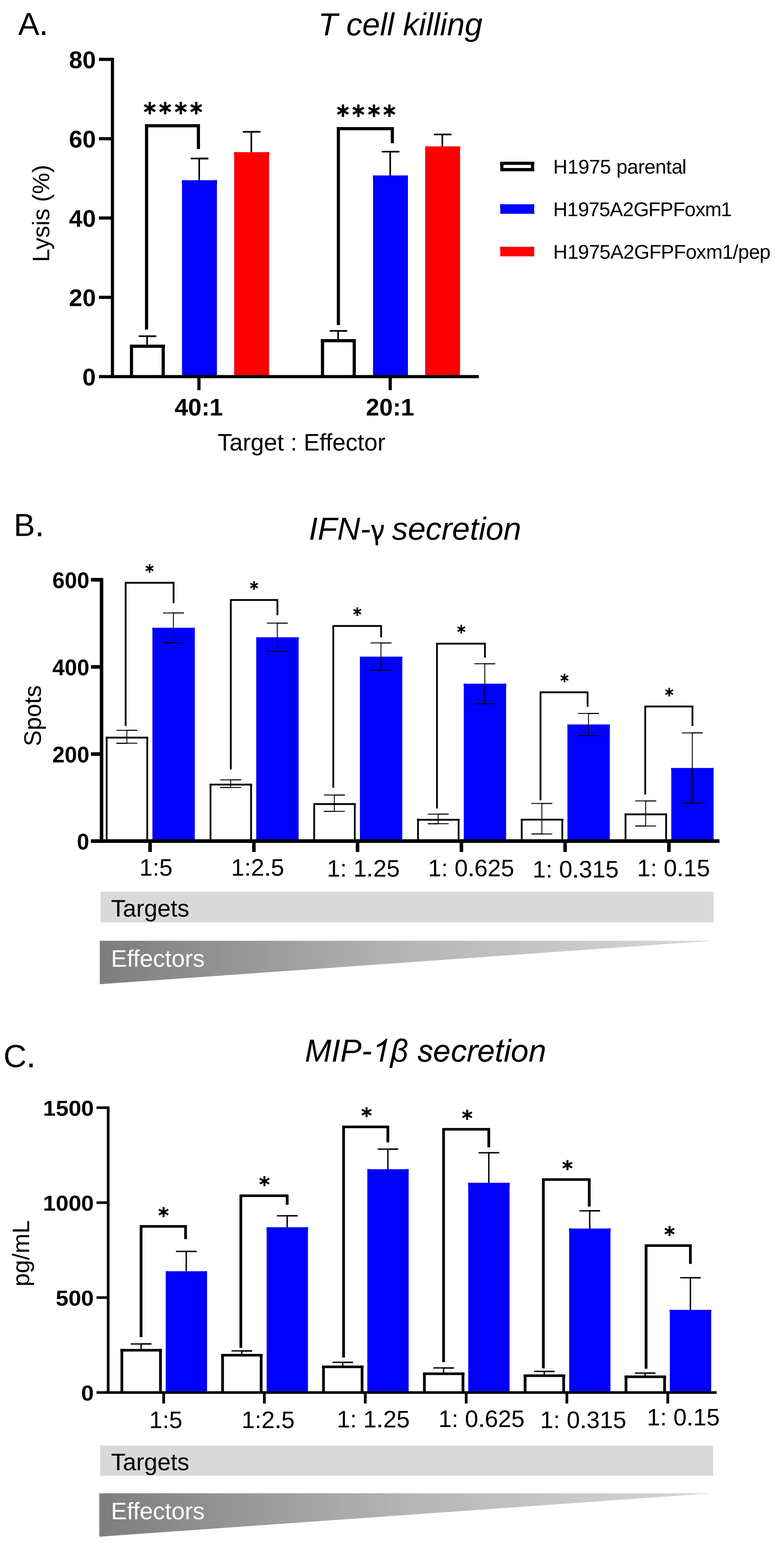

Fig. 4. FOXM1-derived epitopes are targetable via TCR engineering. A) *TCR-2 was successfully engineered into healthy donor PBMCs. Cytotoxicity was assessed by chromium release assay.* ***B)*** *IFN-γ production by ELISpot and* ***C)*** *MIP-1β production by ELISA in response to antigen-expressing target cells.*

Conclusions

- FOXM1-derived epitopes are immunogenic when presented on HLA-A*02:01;
- FOXM1-derived epitopes are naturally endogenously-processed and presented on HLA-A*02:01;
- T cell receptor engineering confers FOXM1-specificity to healthy donor PBMCs.

Acknowledgements

This work was supported by the University of Texas MD Anderson Cancer Center Lung Cancer Moon Shot and the American Association for Cancer Research. We thank the MD Anderson Summer Experience Program Staff for their support and commitment to summer students' success. Most importantly, we thank our patients and their families.