IDENTIFYING CANCER-SPECIFIC T CELLS FOR IMMUNOTHERAPY THROUGH ENGINEERED PROTEINS

Rongyu Zhang*, Institute for Systems Biology, University of Washington, Seattle, WA, USA

Background The identification of cancer-specific T cell receptor (TCR) sequences is paramount to the advancement of cancer immunotherapies. Recent studies and clinical trials have shown that monoclonal T cell therapy is prone to immune evasion of cancer cells by loss of HLA heterozygosity and low antigen heterogeneity. Cocktail T cell therapy which comprises of TCRs corresponding to multiple HLAs and antigens has been proposed to improve the efficacy of adoptive cell transfer therapy. In addition to CD8+ cytotoxic T cells, neoantigen-specific CD4+ T cells, while identified as important for immunotherapy-induced anti-tumor responses, remain a largely untapped therapeutic resources due to the challenging nature of identification and isolation. Hence, a rapid and high-throughput discovery of both CD8+ and CD4+ TCRs against multiples Class I and II HLAs and cancer antigens is an urgent need. We engineered peptide-bound major histocompatibility complex (pMHC) proteins as capture agents for cancer-specific T cells. The design of these single-chain-trimers (SCTs) enables high-throughput multiplexing for identification and isolation of cancer-targeting CD4+ and CD8+ T cells from multiple patients against large panels of cancer antigens. We applied the technology to identify CD8+ and CD4+ TCRs against oncoproteins E6 and E7 from HPV-16, which is the leading cause of cervical cancer.

Methods A panel of 200+ Class I SCTs and 100+ Class II SCTs were designed and expressed in a high-throughput platform. PBMCs from precancerous HPV-16+ patients with cervical lesions were collected and enriched with CD8+ and CD4+ T cells. A large pool of 200+ Class I SCT tetramer pool with barcode as antigen identifier was used to capture cancer-specific CD8+ T cells. A computational analysis pipeline was established to pair TCR α and β. HLA-matching cognate antigen was assigned to each TCR pair after UMI count correction and noise removal. The antigen-specific TCRs are subsequently sequenced, validated for functionality, and analyzed for therapeutic applications.

Results We identified 43 CD8+ TCR pairs against E6 and E7 oncoproteins from HPV-16 and they are in progress for preclinical validation.

Conclusions The SCT platform enables rapid identification of cancer-specific CD+ and CD4+ T cells and allows detailed characterization of anti-tumor T cells for which alternative solutions are extremely limited. We applied the technology to PBMCs extracted from HPV-16 related precancerous patients in a clinical trial and discovered cancer-specific TCRs. In summary, the application of the SCT technology is of high value to the fundamental and clinical immune-oncology studies.

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