A CLONING AND EXPRESSION SYSTEM OF THE NEOANTIGEN-SPECIFIC TCRs FROM TUMOR-INFILTRATING LYMPHOCYTES BY SINGLE-CELL SEQUENCING OF PAIRED TCR α AND TCR β CHAINS

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Background T-cells that target tumor neoantigens arising from cancer mutations are the primary mediators of cancer immunotherapies. Identifying neoantigens and T-cells that recognize them is essential for T-cell-based immunotherapy. However, neoantigen-reactive Tumor-infiltrating lymphocytes (TILs) are highly differentiated or exhausted with a limited proliferative capacity; it is challenging to expand them for a sufficient number to probe their specificity. Therefore, we developed a novel cloning and expression system to examine TCRs discovered by single-cell sequencing of TILs for their neoantigen-specificity.

Methods TILs of lung cancer and sarcoma were analyzed. Surgically removed tumors were divided into several pieces. They were enzymatically digested to prepare fresh tumor digest (FTD) and cryopreserved. They were used to generate TIL cultures and perform WES and RNA-Seq to identify tumor-specific mutations. MHCflurry was used to predict the binding affinity of potential epitopes arising from these mutations to HLA class I. Peptides that were predicted to bind to patients' own MHC class I molecules strongly were then synthesized. Single TILs isolated with the ICELL8[®] cx system (Takara Bio) were dispensed into a nanowell TCR chip containing pre-printed barcodes. Barcoded cDNAs were PCR-amplified in-chip, pooled off-chip, and used as a template in the TCR-specific PCR or for the whole transcriptome library generation of 5' ends of all transcripts. Based on single-cell transcriptome data and TCR profiles of TILs, we predict and prioritize neoantigen-specific TCRs and cloned them into siTCR[®] retrovirus vectors. These TCRs were transduced into SUP-T1-based reporter cells in which ZsGreen fluorescent protein expression is controlled by AP-1 and NFAT binding sites. TCR-expressing reporter cells were cocultured with patient autologous APCs pulsed with a pool of candidate neoantigen peptides. ZsGreen expression indicates that TCRs match their cognate neoantigens.

Results In a lung cancer patient, we set up 18 TIL cultures and obtained 12 TILs. TILs were cocultured with FTD; IFN- γ production was measured by ELISA to evaluate their reactivity to the autologous tumor. NGS identified 197 somatic mutations, 4 fusion genes, and 8 highly expressed cancer-testis antigens. Among them, 339 candidate peptides were synthesized and screened. In addition, we cloned 3 pairs of TCR $\alpha\beta$ chains from most expanded TIL cultures and 4 TCRs from ex vivo TILs with exhausted phenotype. Two reporter cells that express TCRs from exhausted TILs responded to the same neoantigen peptide.

Conclusions Generating TCR expressing cell lines facilitated the identifying neoantigens and their cognate TCR sequences from patients.

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