Engineering high-affinity human single-chain T cell receptors against cancer antigens

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Single-chain T cell receptors consisting of two linked variable regions (Valpha and Vbeta; referred to as scTv) provide a useful alternative engineering format to single-chain antibody fragments (scFv). Previously, we described the engineering of two high affinity human T-cell receptors (TCRs) in which the usage of the highly stable Valpha2 region when properly paired with different Vbeta regions allowed the TCR to be expressed on the surface of yeast as a scTv, and correlated with the ability to express soluble scTv fragments in *E. coli*. In this study, a wild-type TCR specific for melanoma antigen MART1/Melan-A, called INR1-T1, and a wild-type TCR specific for Wilm's tumor antigen, called WT1 P22, which naturally use the highly stable Valpha2 region, were engineered for high-affinity against their respective peptide antigens via yeast display and fluorescence activated cell-sorting. Alanine mutations of various CDR residues demonstrated the key role of particular residues in contacting the alpha helices of HLA-A2. In addition, we have generated a single TCR platform for yeast display-based engineering of designer TCRs with specificities for diverse peptide antigens. This approach would avoid the need to isolate T cell clones against each peptide antigen, and the subsequent characterization of the TCR genes and products.