TCR-LIKE CHIMERIC-ANTIGEN-RECEPTOR TO RECOGNIZE NEOEPITOPES DERIVED FROM DRIVER MUTATIONS

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Background T cell receptor (TCR)-based cell therapy to treat solid tumors by targeting tumor neoantigens is gaining momentum. While there is an urgent need for discovering novel TCRs, one challenge is that the exogenous TCR may compete with the endogenous TCR for CD3 signaling components and form mixed dimers with potential toxicities. Additionally, the activity of T cells highly depends on signaling machinery only present in T cells, which restricts the scope of eligible therapeutic cells. We developed a TCR-like chimeric-antigen-receptor to both overcome the mispairing of TCR α/β chains and to expand the scope of TCR-based cell therapy to other immune cells.

Methods To identify novel TCRs, a monoclonal TCR discovery platform was developed. A high avidity TCR of a neoepitope-specific CD8+ T cell clone (CTL) that was generated from donor PBMCs was isolated and sequenced. A chimeric-antigen-receptor comprising an antigen binding domain (which is a portion of the identified TCR), a CD28 segment containing the hinge/transmembrane domain, and a cytoplasmic signaling domain containing a Dap10 and a Dap12 intracellular domains. To augment the avidity of the antigen receptor, a chimeric co-receptor was constructed by combining HLA-I binding domains of CD8α with the hinge/transmembrane/cytoplasmic domains of CD4. T cell line (J.RT3-T3.5) and NK cell line (KYGH-1) were transduced by a lentivirus encoding the chimeric-antigen-receptor and co-receptor to assess their expression and function.

Results Two novel TCRs specific for CTNNB1 S37F peptide (YLDSGIHFGA) and for p53 R175H peptide (HMTEVVRHC) respectively were identified; both are high-avidity and can recognize the endogenous neoepitope presented by HLA-A2 in tumor cells (figure 1A and B). A chimeric-antigen-receptor specific for CTNNB1 S37F neoepitope/HLA-A2 was expressed on the surface of 293T cells independent of endogenous CD3 (figure 2A). However, its avidity to recognize CTNNB1 S37F neoepitope was approximately 5 folds lower than that of the native TCR (figure 3A). Co-expression of the co-receptor could significantly enhance the avidity of the chimeric-antigen-receptor and make it capable of recognizing endogenously presented neoepitope in tumor cells (figure 3B). In addition, NK cells re-directed with the chimeric-antigen-receptor could also recognize the neoepitope peptide in the context of HLA-A2 (figure 2B and 4).

Conclusions We validated a TCR-like chimeric-antigen-receptor that can be expressed on cells independent of endogenous CD3 and can recognize neoepitope/HLA-A2. We also designed and tested a co-receptor that can enhance the avidity of the chimeric-antigen-receptor. To further leverage the avidity of the chimeric-antigen-receptor, testing of new configurations of the chimeric-antigen-receptor and co-receptor is underway.
Expression of a TCR-like chimeric-antigen-receptor. TCR-like chimeric-antigen-receptor can be expressed on cell surface independent of endogenous CD3 components. Fig. 2A shows that a chimeric-antigen-receptor specific for the CTNNB1S37F neoepitope peptide in the context of HLA-A2 can be expressed on 293T cells. The additional disulfide bond formed between Ca and Cs of the TCR is essential for the stable expression of the chimeric-antigen-receptor while the intracellular domains of CD28 have no effect. Fig. 2B shows that both T cells (J.RT3.5) and NK cells (KYGH-1) can be re-directed to express exogenous TCR-like chimeric-antigen-receptor to bind the epitope peptide/HLA-I.

Function of a TCR-like chimeric-antigen-receptor on T cells. Fig. 3A shows that a chimeric-antigen-receptor specific for the CTNNB1S37F neoepitope peptide in the context of HLA-A2 (JRT-CTN/dap) can be activated by the neoepitope peptide and its avidity can be enhanced by a co-receptor which is a CD8a/CD4 fusion receptor (CD8/4). Fig. 3B shows that with the help of the co-receptor, the avidity of the chimeric-antigen-receptor is comparable to the native TCR and capable of recognize the neoepitope peptide presented by HAL-A endogenously in tumor cells.

Function of a TCR-like chimeric-antigen-receptor on NK cells. TCR-like chimeric-antigen-receptor on NK cells can functionally recognize the neoepitope peptide presented by HLA-I. NK cells (KYGH-1) that re-directed to express a chimeric-antigen-receptor specific for the CTNNB1S37F neoepitope peptide in the context of HLA-A2 (JRT-CTN/dap) can be activated by the neoepitope peptide presented by HLA-A2 on the target T2 cells.