**A novel intracellular tumor antigen-targeted CAR-T therapy utilizing GITR signaling domain for enhanced therapeutic activity towards human solid tumors**

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CAR-T therapy has already shown to have remarkable efficacies for B-cell linage hematological malignancies. Nevertheless, there are no successful cases of CAR-T therapy for solid tumors. One major reason would be due to the scarceness of cell surface molecules that are truly tumor specific. To overcome this issue, we have developed CAR-T therapy by utilizing T cell receptor (TCR)-like antibodies that specifically recognize intracellular tumor antigen-derived peptide/MHC complex (pMHC). We successfully generated CAR-T targeting a complex of HLA-A*02:01 and an epitope peptide derived from MAGE-A4 protein, a cancer-testis antigen highly expressed in, *e.g.* melanoma, lung cancers, and head and neck cancers. From a human scFv phage library, we isolated one scFv antibody with high affinity and specificity towards MAGE-A4$_{p230-239}$/HLA-A*02:01 complex (MAGE-A4 pMHC). We then established a retroviral vector expressing a CAR derived from the anti-MAGE-A4 pMHC scFv antibody. MAGE-A4 pMHC CAR gene-transduced T cells acquired functional capacity to recognize MAGA-A4$^{+}$ tumor cell lines in an HLA-A*02:01-restricted fashion. Furthermore, adoptive transfer of MAGE-A4 pMHC CAR-T cells significantly suppressed tumor growth of MAGE-A4$^{+}$ HLA-A*02:01$^{+}$ tumor cell line in an immune-compromised mouse model. Determination of key amino acid residues in MAGE-A4$_{p230-239}$ peptide necessary for antigen recognition and analysis of cross-reactivity to HLA-A*02:01-binding peptides unrelated to MAGE-A4 but with high homology revealed that MAGE-A4 pMHC CAR likely does not react with pMHCs other than MAGE-A4 pMHC.

Wider application of CAR-T therapy for solid tumors also faces unique challenges posed by limited *in vivo* persistency and immunosuppressive tumor microenvironment. Given that the types of signaling delivered by intracellular domains (ICD) of CAR defines physiological functions of CAR-T cells, there is a chance that CAR with improved ICD confers on T cells superior *in vivo* persistency and resistance to immunosuppression. The most widely used costimulatory signalling domains incorporated into ICD of CAR are derived from CD28 and 4-1BB (CD137) (28z.CAR). On the other hand, we have developed a CAR containing CD3zeta and GITR-derived ICD (zG.CAR). Adoptive transfer of zG.CAR-T cells into tumor-bearing mice resulted in superior tumor growth control as compared to conventional 28z.CAR-T cells. zG.CAR-T cells exhibited prolonged *in vivo* persistency and resistance to regulatory T cell (Treg)- and PD-L1-mediated immunosuppression *in vivo* and *in vitro*.

In summary, CAR-T therapy using intracellular tumor antigen-targeted TCR-like antibody and GITR signaling domain would be a safe and promising treatment for solid tumors. We are planning its future clinical application.