Adoptive immunotherapy using the Chimeric Antigen Receptor (CAR) gene-modified T cells is a promising strategy to treat patients with malignancy and autoimmune diseases. The latest results of CD19 CAR-T cell clinical trials have demonstrated impressive potential in a range of B-cell malignancies. In spite of the recent treat success, serious adverse events observed after infusion of CAR T cells including “on-target off organ” activation, and cytokine release syndrome has been observed in a number of CAR Tcell therapies as a result of excessive Tcell activation. To improve the efficacy and safety of CAR-T cells, selection of the optimal leader, signal peptide or the spacer sequences between VH and VL, that are expressed only on tumor cells, and the appropriate CAR construct with the optimal T cell activities are essential, thereby minimizing the risk of side effects.

In this study, aiming to select the optimal design of CD19 CAR for effective and safe immunotherapy, using anti-CD19 antibody clone FMC63, we performed detailed analysis of non-specific activation and TCR expression of CAR-T cells caused by the design of scFv (e.g. the leader sequences, the order of VH and VL, the spacer sequences between VH and VL, and the extracellular-spacer domains).

Non-specific activation of CAR-T cells

A. Vector constructs used in this study.
B. Flow cytometric analysis of CAR expression level in TCR or CAR-transduced T cells.
C. Relative CD28 expression level of TCR or CAR-transduced T cells compared to the non gene modified T cells.
D. Relative expression analysis of CAR- or TCR-transduced T cells. NGMC, non gene modified cells; CM, central memory; EM, effector memory; TdEM, terminally differentiated effector memory.

Evaluation of hinge

CAR construct having CD8ε hinge represented higher CD25 and CD69 activation without ligand mediated stimulation. Consequently, highly activated CAR-T cells showed lower antigen specific reactivity of intracellular cytokine secretion activity and cytotoxicity activity.

Evaluation of hinge

A. CAR-B construct represented higher CD25 activation without ligand mediated stimulation. Consequently, higher CD25 activation resulted in the reduction of the naive T cell populations.

Evaluation of hinge

Conclusions/Discussions

There was a trend that higher expression of CAR resulted in higher ligand-independent activation and lower CD28 signal. Especially, the CD19-CAR T cells with CD8ε hinge showed lower antigen specific reactivity of intracellular cytokine secretion activity and cytotoxicity activity.

Disclosures


Okamoto et al., poster presentation at the ASCT 18th Annual Meeting, May 13, 2015, New Orleans, Louisiana, USA.