Best CAR design based on the analyses of ligand-independent activation of CD19-CAR T cells

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Background

Adaptive 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 CAR T-cell immunotherapy clinical trials have demonstrated impressive potential in a range of B-malignant malignancies. In spite of the recent great success, substantial safety concerns occurred after infusion of CAR T cells including "on-target off-tumor" activation, and cytokine release syndrome has been observed in a number of CAR T-cell therapies as a result of excessive T-cell activation. To improve the efficacy and safety of CAR T cells, activation of the target tumor-associated antigens, that are expressed only on tumor cells, and the suitable CAR constructs showing the optimal immune responses are essential, thereby minimizing the risk of side effects.

Methods

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 of CAR19 CAR-T cells caused by the design of scFv anti-CD19 leader sequences, the order of VH and VL, the spacer sequences between VH and VL, and the extracellular-space domains. PBMCs from healthy donors were stimulated with anti-CD3 mononuclear antibody (OKT3) together with recombinant fibronectin fragment (Retronectin®) and transduced with CAR T-cell vectors and further expanded. The resultant CAR-T cells were assessed for their non-specific activation via expression analyses of activation markers CD25 and CD69. CD19 ligand specific activation was analyzed by intracellular cytokine secretion such as IFN-γ and TNF-α in the presence of CD19 positive Raji cells.

Evaluation of hinge

Three kinds of hinge sequence were compared with the same backbone of scFv and intracellular signal domain.

A. Vector constructs used in this study. Hinge sequence were chosen from CD28, CD8α and human immunoglobulin G constant region of light chain (hCD8α-CL). B. Anti-CD19-specific activation by measuring the CD25 and CD69 expression level of CAR-transduced T cells compared to the non gene modified T cells (left panel) and Immunophenotype analysis (right panel). C. CD19-antigen specific activation of CAR-transduced T cells. Intracellular cytokine secretion analysis (left panel) and cytokosty assay of caselin labeled tumor killing assay (right panel).

Fig. 4. Evaluation of hinge sequences tested by scFV of clone FMC63

Evaluation of scFv; FMC63 VH/VL and linker

In order to determine the optimal CAR for clinical use, we compared 4 CARs that contained scFv combinations of VH-VL or VH-VL and the linker regions of variable regions.

A. Vector constructs used in this study. B. Anti-CD19-specific activation by measuring the CD25 and CD69 expression level of CAR-transduced T cells. C. CD19-antigen specific activation of CAR-transduced T cells by Intracellular cytokine secretion analysis. D. Intracellular cytokine production analysis of CAR-transduced NSMC, non gene modified cells; CM, central memory; EM, effector memory, TEM, terminally differentiated effector memory.

Highly activated CAR T cells showed lower antigen specific specificity of intracellular cytokine secretion activity. Finally we chose the orde of VL-VH (LH1) flanked by CD28 extracellular domain and CD28 co-stimulatory molecules as our CAR design.

Conclusions/Discussions

There was a trend that higher expression of CAR resulted in higher ligand-independent activation and lower CD19-specific activity. Especially, the CD8α-CAR T cells with CD8 hinge domain were highly activated with CD19-independent manner. The excessive non-specific activation lowered the cytokine secretion and the cytotoxic activities against CD19 expressing tumor cells. Because of the nature of CAR construct by artificial synthesis, the exact mechanism of ligand-independent activation by CD8 hinge domain is still unclear. However, it is desired to select the optimal design of CAR constructs which show high antigen-specific activities with low non-specific activities for the safe and effective CAR-gene modified T cell therapy. A number of ongoing and planned clinical trials in the world will navigate the future direction toward the best CD19-CAR design.

Fig. 5. Evaluation of scFV; FMC63 VH/VL order and linker sequences.

[Disclosure]