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・3題（各5分発表： 3分口演+2分討論）

Design of chimeric antigen receptors affects the characters of CAR-T cells

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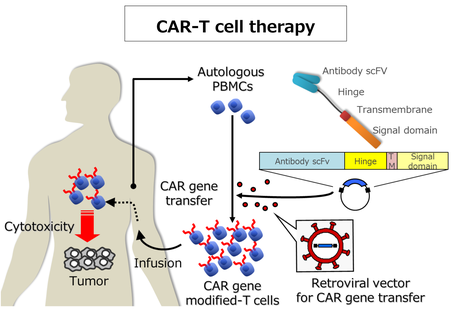
Introduction

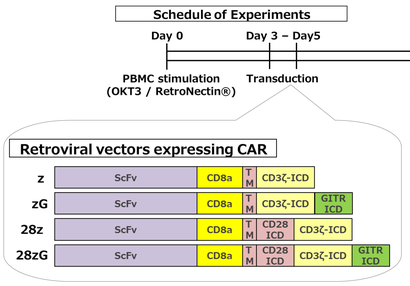
CAR-T cell therapy using chimeric antigen receptor (CAR) transduced T-cells, has recently attracted much attention as one of effective cancer immunotherapies. In particular, CAR-T cell therapy targeting the CD19 antigen of B-cell tumor has been shown to achieve very high response rate to hematologic cancer, and CAR-T targeting CD19 have begun to be approved as new drugs. However, the clinical effect of CAR-T on solid tumors is limited, and a high recurrence rate is pointed out for blood tumors, there is still a need to produce effective CAR-T cells in the body for a long duration while retaining a high therapeutic effect.

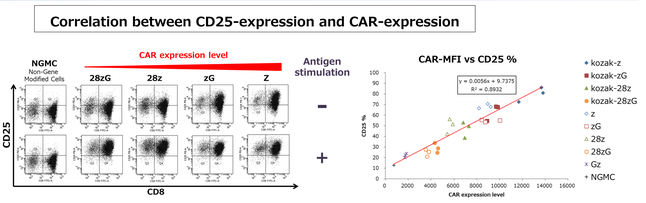
We have been developing CAR constructs focusing on antigen non-specific activation of CAR-T cells due to the design of CARs. Since CAR is an artificial protein in which the single-chain antibody (scFv) and the signal domains are directly linked, CAR-T cells have antigen non-specific activation caused by interaction between CAR and other cellular molecules.

Here, in order to analyze the influence of the extracellular design of CARs on T cells in more detail, we constructed several CARs having different design (the order of scFv variable regions, the linker sequence between the variable regions, the type and length of extracellular spacer region and the transmembrane domains, etc.) and evaluated their characteristics in T cells.



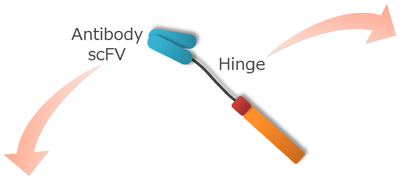
Non-specific activation of CAR-T cells



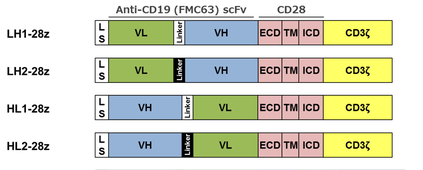


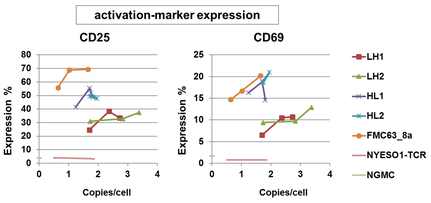
CAR-T cells expressed the activation marker CD25 without antigen stimulation, and CD25 expression levels were correlated with CAR expression levels regardless of the intracellular domain of CARs.

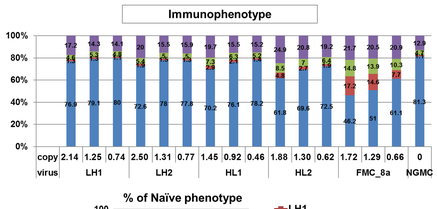
Influence of CAR design on the characters of T-cells

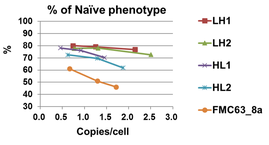


Comparison of ScFv design



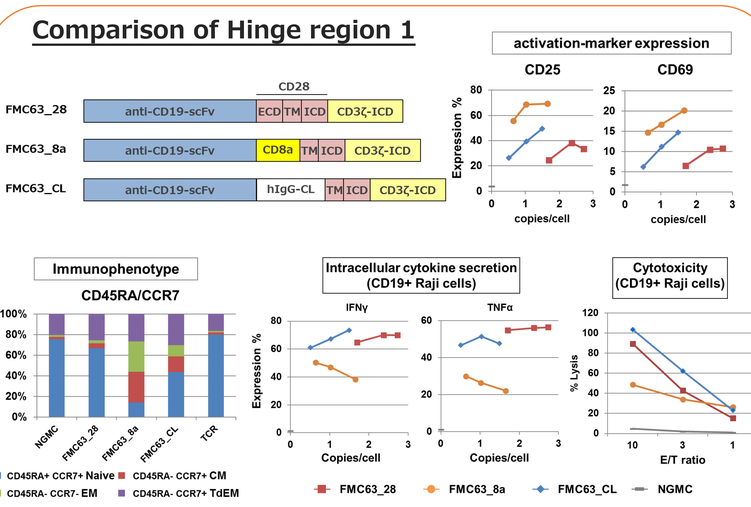




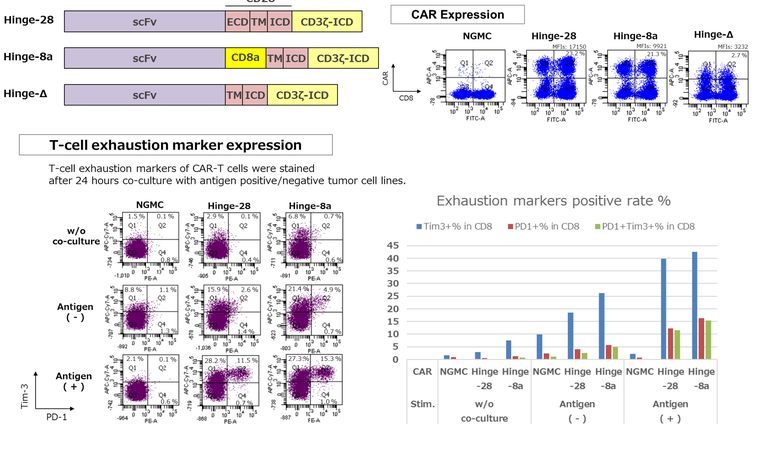


The nonspecific activation and the property of CAR-T cells were also differd depending on the order of V region and the linker sequence in scFv, although the effect was not as great as that of the hinge.

Comparison of Hinge region 1



Comparison of Hinge region 2



Hinge region was crucial for CAR expression on T cells.

The strength of non-specific activation differed depending on the hinge type, highly activated CAR-T cells showed higher expression of exhaustion markers, reduction of naive phenotype, reduction of cytokine production ability, and reduction of cytotoxic activity.

Summary

- CAR-T cells showed antigen non-specific activation which was not detected on TCR-T cells.
- Non-specific activation originated from extracellular design of CARs.
- The non-specific activation affected the properties of CAR-T cells.
  - Low naïve memory subsets and constant expression of exhaustion markers.
  - Cytotoxicity and cytokine production capacity against antigen expressing cells.

⇒ **Need to choose the appropriate CAR design for the effective CAR-T therapy.**

Disclosure of COI

Authors: Yasunori Amaishi, Sachiko Okamoto, Tatsuji Enoki, Junichi Mineno

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