Development of evaluation system for the activity of CD25-targeting CAR-T cells

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Chimeric antigen receptor (CAR)-T cells have demonstrated promising clinical efficacies in relapsed/ refractory B cell malignancies. However, more than 50% of the patients treated with CAR-T cells relapse, often due to CAR-T cell exhaustion, which is caused by excessive activation signals through the strong binding of antibody domain of CAR molecules to target molecules expressed on cancer cells. Therefore, an efficient system to design CAR molecules with optimal affinity is required. We aim to develop a machine learning-based design system of CAR with optimal affinity. In this study, we develop an evaluation system to quantify the activation and exhaustion levels of CAR-T cells.

CD25, an antigen overexpressed on acute myeloid leukemia (AML) cells and immunosuppressive regulatory T (T reg) cells is selected as the target of CAR. By targeting CD25, a synergy effect of AML and T reg cells elimination leading to robust anti-tumor effect can be expected. We first designed 5 CD25-CAR genes based on a clinical-approved CD19-CAR and an anti-CD25 antibody, Daclizumab. After transient expression of the genes in T cell line Jurkat, we identified the most efficiently-displayed CD25-CAR molecule. Then, the conditions for quantification of CAR-T cell activation and exhaustion levels were optimized by flowcytometry. Mitogen-stimulated Jurkat cells were stained with both PE-dye-conjugated anti-CD69 (activation marker) antibody and APC-fire750-dye-conjugated anti-PD-1 (exhaustion marker) antibody, followed by the measurement of median fluorescence intensity of each fluorophore. Now we are constructing mutant CD25-CAR molecules based on binding energy calculation [1] to analyze the relationship between CAR affinity and activation or exhaustion. This evaluation system can be utilized to generate training data for machine-learning to design CAR with optimal binding affinity.

Reference

[1] See, K., Kadonosono, T., Miyamoto, K., et al. Antibody-guided design and identification of CD25-binding small antibody mimetics using mammalian cell surface display. Scientific Reports. 2021; 11(1).