Newly established CAR-T target antigen luciferase reporter cell lines facilitate CAR-T development

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Abstract

Adoptive T cell therapy has emerged as an exciting new approach for cancer treatment. In this novel immunotherapy, T cells are genetically modified to fight tumors. Chimeric antigen receptor (CAR)-T cells have displayed remarkable efficacy in treating malignant cancers, particularly liquid tumors. CAR-T cells have proven to be a new type of “living” therapeutic by harnessing the patient’s immune system to recognize specific tumor associated antigens and redirect the engineered T cells to more specifically target tumor cells. Numerous research efforts have been dedicated to developing new CAR structures to increase the scope of targeted cancers and anti-tumor efficacy. Evaluating the biofunction of CAR-T cells in vitro often involves a series of labor-intensive, co-culture experiments and immunoassays. Reproducibility also remains a challenge during the validation of new CAR-T cells due to factors such as donor-to-donor variation. In this study, we present a panel of luciferase reporter tumor cell lines that can be utilized to examine the function of CAR-T cells. This panel of selected human tumor cell lines naturally express high levels of CAR-T target antigens on cell surface, such as CD19, CD20, and Her2. After the introduction of a Lenti-LUC2 luciferase reporter into the parental cell lines, single cell cloning was performed to isolate stable clones with high luciferase expression. To verify the performance of the target luciferase reporter cell lines, we used commercially available CAR-T cells expressing CD19, CD20, HER2, and empty vector-transduced T cells as controls in co-culture experiments. The cytotoxicity of the CAR-T cells against target tumor cells was measured using both a luciferase assay and cytolytic potency assay. Our results demonstrate that the luciferase reporter system is a relatively simple, robust, and highly sensitive means to measure biological processes. The luciferase reporter cell lines provide an advantage in measuring target cell killing without having to use radioactive ^{51}Cr release assay or pre-labeling the cells in CAR-T functional evaluation. In addition, these reporter cell lines were characterized and authenticated using cell morphology, growth kinetics, and STR analysis. The expression stability of both the target antigen and luciferase was verified by comparing the low passage and the high passage reporter cells. In summary, the well-characterized CAR-T target antigen luciferase reporter cell lines from ATCC provide excellent tools for studying CAR-T biofunction and validating new CAR-T agents for cancer immunotherapy.

Background

Chimeric Antigen Receptor T (CAR-T) Cells

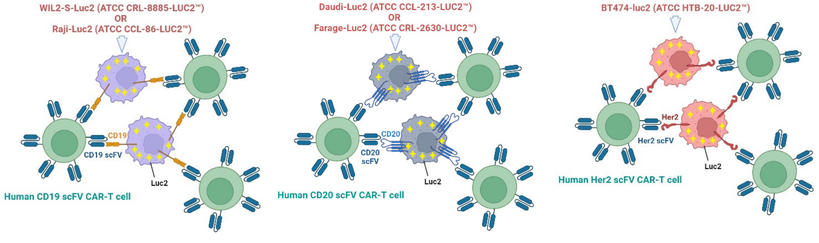


Table 1. ATCC luciferase-expressing cell lines

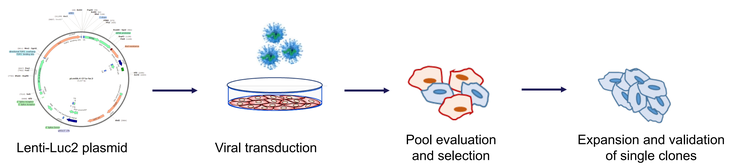
Luciferase Cell Line	ATCC® No.	Tissue/Disease	Target
WIL2-S-Luc2	CRL-8885-LUC2™	B Lymphoblastoid Cell	CD19
Raji-Luc2	CCL-86-LUC2™	Burkitt’s Lymphoma	CD19
Daudi-Luc2	CCL-213-LUC2™	Burkitt's Lymphoma	CD20
Farage-Luc2	CRL-2630-LUC2™	Lymphoma	CD20
BT-474-Luc2	HTB-20-LUC2™	Breast ductal carcinoma	HER2

References

- Jackson HJ, et al. Driving CAR T-cells forward. Nat Rev Clin Oncol 13(6):370-83, 2016. PubMed: 27000958
- Kiesgen S, et al. Comparative analysis of assays to measure CAR T cell-mediated cytotoxicity. Nat Protoc 16(3): 1331-1342, 2021. PubMed: 33589826.

Results

Generation of Luciferase-expressing Cell Lines



Characterization of CAR-T target cell lines

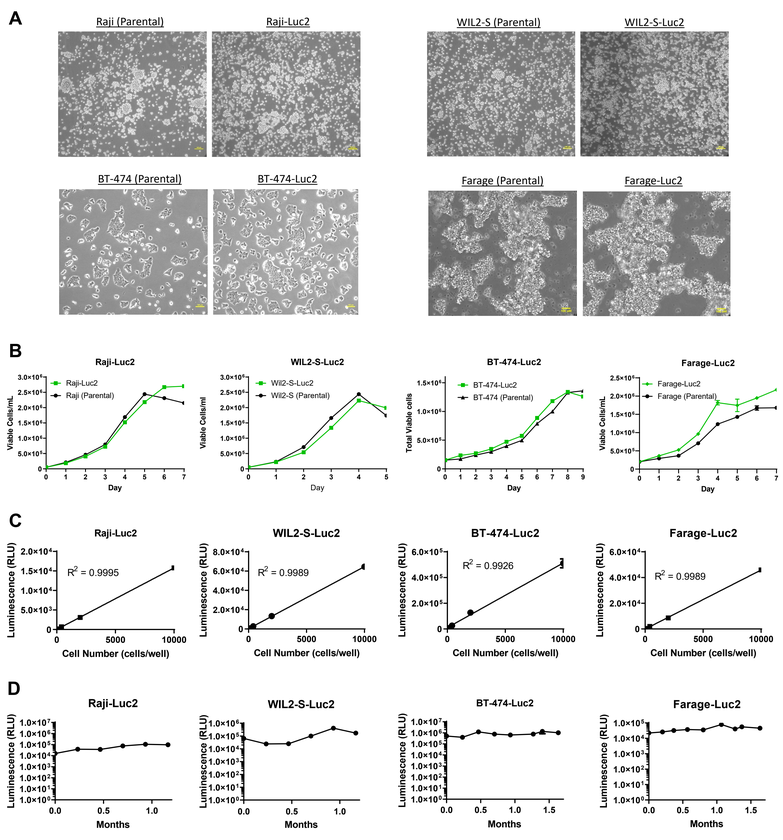


Figure 1. Characterization of CAR-T target cell lines. (A) Cell morphology of the luciferase expressing cell lines was observed under microscopy and images were captured via digital camera (scale bar = 100 mm). (B) 2 x 10^5 cells/mL were seeded into T25 flasks and automated cell counting was used to generate growth curves. (C) Luciferase assay was performed by using Bright-Glo™ (Promega®) Luciferase Assay System and a luminescence plate reader. Data showed a linear correlation between bioluminescence intensity and cell number. (D) To verify the stability of luciferase expression, the cells were maintained in culture for 30 population doublings. The luciferase expression was monitored every week by using the luciferase assay.

Antigen expression in CAR-T Target cell lines

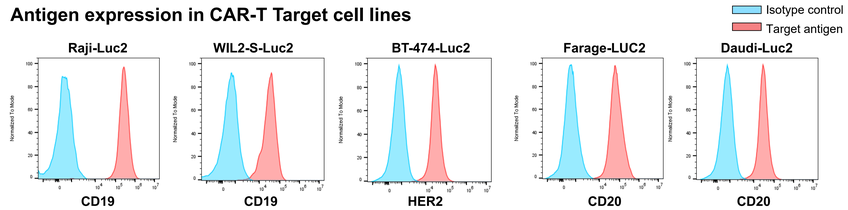


Figure 2. CAR-T Target cell lines. Flow cytometry analysis was performed to assess the CAR-T target antigen expression levels CD19, CD20, and Her2 (pink) on the tumor cell lines compared to isotype controls (blue).

Antigen specific targeting of CAR-T Cells

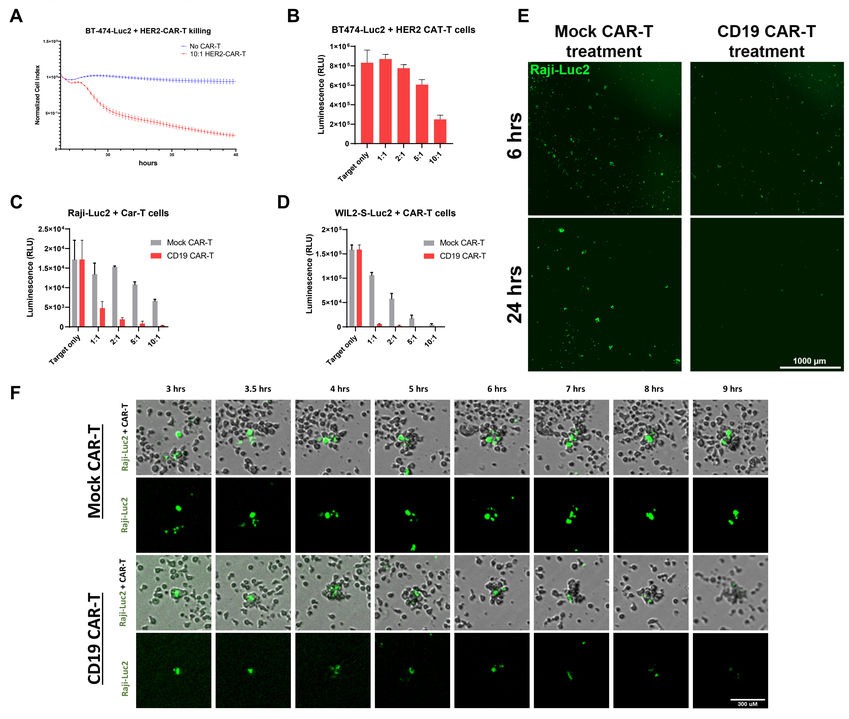


Figure 3. Antigen specific targeting of CAR-T cells. (A) HER2 CAR-T cells were used to target 2 x 10^4 HER2 positive BT-474-Luc2 at a 10:1 ratio and cell killing was measured using the xCELLigence system and (B) 5 x 10^3 cells were targeted by HER2 CAR-T cells at ratios of 1:1, 2:1, 5:1, and 10:1 and cell killing was measured by luciferase assay. (C) CD19 CAR-T cells were used to target CD19 positive 5 x 10^3 Raji-Luc2 and (D) WIL2-S-Luc2 cells at the indicated ratios and cell killing was measured after 24 hours by luciferase assay. Raji-Luc2 cells were stained with Vybrant™ (Thermo Fisher) DiO dye and real-time fluorescent imaging was measured every 30 minutes for 24 hours during the co-culture of Raji-LUC2 cells with CAR-T cells. (E) After 24 hours of co-culture CD19 CAR-T cells showed a decrease in fluorescent cells as compared to 6 hours; in a co-culture with Mock CAR-T cells numerous Raji-LUC2 cells were present. (F) Two stained Raji-Luc2 cells (Green) from the co-culture experiment were tracked for 6 hours and became surrounded by CAR T cells resulting in a decrease of fluorescence when treated with CD19 CAR-T, as compared to co-cultures with Mock-CAR-T cells.

Conclusion

The functionally validated luciferase reporter tumor cells exhibit naturally high expression of the target antigens CD19, CD20, and HER2 and are extremely useful for testing CAR-T activity.

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