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Master of Science HES-SO in Life Sciences

Construction and expression analysis of CAR molecules directed to CD37 and Enolase-1 antigens for cell-based therapy

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DESCRIPTION

Chimeric antigen receptor (CAR) molecules has been developed since late 80's to be introduced on T cells as a novel therapy for cancer patients. In particular, CD19 CAR-T cell therapy had remarkable clinical responses in B-cell acute lymphoblastic leukemia and diffuse large B-cell lymphoma. Additionally to hematological malignancies, the use of CAR-T cells are also being investigated for solid tumors. Enolase-1 (ENO1) and CD37 are both expressed on several cancer types and are potential targets for CAR cell-based therapy.

OBJECTIFS

DNA sequence of antibody fragments directed to ENO1 and CD37 were designed and introduced into a third-generation CAR-construct. Jurkat cells were electroporated with a Sleeping Beauty transposon/transposase vector system carrying the transgene. CAR surface expression analysis for both molecules were performed by flow cytometry. The ENO1 CAR's functionality was also assessed in a western blot by detecting phosphorylation of CD3z Tyr142 after stimulating Jurkat cells with a-enolase protein.

RESULTS

Jurkat cells transfected with the ENO1 CAR showed a positive population for the anti-F(ab')$_2$ antibody staining after 12 days with 76.1% and decreased at 22.5% after 35 days, as illustrated on Figure 1A. Phosphorylation status of CD3ζ in the ENO1 CAR Jurkat cells by Western blot with phosphospecific antibodies for CD3ζ Tyr^{142} was evaluated. All stimulated ENO1 CAR Jurkat cells were phosphorylated in the Tyr^{142} residue CD3ζ at ~19 kDa (Figure 1B).

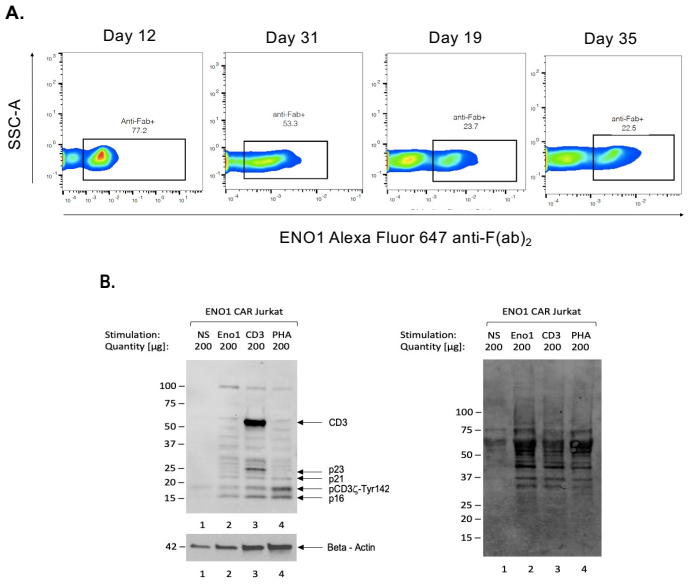

Figure 1 : A. Surface expression of ENO1 CAR stained with Alexa Fluor 647 anti-F(ab')2 antibody at different days after transfection. B. Western Blot for CD3ζ Tyr^{142} detection (Chemiluminescent blot image and stain-free blot total proteins).

The expression of the four constructs with anti- F(ab')2 antibody was analyzed, and relevant expression analysis is shown in Figure 11D. The Jurkat cells transfected with the CD37_1 CAR shows a positive population for the anti-F(ab')2 antibody after 16 days of 12.8%, 40.5% for CD37_2 CAR, 38.0% for CD37_3 CAR and 11.3% for CD37_4 CAR (Figure 11D). Hence, the second construction of CD37 seems to enhance the surface expression of the CAR. Nevertheless, the background staining percentage of the anti-F(ab')2 antibody still needs to be subtracted from these results.

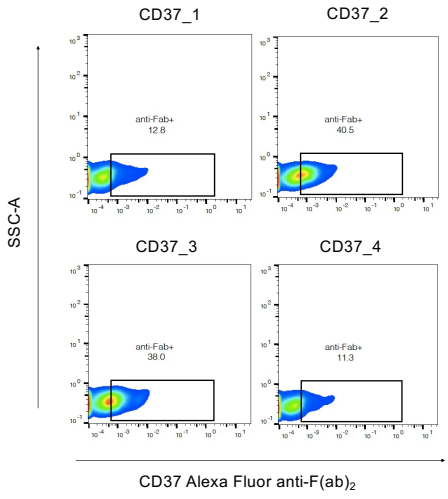

Figure 2 : Surface expression of the different constructions of CD37 CAR stained with anti-F(ab')2 antibody after 16 days of culture.

CONCLUSION

In summary, we designed and constructed a SB transposon vector with ENO1 and CD37 CAR transgene and successfully transfected them into the leukemic Jurkat cell line. We assessed the CAR surface expression by flow cytometry and performed detection of CD3ζ phosphorylation in order to evaluate the ENO1 CAR's functionality. The same evaluation should also be performed for the CD37 CAR. In addition, cytotoxicity assay should also be conducted after transfection of cytotoxic cells, like PBMC, NK or CIK cells, with these two CAR constructions. The overall results show that these two molecules could be promising tools for further development in anti-cancer therapy.

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