ORT_22 - Generation of anti-GD2 CAR-T Cells by sleeping beauty transposon system

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Introduction: Immunotherapy involving T lymphocytes genetically modified with artificial receptors, called Chimeric Antigen Receptors (CARs), is one of the most promising antitumor therapies; once expressed on the T cell, the receptor is able to redirect it to a tumor antigen in a specific way. The CAR-encoding transgene can be inserted into the genome of T cells by means of the Sleeping Beauty system, which is formed by the bicomposite arrangement that is usually two plasmid vectors, one component is a vector containing the CAR in the backbone of the sleeping beauty transposon and the other is the transposase expression plasmid. It is known that some solid tumors commonly express the ganglioside GD2, which makes them a good target for CAR-T cell immunotherapy.

Objectives: The aim of this work was to synthesize and validate the anti-GD2 CAR (14G2A clone) plasmid and generate CAR-T cells from peripheral blood mononuclear cells (PBMCs).

Methodology: Initially the 14G2A encoding sequence was cloned into the PT4 transposon vector; to confirm the cloning, the plasmid was digested by the EcoRV restriction enzyme and visualization was performed using 1% agarose gel electrophoresis. A second validation was performed by electroporation of the chimeric receptor in HEK 293FT cell line and PBMCs.

Results: A frequency of 7.04% of CAR positive cells was observed by flow cytometry 24 hours after the electroporation in HEK 293FT cell line. Then, 30 million PBMCs were electroporated and CAR frequency was assessed by flow cytometry 1, 8 and 12 days later. An average (n=2) of 5.57%, 7.81% and 14.5% of anti GD2 positive CAR-T cells frequency was observed after 1, 8 and 12 days of expansion respectively.

Conclusion: Stable expression of the anti-GD2 CAR plasmid (14G2A) was observed in both HEK293FT cells and PBMCs, following electroporation using the Sleeping Beauty system. The next steps in this work will be functional tests, such as the lysis assay and in vivo validations.

Keywords: CAR-T Cell; GD2; Sleeping beauty