Abstract

Immunotherapy based on chimeric antigen receptor (CAR)-expressing T lymphocytes has proven to be highly successful in the treatment of acute lymphoblastic leukemia (ALL), leading to development of CAR-based immunotherapies for other hematologic malignancies. Currently, efforts are underway to refine T cell modifications to make patient treatment more effective. Each time, this modification then needs to be empirically validated in *in vitro* experiments. We decided to study the effect of the cytokine IL-21 on the antitumor function of CD19-specific CAR T cells using *in vitro* assays. A construct that co-expressed IL-21 under the control of the inducible NFAT promoter together with CARs against CD19 was introduced into T cells. In a series of experiments, the properties of these cells were compared after coculture with tumor B cell lines and CLL cells obtained from patients. The results showed that CAR T cells that express IL-21 proliferate and activate better, even after repeated stimulation with leukemia cells.

In addition to CARs specific against the CD19 molecule, we also investigated CARs specific against the CLL1 molecule, which has been described in the literature as one of the promising targets for the treatment of AML. We prepared CAR T cells against CLL1 producing IL-21. For this purpose, we had to prepare a plasmid that contains the IL-21 gene under the control of the NFAT promoter in addition to the sequence for CAR. The functionality of the plasmid was verified by transfecting PBMCs and culturing CAR T cells expressing the given CAR and IL-21. The concentration of IL-21 was measured by ELISA. The use of interleukin-21 in CAR T therapy could yield positive effects.

Key words: Immunotherapy, CAR T cells, CD19, ALL, CLL1, NFAT, IL-21, hematologic malignancies