Overcoming the challenge of transduction of human T-cells with chimeric antigen receptor (CAR) specific for ERBB2 antigen

ABSTRACT

Breast cancer is one of the most common malignancies among woman. Decades of scientific study have linked the overexpression of ERBB2 antigen to aggressive tumors. To target aggressive breast cancer, chimeric antigen receptor (CAR) technology can be utilized. For this, human T-cells are transduced with a gene sequence encoding a CAR that is specific for tumor-associated antigens (TAAs). These genetically-engineered CAR transduced T-cells (CAR-T cells) are able to target the tumor antigen without the need for major histocompatibility complex (MHC) recognition, rendering it a potentially universal immunotherapeutic option. However, efficient transduction of therapeutic gene into human T-cells and further cell expansion are challenging. In this study, we reported a successful optimization of a transduction protocol using spinoculation on CD3+ T-cells with different concentrations of lentiviral plasmid encoding the CAR gene. CD3+T-cells were isolated from the peripheral blood mononuclear cells (PBMCs). The constructed CAR gene was inserted into a lentiviral plasmid containing the green fluorescent protein (GFP) tag and lentiviral particles were produced. These lentiviral particles were used to transduce activated T-cells by spinoculation. T-cells were activated using Dynabead-conjugated CD3/CD28 human T-cell activator and interleukin-2 (IL-2) before transduction. CD3+ T-cells were selected and GFP expression, which indicated transduction, was observed. Future studies will focus on in vitro and in vivo models to determine the efficiency of CAR-T cells in specifically targeting ERBB2-expressing cells.

Keyword: Breast cancer; CD3+ T-cells; Chimeric antigen receptor (CAR); Immunotherapy