Non-viral Intracellular mRNA Delivery in Human Primary Cytotoxic T Lymphocytes Using Synthetic Lipid-like Nanoparticles

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Statement of Purpose: Engineering patient-derived T cells has become an emerging approach to treat cancer since the trial with chimeric antigen receptor T (CAR-T) cell therapy has produced promising results. The currently used method for engineering T cells in clinical application is using virus due to the "hard-to-transfect" nature of T lymphocytes. However, there are some safety concerns such as tumorigenesis. Furthermore, the current viral method to produce CAR-T cells involves complex manufacturing procedures, which hinders wide spread use of this technology in clinical settings.
Here, we used the combinatorial library of synthetic lipid-like materials (termed "lipidoids") to deliver functional mRNA into human primary cytotoxic T lymphocytes. Using firefly luciferase (FLuc) mRNA as a reporter, we successfully detected the luminescence from T cells after the transfection with lipidoid nanoparticles. Our study may provide non-viral delivery platform of functional mRNA – e.g., CAR or Cas9 - to achieve effective and safe T cell engineering.

Methods: Lipidoids were constructed by the Michael addition of different combination of aliphatic amine head to acrylate carbon tail (1, 2). Lipidoids were fabricated into nanoparticles by sonication in sodium acetate buffer. Lipidoid with formulation was mixed with three excepients – i.e., cholesterol, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-poly(ethylene glycol) (DSPE-PEG) - in ethanol before fabricated into nanopariticles. Commercial lipid reagent, Lipofectamine 2000 (LF2000) (Invitrogen), was used as a control. Human peripheral blood mononuclear cells (hPBMCs) were separated from whole blood (Research Blood Components, MA) using density gradient with Lympholyte-H (Cedarlane Labs, Canada). Human primary cytotoxic T lymphocytes were isolated from hPBMCs using CD8+ T cells Isolation Kit, Human (Miltenyi Biotec, MA). Lipidoid nanoparticles (LNPs) and mRNA were complexed in sodium acetate buffer at room temperature for 15 minutes before treating the cells. Cells were incubated at 37 °C for 20 hours after LNP treatment. The FLuc mRNA (TriLink Biotechnologies, CA) delivery efficacy was quantified using Firefly Luciferase Assay Kit (Biotium, CA) and Synergy luminometer (BioTek).

Results: First, we determined the most effective structure of amine head for T lymphocyte transfection. Second, using the selected amine head, we identified the most effective structure of carbon tail for this purpose. The lipidoid tail containing 17 carbons and one sulfer showed the highest delivery efficacy **(Figure 1)**. Lipidoids with three excipients formulation drastically improved the delivery efficacy (**Figure 1**).

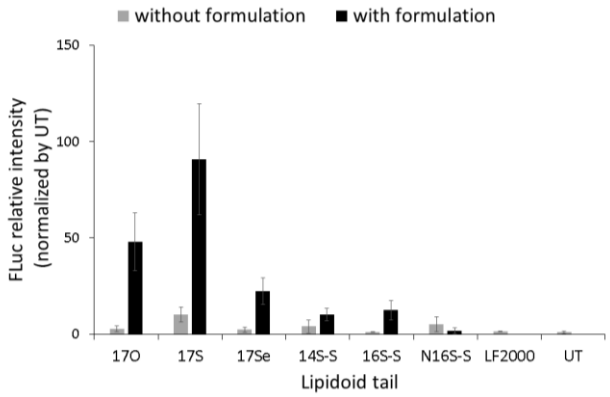

Figure 1. FLuc expression of human cytotoxic T lymphocytes treated with FLuc mRNA loaded LNPs. The length of carbon in lipidoid tail were indicated in the front of the name, followed by a different element (O, S, Se, or disulfide bond) contained in the tail.
LF2000: Lipofectamine 2000, UT: untreated

Conclusions: The results described here establish the non-viral mRNA delivery system of human primary cytotoxic T lymphocytes. Although the current method to produce CAR-T cells is promising, the complex manufacturing procedures still have gaps to be addressed. The use of LNPs eliminates the need for viral system. Moreover, the use of mRNA achieves rapid and transient expression of exogenous protein without mutagenesis to the host genome. This would be beneficial when the exogenous protein has a potential to cause adverse effects on the cells. In addition, LNP-based mRNA delivery system can also be applied to CRISPR/Cas9 gene editing of T cells by using Cas9 mRNA. This is beneficial for producing universal allogenic CAR-T cells or disrupting inhibitory receptors from CAR-T cells.
In summary, there is an increasing demand of developing T cell engineering system using non-viral approach given safety concerns of viral-based delivery, and our study with LNP-based mRNA delivery system has a great potential to provide rapid and safe platform for T cell therapy.

References:

1. MWang. PNAS. 2016;113;2868-2873
2. YLi. Biomaterials. 2018;178;652-662