Improving Non-viral Gene Delivery in Jurkats via Cell Penetrating Peptides

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Statement of Purpose: The aim of this project is to improve the efficiency of non-viral gene delivery for potential use in CAR-T cell therapy. Current therapies use viral vectors to deliver genes to patient's T cells. While viral vectors have high transduction rates the associated safety concerns involving mutagenesis and immunogenicity require continuous patient monitoring as well as the concern for limited packaging space. Non-viral vectors do not suffer from packaging limitations and look to overcome those safety concerns but have yet to achieve the same transfection efficiency results as viral vectors. In this study we propose to complex polyethylenimine (PEI) with various Cell-Penetrating Peptides (CPPs) to take advantage of cell penetrating properties found in viruses and combat any cytotoxicity issues with PEI.

Methods: Clone E6-1 Jurkat (ATCC TIB-152) cells were cultured in X-Vivo 15 and transfected with pEF-GFP. Transfections were carried out using Lipofectamine LTX, polyplex formulated using Linear PEI (10kDa) and polyplex of Linear PEI (10 kDa) and the CPP Penetratin. Expression of GFP was evaluated at 48 hours using FACS analysis (Guava easyCyte HT flow cytometer). Linear PEI and Penetratin was complexed with pEF-GFP based upon a N/P ratio of 5. Incremental percentages of PEI were replaced with Penetratin while maintaining an N/P ratio of 5. These polyplexes were compared against the on the market Lipofectamine LTX and PEI complexes where the same percentage of charge was removed but not replaced to validate if the CPP was responsible for any improvements to transfection. The associated amount of PEI and then Penetratin were added to 4 ul of pEF-GFP and incubated for 10 minutes. 20uL of pEF-GFP was incubated with 1 uL of PLUS reagent for 5 minutes before being incubated with 2.75 uL of Lipofectamine LTX for 5 minutes before transfection. In a 24 well plate, Jurkat cells were seeded with a concentration of 2 x 10^5 cells/well in X-Vivo 1. The time of transfection after seeding the wells was compared to see if there was a difference in transfection efficiencies, immediately versus 24 hour post seed.

Results: Approximately 7-8% transfection efficiency was achieved with pEF-GFP based upon FACS data. Plasmid control acting as a negative control showed less than 0.2% transfection while Lipofectamine our positive control as well as the current on the market product exhibited approximately 10% transfection efficiency for both transfection times, lower than expected for the same day transfection efficiency. The FACS data comparing the complexes as well as time of transfection are shown in Figure 1. For cells transfected 24 hours after seeding, seemed to marginally increase the transfection efficiency. However, for cells transfected the same day they were seeded transfection efficiency increased across the board for our PEI based complexes, achieving 7-8% except for 80% PEI which only got up to 2.4%. Penetratin complexed with plasmid by itself had a transfection efficiency of less than 0.1% regardless of time of transfection.

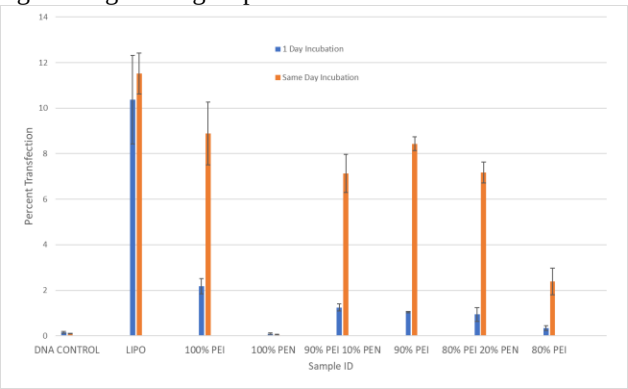

Figure 1. Quantification of percent transfection of Jurkat cells using FACS analysis. Penetratin denoted with PEN. Blue refers to 24 hour wait post seeding before transfection. Orange refers to same day as seeding for transfection.

Conclusions: The increase in transfection efficiency for all samples when transfecting the same day as the plates were seeded indicates that is the optimal time for transfection. Penetratin did not seem to be the reason for increased transfection when replacing 10% of the cationic charge of PEI. However, when replacing 20% of the cationic charge of PEI with Penetratin there was an approximately 5% increase in transfection efficiency. This demonstrates that there may be a beneficial interaction between Penetratin and PEI for transfection at certain ratios. Further working is being done to compare the cytotoxicity of the complexes to determine if Penetratin may or may not lower the amount of cell death. In addition, more Cell Penetrating Peptides are being investigated as well as comparing transfection efficiencies with these peptides against High Molecular Weight branched PEI and Low Molecular Weight branched PEI in an effort to narrow down and determine an optimal complex that may be used to test in primary T cells.

References: (Antonella AB. Molecules. 2018; 23: 295)