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Calcium enhances plasmid gene transfection efficiency in Jurkat cells


Abstract

Jurkat, a leukemic cell line is an excellent surrogate model of primary T-cells for studying T-cell signaling and physiology. Genetic modification, particularly the delivery and expression of an exogenous gene requires efficient transfection. However, Jurkat is hard-to-transfect cell line perhaps due to its human T-cell origin. Efforts have been made to enhance transfection efficiency in Jurkat and primary T-cells. Nevertheless, limitations such as high cytotoxicity and/or low transfection efficiency have not yet been overcome through transfection using non-viral vectors in Jurkat cells. Calcium chloride is a known enhancer of cationic lipid-mediated gene transfection in bovine hamster kidney (BHK) cells. Also, calcium was reported to act as a transfection enhancer in lower mol. wt. poly-l-lysine (PLL)- and low mol. wt. polyethyleneimine (PEI)-mediated gene delivery in A549 lung carcinoma cells. In this report, we show that depending on the type of calcium chloride used in the culture media, calcium enhances the 25kDa branched PEI (P25) - mediated transfection efficiency in Jurkat cells by 4-7 fold when compared to the no-added-calcium control.

Key Findings

1) Calcium enhances high molecular weight (H.M.W) PEI mediated plasmid gene transfection in Jurkat cells.
2) Calcium boosts the association of polyplexes and cell surface.
3) Other divalent cation such as Mg2+ and monovalent cation Na+ proved ineffective in the enhancement of gene delivery.

Explanation

PEI based transfection occurs in a sequence of steps, starting with the association of positively charged PEI-pDNA complexes (polyplexes) and negatively charged cell membrane, uptake of polyplexes through endocytosis, endosomal escape of pDNA and PEI, nuclear entry of pDNA, and finally expression of gene of interest. Unarguably, a major hindrance for non-receptor mediated transfection in the cell membrane itself is the permeability of cell membrane defines the efficiency of transfection. Any reagent that has the potential to enhance the interaction between polyplexes and cell membrane can significantly increase the chances of pDNA uptake and thereby gene expression. In our study, we found that calcium ions as against to chloride ions are responsible to enhance such association (Figure 3). Although magnesium is a valence analog, it did not enhance the association, indicating the capability of calcium in mediating transfection (Figure 3). It is not understood as to how calcium is enhancing the association of polyplexes and cell membrane.

Calcium chloride at concentrations 10mM and 25mM had increased the transfection efficiency in Jurkat cells by 4-7 fold respectively (Figure 1a, 1b). However, such a boost came at the cost of lower cell growth rate including cell death (Figure 1c). It is to be noted that such high toxicity is predominantly calcium chloride (Figure 4) and not soley by the polyplex itself (Figure 1c, CO vs G0). The difference in the expression level of GFP, under 10mM and 25mM could be understood by the level of association of polyplexes with the cell membrane (Figure 3c). It is worth noting that 25mM calcium has decreased the net charge of polyplex (Figure 2c) and yet gave the highest transfection efficiency. Unsurprisingly, although 25mM calcium gave highest gene expression in the tested groups, 10mM calcium was found to be optimal for transfection in Jurkat cells.

Impact

Primary T-Cells from patients are genetically engineered to treat some cancers in adoptive T-cell therapy. Such modifications are achieved by very efficient albeit high risk, viral vectored-based gene delivery. Currently, non-viral methods of gene delivery that pose low risk are highly inefficient in T-Cells. Thus, development of non-viral methods of transfection in T-Cells is necessary. Our study may help in opening doors to future research that takes the non-viral vector-based gene delivery to being the most effective method of transfection in T-Cells.

Conclusion

25kDa bPEI mediated transfection in Jurkat cells can be significantly enhanced by the simple addition of CaCl2 in the culture media.

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Figure 1: Effect of CaCl2 on the transfection of Jurkat Cells analyzed by flow Cytometer at 48h. CO is a cell only control where there is no polyplex and CaCl2. "G0" is a pGFP polyplex + 1mM CaCl2, sample; "G1" is a pGFP polyplex + 1mM CaCl2, sample and so on. "50" is a p-0.18 polyplex + 1mM CaCl2 control; "60" is a p-0.18 polyplex + 1mM CaCl2 control; "G25Mg2" is a pGFP polyplex + 25mM MgCl2 sample. "G25Mg2" is a pGFP polyplex + 25mM MgCl2 sample, and "1F2" is a pGFP bovine only control. (x) Sample codes and their respective transfection efficiencies (y) are shown in each source. (b) Flow view of a GFP expressing Jurkat cell sample "G12" captured with an immersion lens at 60X using Confocal Microscope LSM700. Scale bars - 1um. (c, d, e) All comparisons are made with "G". Results are presented as mean ± SD. (n=3, c, d, e) two-way ANOVA with Dunn’s multiple comparisons, **p = 0.0007 to 0.0011; ***p = 0.0003).

Figure 2: Effect of CaCl2 on the stability of P25-GFP complexes (MW 2) analyzed by Sybr exclusion assay. Size and net charge of polyplexes analyzed by DLS. (a) All comparisons are made with "G". Results are presented as mean ± SD. (n=3, a) two-way ANOVA with Dunn’s multiple comparisons, **p = 0.0068; ***p = 0.0011; ****p = 0.0039.

Figure 3: Effect of CaCl2 on the association of Cy5-labelled pGFP polyplexes and Jurkat cells in a 48-hour culture analyzed by flow Cytometer. "G" is a polyplex only control. "1F2" is a polyplex + 25mM NaCl control. "25 M" is a polyplex + 25mM CaCl2 control, "10 Mg" is a polyplex + 10mM MgCl2 control, "50 Mg" is a polyplex + 50mM MgCl2 control; and "1F2" is a bovine only control. (a) Maximum intensity projection (MIP) of Jurkat cells. Images were captured with an x 200 objective lens at 24h using Confocal Microscope LSM700. Nucleus is stained with DAPI (blue) and pGFP is labeled with Cy5 (red)). (b) 6 hour, (c) 24 hours. Results are presented as mean ± SD (n=3). (d) two-way ANOVA with Dunn’s multiple comparisons, **p = 0.0005 and 0.0006; ***p = 0.0011)

Figure 4: Effect of CaCl2 (mM) on the viability of Jurkat cells in a 2-day culture analyzed by flow cytometer. All comparisons (ANOVA) are made with "G". Results are presented as mean ± SD (n=3). (c) two-way ANOVA with Dunn’s multiple comparisons, **p < 0.001)