International Journal of Healthcare Sciences ISSN 2348-5728 (Online)
Vol. 3, Issue 2, pp: (262-263), Month: October 2015 - March 2016, Available at: www.researchpublish.com

Optimising the Transfection of Mantle Cell Lymphoma Cell Lines with siRNA

[1]Mohammad Al-Jallal, [2]Muhaid Al-Nujidi

Keele-Imam University Research Project

Abstract: **Gene silencing by siRNA (short interfering RNA) represents a valuable tool for basic research in biology and proved to have a major impact on medical sciences. Using siRNA is one of the important techniques that can be used to study gene function and its effects on the cells. There are many techniques that can be used to transfect gene specific siRNAs into the cells but some cells are resistant to most of these methods. The present study aimed at optimising the transfection of the mantle cell lymphoma Jeko-1 cell line, which are reported to be difficult to transfect, with siRNA. Two techniques were used: electroporation and nucleofection. The results showed that the use of both electroporation at 220V and nucleofection with solution T result in the highest transfection efficiency and lower cytotoxicity and therefore can be used for the successful transfection of JEKO-1 cells.**

Keywords: **short interfering RNA (siRNA), transfection efficiency and lower cytotoxicity.**

1. INTRODUCTION

siRNA - mediated gene knockdown is a strong tool that has been used to identify gene function and study biological pathways. Successful siRNA experiments including knockdown of individual genes target require efficient delivery of specific siRNA molecules into suitable cells.Lipid-mediated transfection is a common method for siRNA transfer, however many cell types including suspension cell lines not compatible with this technology. In addition, several of the lipid transfer reagents can cause cytotoxicity. In this study, two methods were investigated in order to optimise the transfection of the mantle cell lymphoma JEKO-1 cells which are resistant to transfection.

2. METHODS

Jeko-1 cells were cultured in RPMI medium supplemented with 5% heat-inactivated fetal calf serum, 2 mM L-glutamine, 50 µg/ml gentamycin. The two transfection techniques used are electroporation and nucleofection. Electroporation was carried out at different voltage 210, 215, 220 and 230 in 0.4 cm cuvettes (Biorad) at room temperature. Nucleofection were carried out according to the manufacturer's guidelines using solutions T, R, V and L. siRNA were labelled with Cy3 in order to assess the transfection efficiency using fluorescent microscopy. Viability was determined using MTS assay and viable cell count. Transfection efficiency and viability were determined 24 or 48h post transfection

3. RESULT

Using elecctroporation with different voltages, transfection efficiency varied between 60-80% and cell viability between 60-85%. Electropration at 220V resulted with the highest transfection efficiency (80%) and lower cytotoxicity (85% viability) (Figure 1). Nucleofection with different solutions resulted in cell viability that ranged between 30-60% and transfection efficiency between 35-60%. Nucleofection with solution T resulted in the highest transfection efficiency (60%) and the highest viability (80%) (Figure 1).

4. CONCLUSION

The results showed that the best voltage for JEKO-1 cell line for electroporation is 220V and the best solution for nucleofection is solution T

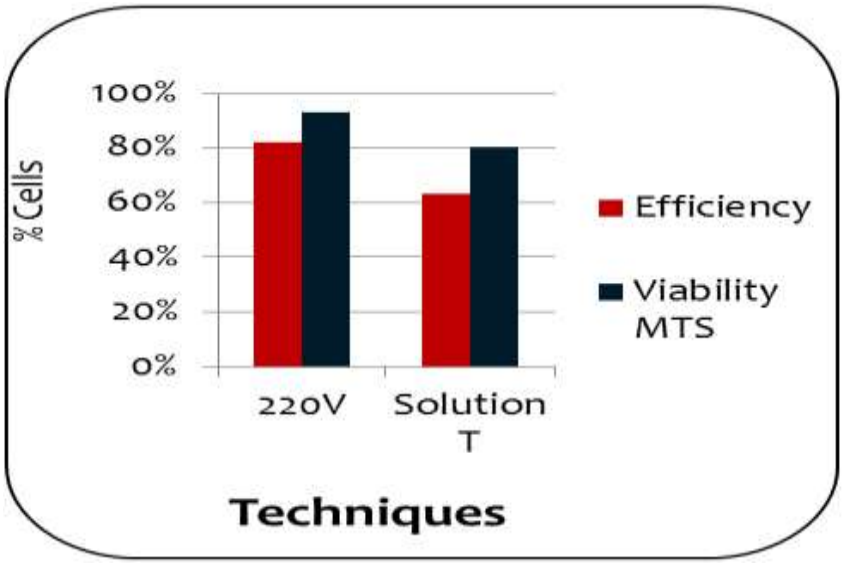

Figure 1: The graph shows the transfection efficience and viability using electroporation 220V and nucleofection with T solution.

REFERENCES

[1] Inomata, Mika, et al. "MicroRNA-17-92 down-regulates expression of distinct targets in different B-cell lymphoma subtypes." Blood 113.2 (2009): 396-402.

[2] Chen, Robert W., et al. "Truncation in CCND1 mRNA alters miR-16-1 regulation in mantle cell lymphoma." Blood 112.3 (2008): 822-829.

[3] Zhao, Jian-Jun, et al. "microRNA expression profile and identification of miR-29 as a prognostic marker and pathogenetic factor by targeting CDK6 in mantle cell lymphoma." Blood 115.13 (2010): 2630-2639.