Original Research Article

Optimization of KYSE-30 Esophagus Cancer Cell Line Transfection Using Lipofectamine 2000

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ABSTRACT

Introduction: There are several methods for delivery of vectors into eukaryotic cell lines. Transfection with liposomes is an easy and accessible way. Lipofectamine 2000 is a transfection reagent with liposome structure. Despite having a specific protocol, the volume of this reagent should be optimized for use in different cell lines. The aim of this study was to optimize transfection of KYSE-30 cell line with pEGFP-NI vector using Lipofectamine 2000. **Materials and Methods:** The vector was purified by plasmid extraction kit. Transfection of the KYSE-30 cell line was done using Lipofectamine 2000 and different concentrations of the vector. Expression of green fluorescent proteins (GFP) was evaluated by fluorescence microscopy, and later analyzed with ImageJ software. **Results:** Optimized concentration of plasmid (5µg) and volume of Lipofectamine 2000 (6µl) were determined for KYSE-30 cell line. GFP plasmid transfection using the determined values showed more than 65% efficiency in the KYSE-30 cell line. The quantity of DNA per transfection and volume of reagent were identified as essential factors for a successful transfection. **Conclusions:** This study shows that lipofection with lipofectamine 2000 is an efficient method of gene delivery into KYSE-30 cell line.

KEYWORDS: Optimization, Esophageal cancer, Lipofectamine 2000, KYSE-30 cell line

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INTRODUCTION

There are different methods to transfect eukaryotic cells [1]. The requirements for efficient transfection includes simplicity, having strong protocols, ability to transfect a variety of eukaryotic cells and low cell toxicity [2].

Nucleic acid and cell surface membrane have a negative charge under physiological conditions. A cationic reagent is required for successful delivery of nucleic acids into cells. Lipofectamine 2000 is a positively charged liposome that can be used for nucleic acid transfection [2, 3]. Although several fat molecules are able to mediate nucleic acid delivery, they all share some features. One is a positively charged head group that usually includes one or more nitrogen atoms, causing an interaction between the positively charged transfection agent and sugar-phosphate molecules of nucleic acid. Another is usually a spacer, linking head group to one, two or three hydrocarbon chains. In some cases, this spacer may play a role in increasing contact between cationic lipids and nucleic acids [4-6]. Considering the mentioned features, Lipofectamine 2000 is considered an effective transfection reagent for the transfer of nucleic acids into eukaryotic cells. However, weight and size of nucleic acids, as well as type of target cell are important factors that need to be optimized [7]. This study aimed to optimize plasmid DNA delivery into KYSE-30 cell line using Lipofectamine 2000.

MATERIALS AND METHODS

Plasmid preparation

Green fluorescent protein expression vector, pEGFP-NI (4.7 Kbp. Clontech Laboratories, USA) was transformed into DH5-α competent *Escherichia coli* cells by heat shock method, and grown under kanamycin (30 µg/ml) treatment. After bacterial growth, plasmids were extracted using Plasmid MidiPrep Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. For plasmid confirmation, the extracted products were electrophoresed on 0.8% agarose gel.

Cell line propagation

Esophageal cancer KYSE-30 cell line (Pasteur Institute, Iran) was cultured in RPMI1640 medium (gibco, UK) supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100µg/ml) (Gibco, UK). Cells were grown at 37 °C and 5% CO_2.

Plasmin transfection

On day one, 10^6 cells were seeded onto 6-well plates containing RPMI-1640 medium supplemented with 10% fetal bovine serum without antibiotics. After reaching cell confluency of 70%, we assessed 2 µg of plasmid DNA along with 3, 6, 9 and 12 µl of Lipofectamine 2000 reagent (Invitrogen, USA) according to the protocol provided by the kit's manufacturer. After determining the efficient amount of the reagent, 2, 3 and 5µg of plasmid DNA were evaluated. Plasmid DNA and Lipofectamine 2000 reagent were diluted with serum and antibiotic-free RPMI-1640 separately to reach a final volume of 250 µl. The mixture was stored at 37 °C for 10 minutes. Supernatant was removed and replaced with 500 µl of RPMI-1640 medium containing no serum and antibiotic. Then, prepared solutions were added to the cells and incubated at 37 °C and 5% CO_2 for 6 hours. Finally, the transfection solution was aspirated and replaced with 1 ml of complete medium per well.

Fluorescent microscopy and image analysis

After 24 hours, the wells were irradiated with UV (wavelength 450-490 nm). Green spots of green fluorescent protein (GFP) were observed under a microscope with 40X magnification. Images were randomly taken from cells in three regions per well by fluorescence microscopy (Olympus BX51, London, UK). Fluorescent images were studied by ImageJ software package [8]. Number of cells and amount of fluorescent light in transfected cells were compared to the total cells. The percentage of transfected cells was calculated as follow:

$$Tra(\%) = \frac{transfected\ cells}{whole\ cells} \times 100$$

Statistical analysis

Transfection efficiency of the serially diluted reagent and transferring vector was compared and analyzed using ANOVA and t-test in Microsoft Excel.

RESULTS

Different volumes of Lipofectamine 2000 were prepared according to the manufacturer. After optimization, volume of 6 µl was found as the most efficient and chosen for later experiments. Then, three different concentrations of DNA were selected. Intensity of the fluorescent vector was analyzed using the ImageJ software. Data was normalized and analyzed with ANOVA and t-test. After 24 hours, the percentage of transfected cells was found as 30%, 42.56% and 69% for 2 µg, 3 µg, and 5 µg of DNA, respectively. Transfection efficiency of the KYSE-30 cells increased significantly (P-value=0.033) when using more plasmids. As it is shown in figure 2, the percentage of transfected cells correlates directly with the plasmid concentration. The

highest transfection level (69%) was observed when using 5 µg of plasmid (Figure 1). However, there was no significant difference in transfection efficiency between concentrations of 2 µg and 3 µg DNA (P>0.05) (Figures 2 and 3).

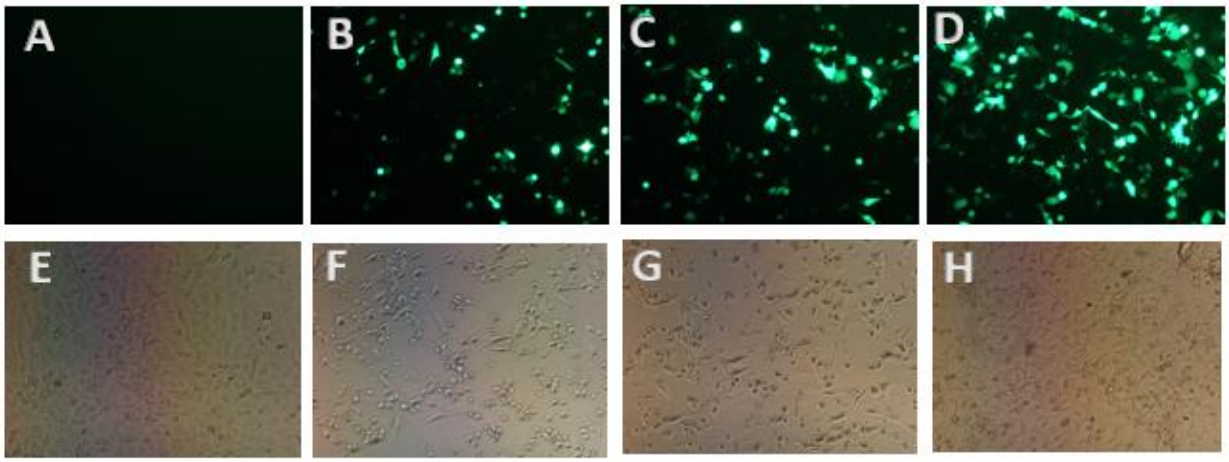

Figure 1: GFP expression profiles in KYSE-30 cell line transfected by Lipofectamine 2000. A&E: non-transfected control cell, B&F: 2 µg of transfected GFP plasmid, C&G: 3 µg of transfected GFP plasmid, and D&H: 5 µg of transfected GFP plasmid. Images in the upper raw are taken by blue filter of a florescent microscope. Images in the bottom are taken using a light microscope

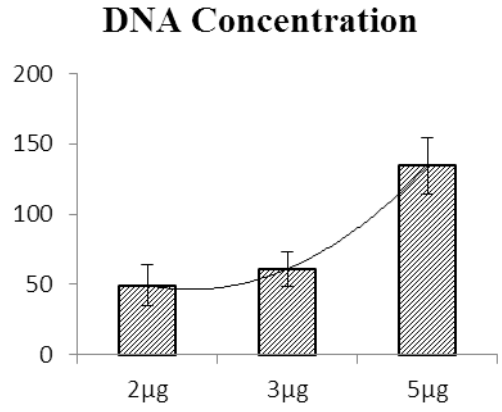

Figure 2: Mean number of transfected cells when using different concentrations of DNA (counted by imageJ package).

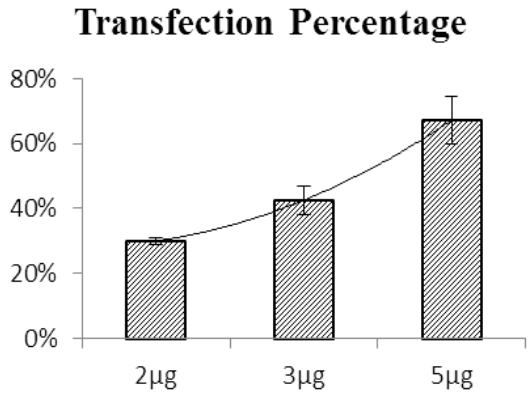

Figure 3: Transfection efficiency of different concentrations of plasmid.

DISCUSSION

Lipofectamine 2000 is a known transfection reagent used for nucleic acids delivery. There are several pathways for up-taking materials into eukaryotic cells, but Lipofectamine 2000 benefits from the clathrin and caveolae-mediated endocytosis [9]. According to several studies, transfer of nucleic acids (plasmid and siRNA) into eukaryotic cells has been successful when using Lipofectamine 2000 [10-12]. In addition, some studies suggested that this reagent increases rate of bacterial transfer into eukaryotic cells [13]. In this study, we have used pEGFP-NI vector for nucleic acid transfection. This vector acts under the cytomegalovirus promoter, which can be used in various cell types. Study of Ward and Stern reported that utilizing plasmid pEGFP-NI with cytomegalovirus promoter increases GFP expression in five mouse cell lines by more than 50%. The mentioned study also used Lipofectamine for transfection [14]. Shaban et al. transfected five mice myeloma cancer cell lines with pEGFP-N1 vector using Lyovac, jetPEI and Lipofectamine 2000. According to the mentioned study, transfection of cells with Lipofectamine 2000 has the highest efficiency compared to other reagents [15]. Salimzodeh et al. also transfected lung cancer cell lines with the GFP vector using CaP, DEAE-dextran, superfect, electroporation and lipofection methods. They reported that lipofection with Lipofectamine 2000 has the highest effect (40.1%) on Mehr-80 cell line [16]. Hashemi et al. transfected Huh_7 and Vero cells with pEGFP_N1 vector using three methods of electroporation, and transfection with Lipofectamine 2000 and jetPEI. Their results showed that Lipofectamine 2000 has efficiency of about 63% in Huh-7 and 73% in Vero cells, which are higher compared to others methods [17]. In the present study, transfection efficiency increased by increasing the concentration of plasmid when using 6 µl of Lipofectamine 2000. Using 5 µg of plasmid showed more than 65% transfection efficiency in the KYSE-30 cell line. Considering the differences in results obtained for different cells, it is suggested to optimize this method of transfection for different cell lines.

CONCLUSIONS

In this study, we have optimized the efficient concentration of DNA and volume of Lipofectamine 2000 for transfection of KYSE-30 cell line. Our findings can be used in future studies.

ACKNOWLEDGMENTS

The study has been derived from a Master's thesis, funded by Golestan University of Medical Sciences, Gorgan, Iran (grant number: 940819199).

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