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Evaluation of transfection effectiveness using fluorescein-labelled oligonucleotides and various liposomes

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Silencing of genes using siRNA represents a generally used technique aimed at inhibiting expression of proteins in cells. Results have frequently not met expectations and this has posed problems in association with this technique. The phenomenon might reflect an incorrect sequence of RNAi, poor penetration of the cells by the nucleotides or insufficient knowledge of the protein in question. The present study is aimed at evaluating the effectiveness of the transfection of selected cell lines using various liposomes.

The studies were performed using 9 cell lines: EPG 257/85 RNOV, EPG 257/85 RDB, W 181/A17, A 2780P, A 2780 RCIS, MEWO CIS, 181 RDB, 181 P and MCF-7/MX. The lines were transfected with fluorescently labelled oligonucleotides. Two parallel experiments were performed. In one oligofectamin and in the other DMRI were used as oligonucleotide carriers.

The studies demonstrated that in every case nucleotides penetrated more than 90% of the cells. In 4 cell lines the signal was stronger when oligofectamin was used, in 4 cell lines when DMRI was employed and in one case the signal strength was comparable using any carrier. The studies showed that various liposomes demonstrated distinct transfection efficiency, depending on the cell line used, and that application of fluorescently labelled nucleotides may be helpful in the optimisation of transfection conditions.

key words: transfection, effectiveness, liposomes, labelled oligonucleotides

INTRODUCTION

Gene silencing involves transcriptional and post-transcriptional inhibition of protein expression. At present, one of the most frequently used methods involves silencing using RNA. The technique relies on RNA interference, as observed in *Drosophila melanogaster* and *Caenorhabditis elegans* [2, 3].

Progress in genetics has resulted in the recognition of sequences of a significant number of genes, although, unfortunately, the function of the genes in the body remains unknown. In recent years we have witnessed the development of so-called "reverse genetics". In order for the function of a gene in the cell to recognised, it is silenced. To recognise the significance of its expression in an entire body, studies are performed on genetically modified animals, mainly mice [7].

Numerous publications describe the application of the siRNA phenomenon to silence oncogenes,

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genes linked to multidrug resistance, or in attempts to inhibit viral infections [4, 6].

A frequent problem linked to attempts to silence a gene is that of results falling short of expectations. This may reflect an incorrect sequence of RNAi, poor penetration of oligonucleotides to the cells under study or insufficient knowledge of the protein in question [1].

The present study aimed at evaluation of transfection efficiency in selected cell lines using various liposomes.

MATERIAL AND METHODS

The studies were performed on 9 cell lines: EPG 257/85 RNOV, EPG 257/85 RDB, W 181/A17, A 2780P, A 2780 RCIS, MEWO CIS, 181 RDB, 181 P and MCF-7/MX. The examined cells were placed on 2 chamber slides (Nunc, USA), at 100,000 per chamber. They were then incubated for 24 hours in Leibovitz L-15 medium (Sigma, USA) at 37°C and at 5 % CO_2. The cells were then washed twice using OPTI-MEM medium (Gibco, USA) and left in the medium for 1 hour in the above conditions. The cells were transfected using the same amounts of fluorescein labelled oligonucleotides (FITC, Sigma, USA) in 2 parallel series. In one of the series oligofectamin, while in the other DMRI (Qiagen, USA) were used as nucleotide carriers. After 3 h the studied cells were washed twice in PBS and stained with DAPI. The preparations thus obtained were evaluated under a fluorescence microscope. The intensity of the signal was evaluated using the IRS scale, which took into account the percentage of positive cells and the intensity of the signal. The parameters yielded an appropriate score and the final result represented the product of the scores [5]. In every case 4 visual fields were evaluated.

RESULTS AND DISCUSSION

The reactions performed resulted in doubly stained cells, namely DAPI-stained cell nuclei (Fig. 1A, 2A), while FITC-labelled nucleotides were localised both in the cell nuclei and in the cytoplasm of the cells studied (Fig. 1B, 2B). In every case more than 90% of cells demonstrated the green fluorescence typical for FITC, indicating that the nucleotides being tested had penetrated the cells.

In the case of EPG 257/85 RDB cells, signal intensity was the same for both types of liposome. Oligofectamin proved to be a more effective carrier in cases of EPG 257/85 RNOV, A 2780P, MEWO CIS and 181 P cell line, while DMRI was more efficient in cases of W181/A17, A 2780 RCIS, 181 RDB and MCF-7/MX cell lines (Fig. 3).

The studies showed that various liposomes exhibit a distinct efficiency of transfection, depending on the cell line, and that application of fluorescein labelled oligonucleotides may prove useful for optimisation of transfection conditions.

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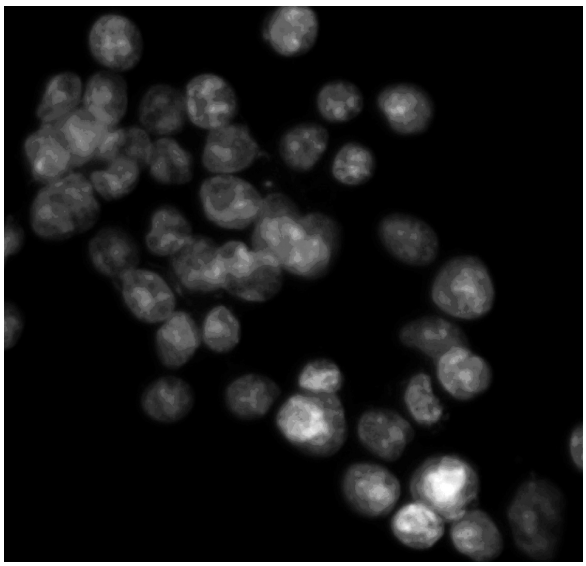

Figure 1A. EPG 257/85 RNOV cells stained by DAPI technique (× 400).

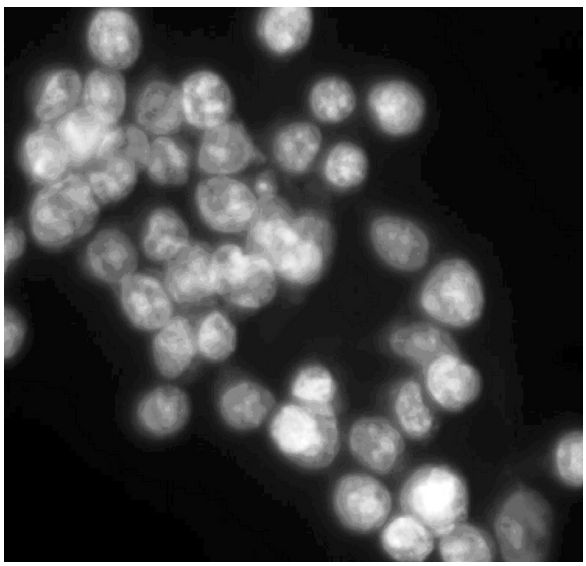

Figure 1B. EPG 257/85 RNOV cells transfected with fluorescein-labelled oligonucleotides. Transfection using oligofectamin (× 400).

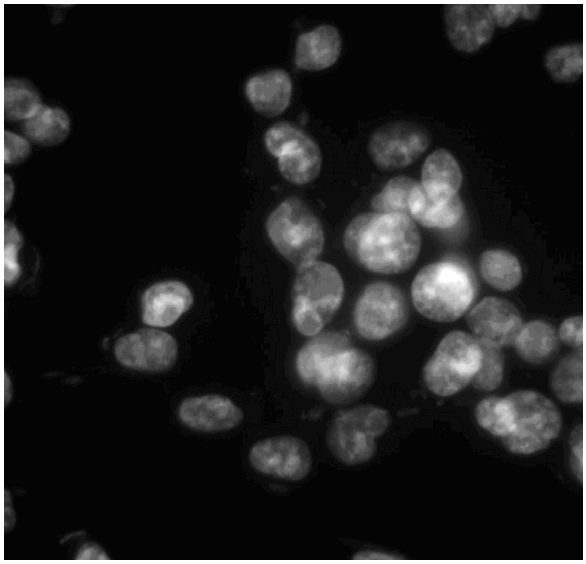

Figure 2A. EPG 257/85 RNOV cells stained by DAPI technique (× 400).

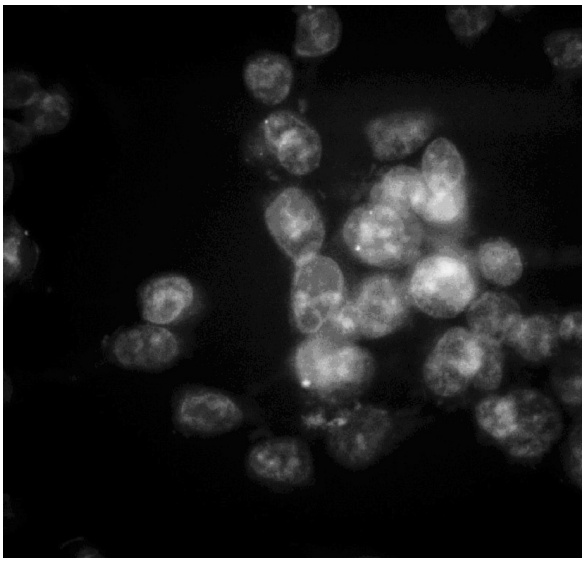

Figure 2B. EPG 257/85 RNOV cells transfected with fluorescein-labelled oligonucleotides. Transfection using DMRI (× 400).

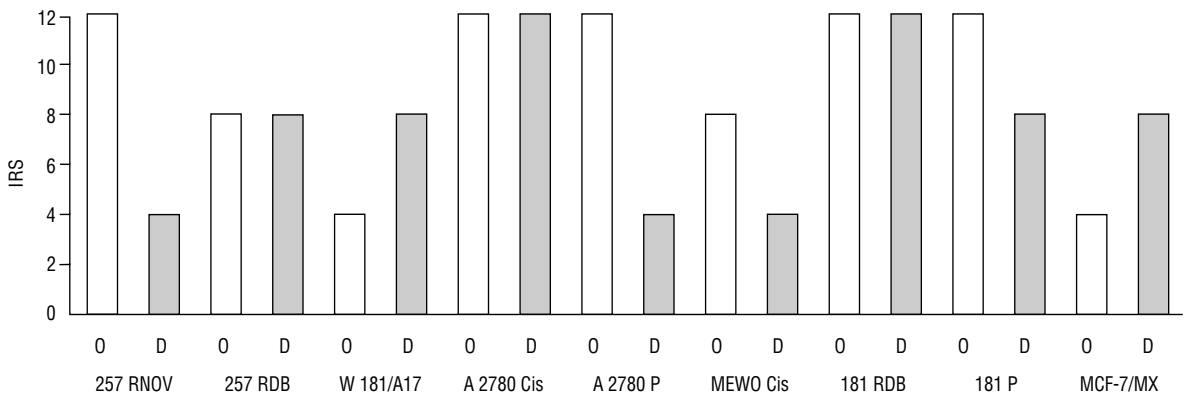

Figure 3. Intensity of signal (IRS) obtained due to transfection of studied cells with FITC-labelled oligonucleotides and various liposomes: O — oligofectamin, D — DMRI.

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