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Cell type-specific gene expression, mediated by TFL-3, a cationic liposomal vector, is controlled by a post-transcription process of delivered plasmid DNA

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Abstract

The issue of whether the TFL-3, a recently developed cationic liposome, achieves efficient gene expression in different mammalian cell lines (NIH/3T3, LLC, A431 and HeLa cells) was examined. The issue of whether gene expression is related to the amount of plasmid DNA (pDNA) delivered in cells or nuclei following transfection was also examined. The cells were transfected for 1 h with pDNA/TFL-3 lipoplexes, and the transfection efficiency was determined by means of a luciferase activity assay. The amount of intracellular and intranuclear pDNA following the transfection was also quantitatively determined. Successful transgene expressions in all cell lines we tested were observed under our experimental conditions, suggesting that the TFL-3 represents a suitable nonviral vector system for the successful gene expression in mammalian cells in vitro. The degree and rate of gene expression were dependent on the type of cells used as well as the incubation time after transfection, but these parameters were independent of the amount of gene delivered to cells and nuclei. These results suggest that TFL-3 mediated gene expression is largely controlled by the process of post-transcription of the delivered pDNA, and not by the process of cellular entry of pDNA and cytoplasmic trafficking of pDNA into nuclei, which is dependent on the cell type. Therefore, the results obtained here clearly suggest that the cell type-specific improvement in transcription efficiency of pDNA and translation of the derived mRNA, together with an improved delivery system to enhance the nuclear delivery of pDNA, is necessary to achieve efficient transgene expression in mammalian cells.

Keywords: Gene delivery; Cationic liposome; Lipoplex; DNA; Transfection

Abbreviations: CCLR, cell culture lysis reagent; CHOL, cholesterol; DOPE, dioleoylphosphatidylethanolamine; FBS, fetal bovine serum; PBS, phosphate buffered saline; pDNA, plasmid DNA; TFL-3, a cationic liposome composed of DC-6-14, with helper lipids dioleoylphosphatidylethanolamine (DOPE) and cholesterol (CHOL) (1:0.75:0.75 at a molar ratio)

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1. Introduction

The success of gene therapy is predicated on the development of gene transfer vectors that are safe and efficacious. The application of viral vectors in clinical therapy is limited because they suffer from numerous problems such as immunogenecity, oncogenic effects, feasibilities of endogenous virus recombination (Temin, 1990; Byrnes et al., 1995), although superior transfection efficiency is generally observed when viral vectors are used. Considering these limitations of viral vectors, non-viral vectors such as cationic liposomes offer an attractive alternative. Non-viral vectors are developed under the assumption that they will overcome most of the problems associated with viral vectors. Indeed, cationic liposomes have been proven to be useful tools for gene delivery to cultured cells and for gene therapy in preclinical and clinical trials (Hofland et al., 1996; Li and Huang, 2000).

In the earlier studies, TFL-3, a cationic liposome, composed of a newly developed cationic lipid, DC-6-14, with helper lipids dioleoylphosphatidylethanolamine (DOPE) and cholesterol (CHOL) (1:0.75:0.75 at a molar ratio), showed a higher transfection efficiency in dividing or non-dividing cells in vitro, even in the presence of serum (Kikuchi et al., 1999; Nguen et al., 2003). TFL-3 in pDNA-liposome complexes retained their morphology in the presence of serum, and this property may account for the high activity in serum-containing media (Serikawa et al., 2000). In addition, TFL-3 showed an effective gene transfection activity in vivo, i.e., intraperitoneal injection in nude mice (Kikuchi et al., 1999). From these features, TFL-3 would be expected to be superior non-viral vector in gene transfection, both in vitro and in vivo. Nevertheless, experimental data on gene expression by means of TFL-3 is limited at present. The accumulation of results with respect to TFL-3 will further increase their utility as a non-viral vector of gene transfection in vitro and in vivo. In this study, therefore, we examined the issue of whether TFL-3 increases the efficacy of transgene expression in various types of cells, i.e., mouse fibroblast (NIH/3T3 cell), mouse lung cancer (LLC cell), human epidermoid (A431 cell) and human cervix (HeLa cell). These cell lines are adhesive-type and widely used for studying exogenous gene expression with non-viral and viral vector systems. The amount of intranuclear intact plasmid DNA (pDNA) delivered by TFL-3 was determined, and the relationship between amount of pDNA delivered to cells or nuclei and gene expression was examined.

2. Materials and methods

2.1. Materials

TFL-3 composed of *O*,*O*′-ditetradecanoyl-*N*-(α-trimethyl ammonioacetyl) diethanolamine chloride/DOPE/CHOL (1/0.75/0.75 mol/mol) was a generous gift from Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). Plasmid pGL3-Control (pDNA) containing the cDNA of firefly luciferase driven by the SV40 promoter, the luciferase assay kit and cell culture lysis reagent (CCLR) were purchased from Promega (WI, USA). [α-^{32}P] dCTP was purchased from Amersham (NJ, USA). Opti-MEM I medium was purchased from Life Technologies (MD, USA). Other cell culture regents were obtained from Nissui Pharmaceutical Co. Ltd. (Tokyo, Japan). All other reagents were of analytical grade.

2.2. Cell culture

AH431 cells (human epidermoid), HeLa cells (human cervix) were obtained from Dr. Y. Shinohara (Division of gene expression, Institute for Genome Research, The University of Tokushima, Japan). NIH/3T3 cells (mouse fibroblast) were provided by Dr. S. Sone (Department of Internal Medicine and Molecular Therapeutics, School of Medicine, The University of Tokushima). LLC cells (mouse lung cancer) were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Japan). NIH/3T3, A431, HeLa and LLC cells were cultured in DMEM medium supplemented with 10% heat-inactivated FBS, 10 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. For the luciferase assay, the cell lines were plated in a well of a 12-well plate at density of 2×10^5 cells for A431, HeLa or LLC cells, or at density of 1.5×10^5 cells for NIH/3T3 cells. For a Southern blot analysis, the cell lines were plated in a 100 mm dish at a density of 2×10^6 cells for A431, HeLa or LLC cells, or at a density of 1.5×10^6 cells

for NIH/3T3 cells. Cultures were maintained at 37 °C in a 5% CO_2 air incubator.

2.3. Transfection (lipofection) and liciferase assay

pDNA/TFL-3 complexes (lipoplexes) were prepared at a ratio of 1 μg of pDNA to 5 μmol of cationic lipid (the molar charge ratio (N/P) was 1.33) in Opti-MEM I medium, which is the optimal ratio of lipoplex, as shown previously (Almofti et al., 2003a). The size of lipoplexes was approximately 2 μm. After an over night pre-culture, the lipoplexes (2.5 μg pDNA/ml) were added to the cells at a final volume of 400 μl in a well of a 12-well plate and 5 ml per 100 mm dish. After 1 h of lipofection, the medium was replaced with appropriate medium supplemented with 10% heat-inactivated FBS after washing twice with cold phosphate buffered saline (PBS). The cells were further incubated for 3–47 h until being used in the luciferase assay. The cells were lysed by the addition of 400 μl of CCLR. The cell lysate was collected and centrifuged for 2 min at 12,000 × *g* to give a clear supernatant for the assay. A 20-μl aliquot of the supernatant was used to determine the luciferase activity using luciferase assay reagent according to the manufacturer's recommended protocol (Promega, WI, USA). The protein content of the lysate was measured with the DC protein assay kit (Bio-Rad Laboratories, CA, USA), and the data are expressed as light counts/min/mg of protein.

2.4. Internalized pDNA associated with the TFL-3 by cells

In order to evaluate lipoplex-cell binding and uptake, the lipoplex was prepared with TFL-3 and ^{32}P-labeled pDNA at a ratio of 1 μg of pDNA to 5 μmol of cationic lipid in Opti-MEM I medium. ^{32}P-labeled pDNA was prepared using [α-^{32}P] dCTP with a nick translation kit (Amersham, NJ, USA). Cells were plated in a well of a 12-well plate at the density mentioned above. Four-hundred microliter of the lipoplex was added to a well and incubated at 4 °C for binding and at 37 °C for uptake, for 0–60 min. After incubation, the cells were washed three times with cold PBS and lysed by the addition of 400 μl of CCLR to each well. The radioactivity of the cell lysate was determined by a liquid scintillation counter (LSC, Tokyo, Japan). The total radioactivity in a 400 μl of lipoplex added to the well was also determined as the total dose. The percentage of internalized pDNA was calculated by dividing the radioactivity of pDNA (37 °C) taken up minus bound pDNA (4 °C) by the total radioactivity of the added pDNA as lipoplex.

2.5. Quantification of pDNA delivered to the nucleus with the TFL-3 as determined by Southern blot analysis

The pDNA delivered to nuclei following transfection was quantitatively determined using a method recently developed in our laboratory (Tachibana et al., 2002) with minor modifications. Briefly, the cells, plated in a 100 mm dish, were transfected with the pDNA/TFL-3 lipoplex. At various time points post-transfection, the cells were washed twice with 5 ml of cold PBS, and then harvested by scraping and subsequent centrifugation (3000 rpm, 5 min, 4 °C) after treatment with 3 ml of Tris–HCl buffer (40 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA (pH 7.4)) for 10 min at 25 °C. The collected cells were suspended in 0.5 ml of lysis buffer (0.5% Nonidet P-40, 10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris–HCl; pH 7.4) to dissolve the plasma membrane, and the nuclear fraction was then isolated by centrifugation at 1400 × *g* for 2 min at 4 °C. The precipitate was used as the nuclear fraction, and the nuclear fraction was treated with SalI (Nippon Gene, Tokyo, Japan), a restriction enzyme of pGL3 to prevent the contamination of extranuclear pDNA.

To extract nuclear DNA (containing pDNA), proteinase K (Merck, Frankfurter, Germany) was added to the nuclear fraction to a final concentration of 0.1 mg/ml. After incubation at 37 °C for 4 h, proteins were eliminated by phenol/chloroform treatment; and the DNA was precipitated by the addition of ethanol. The precipitate was dissolved in TE buffer (10 mM Tris–HCl, 1 mM EDTA; pH 8.0) and used as a DNA sample. Concentrations of DNA were determined by measurement of the absorbance at 260 nm with a Shimadzu UV-1200 spectrophotometer (Shimadzu, Kyoto, Japan). The number of nuclei in the samples was calculated from the amount of genomic DNA, based on the assumption that the nucleus of a cell contains 6×10^9 nucleotide pairs of DNA. Namely, a nucleus contains 6.6×10^{-6} g of DNA because the average molecular weight of one nucleotide pair is 660.

For Southern analysis, aliquots of the extracted DNA sample (approximately 1–10 μg) were digested with 120 units of *Hin*dIII (Nippon Gene, Tokyo, Japan) at 37 °C overnight, and then subjected to agarose gel electrophoresis. After the electrophoresis, the DNA samples were transferred to a nitrocellulose membrane. For the detection of plasmids, a DNA fragment corresponding to the open reading frame of luciferase, prepared by PCR with RHY008 and RHY010 as primers, was prepared according to a previously described method (Sambrook et al., 1989). The DNA fragment was radiolabeled with ^{32}P using a nick translation kit (Amersham, NJ, USA) and the ^{32}P-labeled fragment was used as a probe. Southern hybridization was carried out using a standard protocol (Sambrook et al., 1989). In brief, the membranes were pre-hybridized at 42 °C overnight in a solution consisting of 0.01 mg/ml salmon sperm DNA, 5× SSPE, 5× Denhardt's, 0.1% SDS, and 50% formamide and then hybridized at 42 °C overnight in the same solution in the presence of the ^{32}P-labeled probe. After hybridization, the membranes were washed three times with 2× SSC containing 0.1% SDS at 60 °C for 60 min. After washing, the membranes were exposed to the imaging plate of a bioimage analyzer Fuji-BAS 1500 (for quantification).

3. Results and discussion

3.1. Effect of dose of lipoplexes on the gene expression in the different cell lines

The effect of the dose of lipoplexes (pDNA/TFL-3) on gene expression in different cell lines is summarized in Table 1. TFL-3 showed efficient gene transfer in four different cell lines. In HeLa and A431 cells, gene expression tended to increase with an increase in the amount of lipoplexes administered. To the contrary, in LLC and NIH/3T3 cells, gene expression gradually increased and then decreased over the dosage range of lipoplexes administered. It has been reported that one of major limitations associated with cationic liposomes is cytotoxicity, presumably due to the synthesized cationic lipid included (Lappalainen et al., 1994; Aberle et al., 1998), and the cytotoxicity is closely associated with the dose of lipoplexes administered (Dass et al., 2002). Therefore, the decreased gene expression in the higher dose range observed in the case of LLC and NIH/3T3 cells is likely due to TFL-3 related cytotoxicity against these cells. It appears that the extent and degree of gene transfer and cytotoxicity mediated by TFL-3 are cell type-specific.

We recently reported that the TFL-3 achieved efficient gene expression in non- or less frequently dividing cells, primary cultured rat hepatocytes, in the presence of 5% FBS (Nguen et al., 2003). The present findings demonstrate the stable transfection activity of the TFL-3 in four different mammalian cells (Table 1) that are actively dividing. A similar stable gene transfer activity of TFL-3 in actively dividing mammalian cells has also been reported in the presence of 10% fetal calf serum (Kikuchi et al., 1999; Serikawa et al., 2000). Collectively, these results clearly suggest that TFL-3 is a suitable nonviral vector system for successful gene expression in all types of mammalian cells in vitro.

3.2. Effect of time for culture post-transfection on gene expression in different cell lines

The results shown in Table 1 indicate that the optimal dose of pDNA is 1 μg per well, corresponding to 5 nmol of total lipids, for achieving efficient gene expression and less cytotoxicity under our experimental conditions. Hence, subsequent experiments were carried out using these conditions.

The time course of gene expression mediated by lipoplexes was determined (Fig. 1). Over the period of time tested, the gene expression in A431 and HeLa cells was lower relative to that for in LLC and NIH/3T3. In LLC and NIH/3T3 cells, gene expressions increased transiently with an increase in the time of culture post-transfection, reaching a maximum level between 24 and 48 h post-transfection. It is likely that the level of gene expression is dependent on the time of culture post-transfection. This indicates the importance of culture time after transfection in achieving efficient transgene expression. In addition, the longevity of protein activity was cell type-specific. It appears that the stability of the expressed protein itself is dependent on the type of cell lines.

3.3. Percentage of pDNA internalized by cells

The percentage of internalized pDNA mediated by the TFL-3 was calculated, as described in Section 2

Table 1
Effect of pDNA dose on gene expression mediated by TFL-3 in different cell lines

Cell line	Luciferase activity ($\times 10^5$ cpm/mg of protein)						
	Dose of pDNA (μg per well)						
	0.25	0.5	1	1.25	1.5	2	3
A431	0.8 ± 0.3	2.6 ± 1.0	4.0 ± 1.1	3.8 ± 0.6	5.2 ± 0.9	7.0 ± 1.6	6.8 ± 1.1
HeLa	0.8 ± 0.2	1.5 ± 0.5	3.1 ± 0.9	3.5 ± 0.6	5.0 ± 0.5	–	–
NIH/3T3	4.4 ± 1.6	5.4 ± 0.9	8.8 ± 2.4	8.2 ± 1.1	5.5 ± 1.8	–	–
LLC	0.8 ± 0.2	3.4 ± 0.4	5.7 ± 0.6	5.2 ± 0.8	4.5 ± 0.3	4.6 ± 0.2	3.2 ± 0.1

A431, HeLa, NIH/3T3 and LLC cells were transfected for 1 h with different amounts of pDNA (0.25–3 μg per well) at a ratio of 5 nmol lipid/μg plasmid. After removal of lipoplexes, the cells were further incubated for 23 h. The activity of luciferase (cpm/mg of protein) in the cells was measured as described in Section 2. Data are mean ± S.D. ($n = 3$). (–) not measured.

(Fig. 2). The TFL-3 successfully transported pDNA into the cells but the transport activity of the TFL-3 appears to be independent on the type of cell lines used. Various efforts have been undertaken to understand the mechanism of the internalization of lipoplexes internalization into the cytoplasm. Initial studies suggested that lipoplexes fused with the plasma membrane and that the fusion process mediated the entry of pDNA into cells (Felgner et al., 1987, 1995). Other studies indicated that endocytosis of the lipoplexes is another possible mechanism of entry of pDNA (Felgner et al., 1987; Zabner et al., 1995; Wrobel and Collins, 1995; Farhood et al., 1995). We recently reported that the entry of pDNA mediated by the TFL-3 into cells occurs via a lipoplexe-plasma membrane fusion

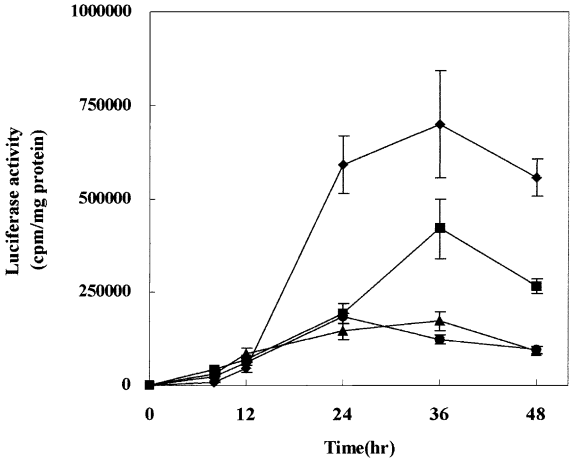

Fig. 1. Effect of time for culture post-transfection on gene expression in different cell lines A431 (●), HeLa (▲), LLC (■) and NIH/3T3 (◆) were plated in a well of a 12-well plate. After an overnight pre-culture, the cells were transfected for 1 h with lipoplexes at a dose of 1 μg pDNA per well, corresponding to 5 nmol of lipids. The cells were washed three times with cold PBS to remove unassociated lipoplexes and then further incubated. During the incubation, at the indicated time points, the luciferase activity (cpm/mg of total protein) in the cells was determined according to the method described in Section 2. Data are mean ± S.D. ($n = 3$).

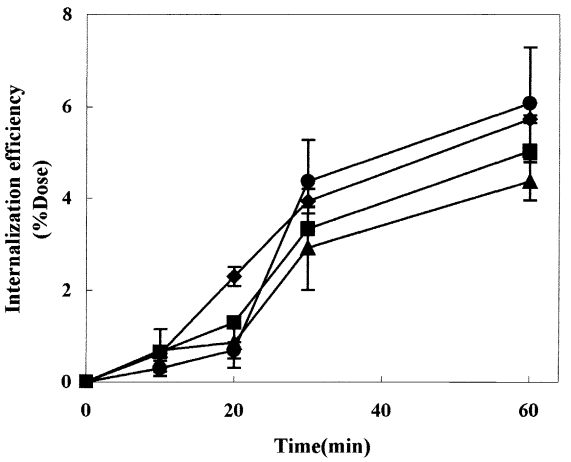

Fig. 2. Amount of internalized pDNA mediated by TFL-3. A431 (●), HeLa (▲), LLC (■) and NIH/3T3 (◆) were plated in a well of a 12-well plate. After an overnight pre-culture, the cells were incubated at 4 °C for binding and at 37 °C for uptake, for 0–60 min, with lipoplexes containing radiolabeled pDNA (^{32}P-pDNA) and TFL-3 at a dose of 1 μg pDNA per well corresponding to 5 nmol of lipids. Four hundred microliters of the lipoplex was added to a well and incubated, for 0–60 min. After incubation, the cells were washed three times with cold PBS. Radioactivity of the cell lysate was determined using a liquid scintillation counter (LSC, Tokyo, Japan). The percentage of internalized pDNA was calculated by dividing radioactivity of uptaken pDNA (37 °C) minus that of bound pDNA (4 °C) by total radioactivity of administered pDNA as lipoplexes. Data are mean ± S.D. ($n = 3$).

(Almofti et al., 2003b). In this case, the efficiency of entry of pDNA mediated by the TFL-3 should be related to efficiency of cellular association of the pDNA/TFL-3 lipoplexes. Generally, cellular association is thought to be governed mainly by electrostatic attractions between the positively charged cationic lipid in the lipoplexes and the negatively charged cell membranes (Pires et al., 1999; Sakurai et al., 2000). In this study, the ratio of pDNA to cationic lipid was fixed (1 μg of pDNA to 5 μmol of cationic lipid). Thus, the observed similar percentage of internalized pDNA following transfection in our experiments (Fig. 2) is possibly due to less difference in the extent of negative charges on the cell surface, probably related to the presence of sialic acid residues.

In addition, the efficiency of internalization of pDNA mediated by the TFL-3 was increased with the time used in the incubation of cells with the lipoplexes (Fig. 2). Approximately 4–6% of the pDNA administered was internalized during the 60 min incubation. A longer incubation in the presence of lipoplexes may enhance the transport of pDNA and consequently increase gene expression. On the other hand, a prolonged incubation time may serve to maximize the cationic liposome-related cytotoxicity against donor cells and consequently decrease gene expression. These issues indicate the importance of optimization of the experimental conditions in achieving an increased gene expression by our vector system.

3.4. Amount of pDNA delivered into nucleus as a consequent of transfection

Studies on the fundamental mechanism of gene delivery are very important in the terms of the development of nonviral vectors which can be successfully used in clinical setting. In early reports, radio-labeled or fluorescence-labeled pDNA were used for the quantitative analysis of pDNA delivered to cells (Lee and Huang, 1996; Farhood et al., 1995). However, in terms of following the intracellular disposition of intact, undegraded pDNA after transfection have several disadvantages. The chemical modification of pDNA such as fluorescence labeling may change their physicochemical properties, resulting in an alteration of their intracellular disposition. In addition, it is difficult to distinguish intact pDNA from partially or completely degraded plasmids. Furthermore, the possibility that the free label, cleaved from pDNA, is also measured cannot be excluded. In order to overcome these disadvantages, we recently developed a quantitative method by means of Southern blot analysis (Tachibana et al., 2002). In this study, we employed this method to differentiate and quantify only intact pDNA delivered into nuclei as a consequence of transfection.

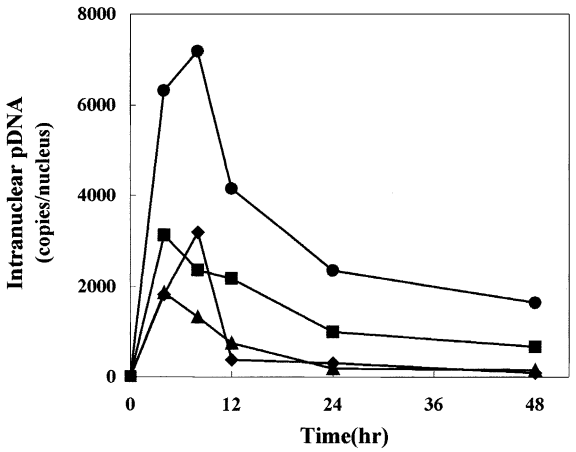

Fig. 3. Amount of pDNA delivered to the nucleus as a consequent of transfection. A431 (●), HeLa (▲), LLC (■) and NIH/3T3 (◆) were plated in a 100 mm dish. After overnight pre-culture, the cells were transfected for 1 h with lipoplexes at a dose of 1 μg pDNA per well. After removal of the complexes, the cells were further incubated for periods of up to 48 h. At the indicated time points, the amount of intranuclear pDNA (copies/nucleus) in the cells was determined according to the method described in Section 2. A similar tendency was observed in three independent experiments and a typical result is shown.

In all cell lines tested, amount of pDNA delivered into nuclei gradually increased and then decreased up to 48 h post-transfection (Fig. 3). Approximately, 0.1–0.4% of the intact pDNA administered was delivered to nuclei by the TFL-3. The rank order of amount of nuclear pDNA taken up was A431 $>$ NIH/3T3 $\approx$ LLC $\geq$ HeLa cells, although a similar uptake of pDNA by the cells was observed in Fig. 2. These findings suggest that the efficiency of cytoplasmic trafficking of pDNA to nucleus following the entry of pDNA mediated by the TFL-3 is cell type-specific. The time profiles of the amount of delivered pDNA to nuclei indicate that a different rate-limiting step exists in each cell line. In the case of A431 cells, the rate of transport of pDNA to nuclei is likely to be much faster than the elimination of pDNA from

nuclei related to degradation and/or the excretion of pDNA. To the contrary, in the case of LLC, HeLa and NIH-3T3 cells, the transport rate of pDNA is likely to be slower than that for A431 cells but still faster than the elimination of pDNA from nuclei related to degradation and/or excretion of pDNA. Therefore, the peak in the LLC, HeLa and NIH/3T3 cases might be lower than that for A431 cells.

Interestingly, in A431 cells, the TFL-mediated nuclear delivery of pDNA was successfully achieved compared to other cell lines (Fig. 3), whereas the gene expression was lower than those in other cell lines (Fig. 1). To the contrary, in NIH/3T3 and LLC cells, the nuclear delivery of pDNA was lesser than that for A431 (Fig. 3), whereas significant gene expression was observed compared to that in A431 cells (Fig. 1). These findings suggest that the efficiency of gene expression after transfection is not necessarily related to the amount of pDNA delivered to the nucleus by TFL-3, a nonviral vector, although the delivery of a sufficient amount of pDNA to a nucleus to permit gene expression is a requisite. A similar result was observed in non- or frequently lesser-dividing mammalian cells, primally cultured rat hepatocytes (Nguen et al., 2003).

It is generally accepted that the uptake of pDNA by target cells, its release from intracellular vesicles such as endosomes or lysosomes (if necessary), transport to the nucleus where transcription takes place, transcription and translation of derived mRNA are limiting steps in obtaining highly efficient gene expression. The results reported here clearly indicate that the process of the transcription of pDNA and translation of derived mRNA, not for entry, intracellular trafficking of pDNA and transport of pDNA via the nuclear membrane, is a major obstacle in achieving efficient gene expression by means of TFL-3. Thus, for the further optimization of our gene transfer system, TFL-3, it will be important to develop a better understanding of the essential biological characteristics of targeted cells, such as cell cycles and activity of transcription.

4. Conclusion

The study described here indicates that TFL-3 mediated gene expression is cell type-specific and is largely controlled mostly by the process of post-transcription of the delivered pDNA to the nuclei. This suggests that an improved transgene expression would be attained by increasing the transcription efficiency of pDNA in nuclei and the translation of derived mRNA. Many efforts have been made to increase the transcription and translation efficiency of pDNA. Strong promoters and various poly(A) signal sequences have been used for this purpose. Kamiya et al. (2002) recently indicated that the structure and size of pDNA molecules affect the transcription: smaller linearized DNA dumbbells showed a higher transgene expression in mammalian cells. It is generally understood that improvements of cellular and nuclear entry of pDNA associated with nonviral vectors by various methods are necessary to enhance transgene expression efficiency (Gao and Huang, 1996; Liang and Hughes, 1998; Schoen et al., 1999), since the delivery of a sufficient amount of pDNA to a nucleus to permit gene expression is a requisite. Therefore, we propose that the cell type-specific improvement of transcription and translation efficiency of pDNA, along with an improved delivery system, is important in achieving efficient transgene expression in mammalian cells.

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