Biotechnol Lett
https://doi.org/10.1007/s10529-018-2551-2

ORIGINAL RESEARCH PAPER

Delivery of molecular cargoes in normal and cancer cell lines using non-viral delivery systems

Sepideh Shahbazi · Nooshin Haghighipour · Sepehr Soleymani · Seyed Alireza Nadji · Azam Bolhassani

Received: 26 January 2018 / Accepted: 3 April 2018

Abstract
Objective In this study, transfection efficiency of human papillomavirus (HPV) E7 DNA and protein constructs into HEK-293T normal cell line, and A549 and TC-1 tumor cell lines was evaluated by four delivery systems including supercharge GFP, hPP10 cell penetrating peptide, TurboFect and Lipofectamine using fluorescence microscopy and flow cytometry.
Results The results indicated that Lipofectamine 2000 and TurboFect produced more effective transfection for GFP and E7-GFP DNA constructs in HEK-293T cells compared to in A549 and TC-1 cells ($p < 0.05$). In contrast, the supercharge GFP was efficient for E7 DNA and E7 protein delivery in both normal cell (~ 83.94 and ~ 77.01% for HEK-293T), and cancer cells (~ 71.69 and ~ 67.19% for TC-1, and ~ 73.86 and ~ 67.49% for A549), respectively. Indeed, in these cell lines, transfection efficiency by +36 GFP reached ~ 60–80%. Moreover, the hPP10 produced the best transfection result for E7-GFP protein in HEK-293T cells (~ 63.66%) compared to TurboFect (~ 32.95%); however, the efficiency level of hPP10 was only ~ 17.51 and ~ 16.36% in TC-1 and A549 cells.
Conclusions Our data suggested that the supercharge GFP is the most suitable transfection vehicle for DNA and protein delivery into TC-1 and A549 tumor cell lines compared to other carriers.

Keywords Human papillomavirus · E7 protein · Delivery system · hPP10 · +36 GFP · TuboFect · Lipofectamine

Sepideh Shahbazi and Nooshin Haghighipour are the first authors.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10529-018-2551-2) contains supplementary material, which is available to authorized users.

S. Shahbazi · S. Soleymani · A. Bolhassani (✉)
Department of Hepatitis and AIDs, Pasteur Institute of Iran, Tehran, Iran
e-mail: azam.bolhassani@yahoo.com; A_bolhasani@pasteur.ac.ir

N. Haghighipour
National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran

S. A. Nadji (✉)
Virology Research Center (VRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran
e-mail: sarnadji@yahoo.com

Introduction

Many bioactive molecules are not able to overcome the membrane permeability barrier as a major problem in gene, protein and drug delivery for diagnostic and therapeutic purposes. Thus, viral and non-viral

Published online: 09 April 2018

delivery systems have been developed in the recent years (Chugh et al. 2010). The studies showed that the uptake of DNA in cancer cells might be different from normal cells in vivo due to gene mutations and the ability of endocytosis (Kong et al. 2017). DNA is commonly packaged into a viral, polymer or lipid particle to be transferred into cells (Kong et al. 2017). Recently, novel classes of cell-penetrating peptides (CPPs) were determined to deliver a variety of cargoes that are unable to overcome the permeability barrier either by covalent or non-covalent binding (Arukuusk et al. 2013; Chugh et al. 2010). In general, CPPs are less than 30 amino acids, rich in arginine and lysine, positively charged or amphipathic, easy to prepare, and non-toxic (Huang et al. 2015). For example, hPP10, a CPP derived from human cell nucleoproteins (KDM4A protein) has the ability to penetrate the cell membrane which can be used for carrying proteins in a covalent fashion (Wang et al. 2016). Recently, super-positively charged green fluorescent proteins including a variant with a theoretical net charge of +36 (+36 GFP) have been introduced to penetrate a variety of mammalian cell lines. The use of +36 GFP requires simple mixture of nucleic acids together (McNaughton et al. 2009). However, the type of cell lines was involved to determine a transfection efficacy, because there is different sensitivity to cargoes and transfection reagents. The experimental conditions are important and usually identical in all the cells treated at time of transfection such as 80% confluency, mycoplasma free and optimal cell viability. On the other hand, GFP is common reporter gene, broadly used in biological research for delivery detection in the cells (Kim and Eberwine 2010; Tong et al. 2014). In this report, we compared the uptake of E7 DNA and protein as well as GFP DNA and protein in normal and cancer cells using four transfection vehicles including TurboFect, Lipofectamine, +36 GFP and hPP10 CPP for selection of the best delivery system.

Materials and methods

Cells and transfection reagents

The normal human HEK-293T cells (CRL-3216), the carcinoma cell line HeLa (CCL-2, ATCC) and A549 (CCL-18, ATCC) were grown in complete RPMI-1640 medium (Sigma, Germany) supplemented with 10% heat-inactivated fetal calf serum (FCS, Gibco, Germany) at 37 °C in an atmosphere containing 5% CO_2. Lipofectamine 2000 (Invitrogen) and TurboFect (Fermentase) are cationic lipid and polymeric vectors, respectively.

Preparation of plasmid DNAs and proteins

The pEGFP-N1 (Clontech), pEGFP-E7 and pcDNA-E7 constructs previously provided in our laboratory (Bolhassani et al. 2008), were generated to transfect into the cells in large scale using DNA extraction Midi-kit (Qiagen). Moreover, the generation of the recombinant (r) E7, wild type (wt) GFP, supercharges +36 GFP and E7-GFP proteins was performed as previously reported (Bolhassani et al. 2009; Kadkhodayan et al. 2016; Motevalli et al. 2017; Shahbazi et al. 2018). Briefly, the *E. coli* M15 strain was transformed with the recombinant pQE-E7-GFP or pQE-E7 vectors. The transformants were grown to an optical density of 0.7–0.8 at 600 nm in Ty2x medium. The E7 or E7-GFP protein expression was induced with 1 mM IPTG and incubated for 3 h at 37 °C. Moreover, to obtain the recombinant wt GFP and +36 GFP, induction of protein expression was performed in *E. coli Rosetta* strain (*i.e.,* pET/*E.coli* system) with 1 mM IPTG and incubated for 3 h at 37 °C. On the other hand, the *E. coli* Rosetta strain was transformed with the recombinant pET-hPP10-GFP and pET-hPP10-E7-GFP plasmids. The transformants were selected on LB agar plate and grown to an optical density of 0.7–0.8 at 600 nm in Ty2x medium. Induction of protein expression with 1 mM IPTG was performed at 37 °C and 16 h after induction.

Finally, all cell pellets were harvested, analyzed by 12% SDS-PAGE and purified by affinity chromatography using a Ni–NTA agarose column under native conditions (i.e., 300 mM imidazole buffer, pH 8) according to the manufacturer's instructions (Qiagen). The purified proteins were dialyzed against PBS1X. Their concentrations were measured by NanoDrop spectrophotometry, and stored at − 70 °C until used.

Complexation of non-viral gene vector with plasmid DNA

For preparation of the GFP/DNA nanoparticles, the +36 GFP solution was added to 1 µg of plasmid DNA (pcDNA-E7) at an N/P ratio of 10:1 in PBS (pH 7.4)

and incubated for 15 min at room temperature (Motevalli et al. 2017). The size and morphology of complexes were analyzed with a scanning electron microscope (SEM; KYKY-EM3200 model, China). Furthermore, TurboFect and Lipofectamine 2000 transfection reagents were used to deliver the pEGFP-N1 and pEGFP-E7 into the cells and investigate the expression of proteins according to the manufacturer's protocols.

Complexation of non-viral vector with protein

TurboFect was used as a protein transfection reagent. TurboFect/GFP or Turbofect/E7-GFP complexes were prepared according to manufacturer's instructions (Pro-Ject^{TM} Reagent, Germany). Briefly, 2.5 µl of TurboFect protein transfection reagent was used for preparation of complexes containing 1.0 µg of GFP or E7-GFP. Moreover, the +36 GFP/E7 protein complex with molar ratio of 10:1 (1 µg E7 protein) were formed in 100 µl PBS and incubated for 30 min at room temperature (Motevalli et al. 2017). The size and morphology of complexes were analyzed with a scanning electron microscope (SEM; KYKY-EM3200 model, China).

In vitro transfection of normal and cancer cell lines

For transfection assay, the HEK-293T, A549 and TC-1 cells were seeded onto 24-well culture plates (Greiner, Germany) at a density of 0.5×10^5 cells/well and incubated overnight in RPMI containing 10% FCS. After growth of the cells to 80% confluency, the medium was replaced by serum-free RPMI and then 100 µl of each complex (*i.e.,* TurboFect/pEGFP-N1, Lipofectamine/pEGFP-N1, Lipofectamine/pEGFP-E7, TurboFect/pEGFP-E7, TurboFect/rGFP, TurboFect/rE7-GFP, 1 µg of hPP10-E7-GFP, 1 µg of hPP10-GFP, E7 DNA/+36GFP and rE7/+36GFP) was applied to each well. After certain times (i.e., 3 h for hPP10 and +36 GFP, and 6 h for TurboFect and Lipofectamine), the cells were grown in complete medium at 37 °C. The transfection efficiency of cargoes/carriers was monitored by fluorescence microscopy (Envert Fluorescent Ceti, Korea) and quantified by a FACS Calibur flow cytometer (Partec, Germany) at 24 h post-transfection. For each individual sample, 10,000 cells were counted.

Statistical analysis

Statistical analysis (Student's *t* test) was performed by Prism 5.0 software (GraphPad, San Diego, California, USA) to analyze the flow cytometry results. The value of $p < 0.05$ was considered statistically significant. Similar results were obtained in two independent experiments.

Results

Generation of the recombinant proteins

The expression and purification of HPV E7, E7-GFP, wt GFP, +36 GFP, hPP10-GFP and hPP10-E7-GFP proteins was performed in bacterial systems. The recombinant E7, E7-GFP, wt GFP, +36 GFP, hPP10-GFP and hPP10-E7-GFP proteins migrated as clear bands of ~ 23, ~ 50, ~ 27, ~ 27, ~ 30 and ~ 50 kDa in SDS-PAGE, respectively. The results indicated that all the recombinant proteins could be successfully purified under native conditions (data not shown). The recombinant proteins had a concentration range between 0.6 and 1.2 mg/ml.

Confirmation of the DNA constructs

The presence of HPV16 E7 gene in pEGFP-N1 and pcDNA3.1 (−) vectors was confirmed using digestion as a clear band of ~ 300 bp migrated in agarose gel (data not shown). The purified plasmids had a concentration range between 0.3 and 0.6 µg/µL. The 260:280 UV absorption ratios ranged from 1.8 to 2.0.

Formation of E7 DNA or rE7 protein/+36 GFP complexes

The size and morphology of nanoparticles (E7 DNA/+36GFP and rE7/+36GFP) were analyzed by SEM as shown in Fig. 1. The rE7/+36 GFP complexes formed the particles with an average diameter of 250–300 nm at a molar ratio of 1:10. Moreover, SEM analysis of E7 DNA/+36 GFP nanoparticles at an N/P ratio of 10:1 showed a spherical and regular shape with a size of ~ 200–250 nm at 25 °C.

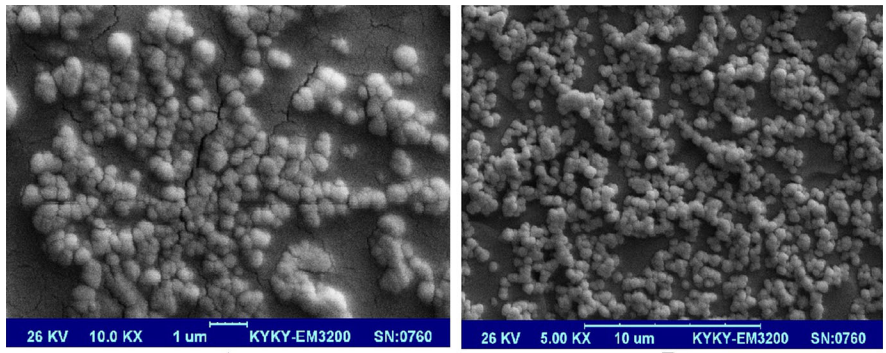

Fig. 1 **a** SEM electron microscopy of rE7/+GFP complexes at a molar ratio of 1:10 with ×10,000 magnification. An average diameter of 250–300 nm was observed for E7/GFP complexes; **b** The SEM micrograph of the spherical nanoparticles formed at N/P = 10:1 (+36GFP/E7 DNA) with ×10,000 magnification: A size of ~ 200–250 nm was observed for nanoparticles at 25 °C

DNA and protein delivery by different carriers in vitro

The ability of the supercharged GFP and hPP10 was evaluated to penetrate the eukaryotic cell lines (*e.g.,* HEK-293T, A549 and TC-1) using fluorescence microscopy and flow cytometry for GFP fluorescence at 24 h post-transfection. The data indicated that +36 GFP is able to deliver E7 DNA and also E7 protein effectively into the normal and tumor cells in vitro (Figs. 2, 3, 4**).** The cellular uptake of the E7 DNA and E7 protein into the HEK-293T cells (i.e., fluorescence intensities) was ~ 83.94 and ~ 77.01%, into the TC-1 cells was ~ 71.69 and ~ 67.19%, and into the A549 was ~ 73.86 and ~ 67.49%, respectively. On the other hand, the efficiency of wt GFP or E7-GFP protein delivery by hPP10 into the HEK-293T cells was ~ 77.77 and ~ 63.66%, into the TC-1 cells was ~ 26.01 and ~ 17.51%, and into the A549 was ~ 21.6 and ~ 16.36%, respectively. Flow cytometry analysis showed that the fluorescence intensities of wt GFP and E7-GFP proteins was ~ 57.96 and ~ 32.95% for HEK-293T, ~ 14.49 and ~ 14.09% for TC-1, and ~ 18.49 and ~ 14.54% for A549 at 24 h after transfection using TurboFect, respectively (Figs. 2, 3, 4**).** Moreover, the pEGFP-N1 and pEGFP-E7 delivery were detected in approximately ~ 99.1 and ~ 80.63% of HEK-293T cells, ~ 5.04 and ~ 4.47% of TC-1 cells, and ~ 6.66 and 5.95% of A549 cells treated with TurboFect, respectively. In contrast, the E7 DNA (pEGFP-E7) and GFP (pEGFP-N1) delivery were detected in approximately ~ 98.1 and ~ 74.43% of HEK-293T cells, ~ 14.54 and 8.11% of TC-1 cells, and ~ 19.31 and 16.36% of A549 cells treated with Lipofectamine, respectively. Generally, the data indicated that the transfection efficiency of +36 GFP-based nanoparticles was higher than TurboFect, Lipofectamine and hPP10 in tumor cell lines ($p < 0.05$). Indeed, the spreading green regions were observed for DNA and protein delivery using supercharge GFP carrier by fluorescent microscopy in these cells (data not shown). Our flow cytometry results demonstrated that +36 GFP delivers E7 DNA slightly more than E7 protein in all the cells indicating different nature of the cargo-related cell uptake. Moreover, there is no significant difference for protein delivery into TC-1 cells and A549 cells using hPP10 and TurboFect ($p > 0.05$). However, the expression of DNA in cancer cells was significantly less than that in normal cell using transfection reagents and hPP10 CPP ($p < 0.05$) indicating the importance of cell type and selection of suitable carriers (Figs. 2, 3, 4).

Discussion

In the current study, HPV E7 DNA and protein constructs were transfected into HEK-293T normal cell line, and A549 and TC-1 tumor cell lines using two delivery systems such as supercharge GFP and hPP10 cell penetrating peptide, and two commercial transfection reagents including TurboFect and Lipofectamine. Prior to transfection, the E7 gene was cloned into a eukaryotic expression vector (*i.e.,* pEGFP-N1) and then sub-cloned into a prokaryotic

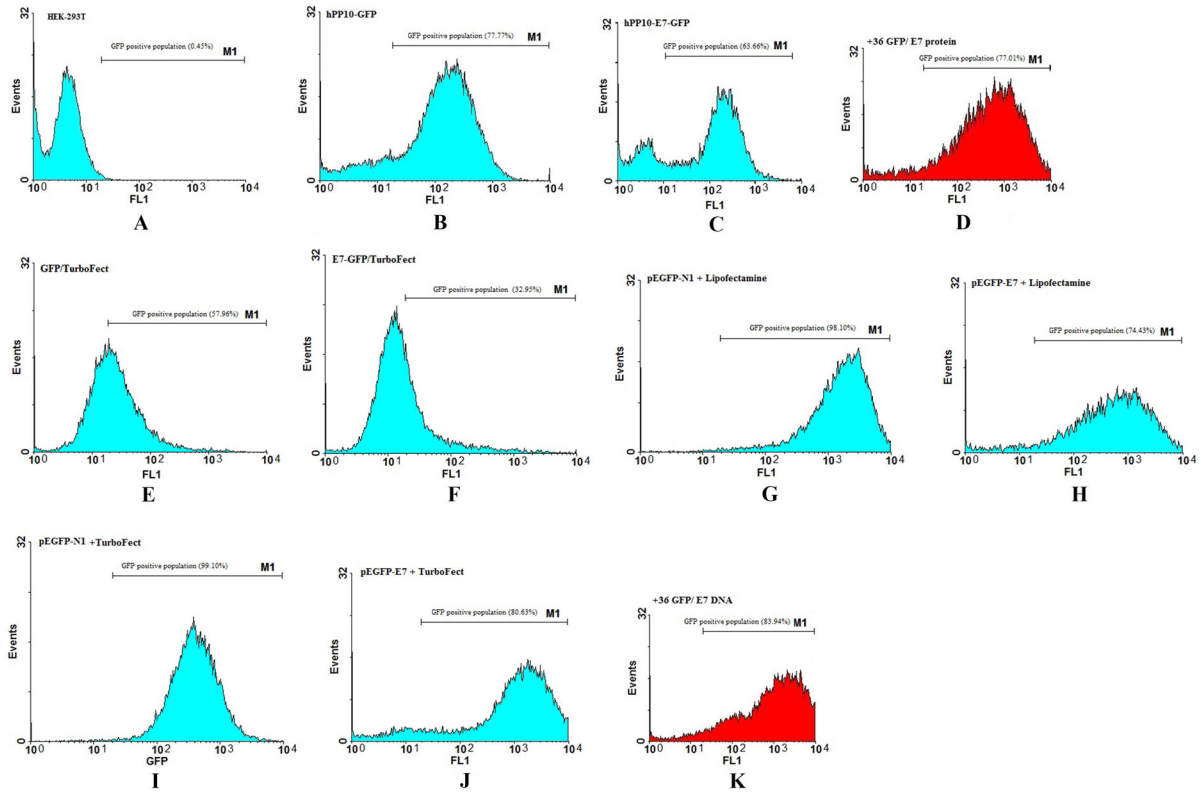

Fig. 2 Evaluation of supercharged +36 GFP (**d**, **k**), hPP10 (**b**, **c**), TurboFect (**e**, **f**; **i**, **j**) and Lipofectamine (**g**, **h**) for DNA and protein delivery into HEK-293T normal cells. Transfection efficiency was monitored by flow cytometry at 24 h post-transfection as compared to the negative control (**a**)

expression vector (*i.e.,* pQE-30) in order to express a green fluorescent protein tag at the 3′ end of E7. Moreover, the pEGFP-E7 and pcDNA-E7 constructs were provided in large scale for DNA transfection. Then, Flow cytometry and fluorescent microscopy were used to analyze transfection efficiency of DNA and proteins into different cell types.

In DNA transfection, viral vectors were usually most efficient for cell transduction, but they showed several disadvantages related to immunogenicity, inflammation and limitation of DNA size. In contrast, non-viral vectors were generally less effective in delivering DNA and initiating gene expression as compared to viral vectors (Horibe et al. 2014). Thus, transfection of reporter plasmid DNA into the cell lines is an important research, because the transfection efficiency of DNA varies among various cell lines including normal and cancer cell lines (Horibe et al. 2014). For instance, a study indicated that hepatocellular carcinoma cells possess the ability to deliver large DNA fragments directly without a transfection reagent, whereas normal liver cells cannot. Indeed, the efficiency of uptake was related to the DNA size. On the other hand, some cell lines of lung cancer and breast cancer showed similar uptake of DNA (Kong et al. 2017). The reports indicated that Lipofection was widely used to transfer genes into various cell lines. However, the rate of DNA integration into the genome following lipofection is relatively low as compared to other methods such as retroviral systems (Pipes et al. 2005). A study indicated that the transfection efficiency of NT2/D1 and HeLa cells with Lipofectamine and Effectene transfection reagents using the pCH110 eukaryotic vector could be augmented by increasing the amount of plasmid DNA about 1.5–3 times more than the recommended concentration by the manufacturers without any visible cytotoxicity. Indeed, the highest transfection efficiency by the reagents was dependent on plasmid DNA concentrations (Nikcevic et al. 2003).

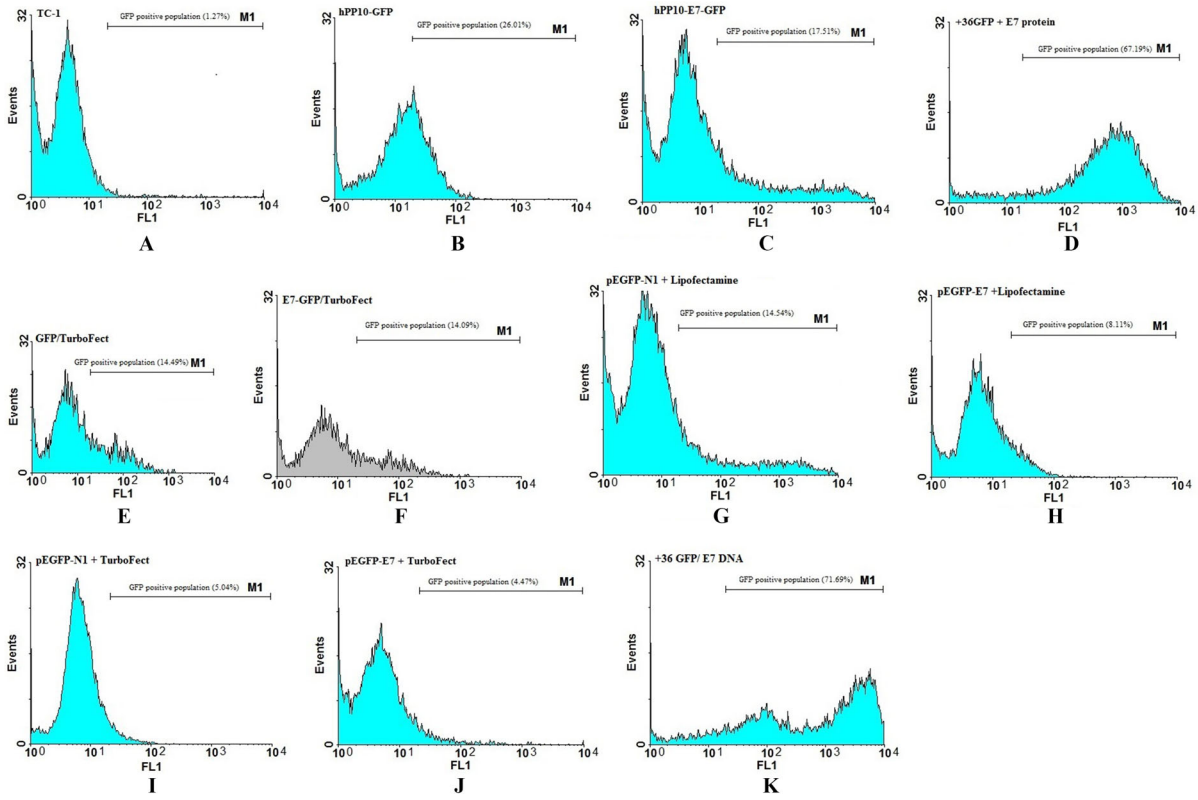

Fig. 3 Evaluation of supercharged +36 GFP (**d**, **k**), hPP10 (**b**, **c**), TurboFect (**e**, **f**; **i**, **j**) and Lipofectamine (**g**, **h**) for DNA and protein delivery into TC-1 tumor cells. Transfection efficiency was monitored by flow cytometry at 24 h post-transfection as compared to the negative control (**a**)

Based on our data, the transfection efficacy of the plasmids by three delivery systems in HEK-293T normal cells was different from that in TC-1 and A549 tumor cells after their expression. The expression of DNAs was significantly higher in HEK-293T cells compared to that in TC-1 and A549 cells. Moreover, the efficiency of transient transfection into the tumor cells monitored by flow cytometry using the enhanced green fluorescent protein (EGFP) was importantly increased by +36 GFP compared to other delivery systems. Other studies also demonstrated that simple mixing of siRNA or plasmid DNA with +36 GFP led to the formation of electrostatic complexes and their delivery even into cell lines resistant to lipid-mediated transfection (Thompson et al. 2012). On the other hand, in a study, the viral plasmid DNAs were transfected into A549 cells (Giard et al. 1973) using Biontex K2 transfection system. The fluorescence microscopy showed that K2 has higher transfection efficiency than the PEI and Lipofectamine (http://www.biontex.com) suggesting the importance of carriers. Herein, the recombinant E7, E7-GFP, wt GFP, hPP10-GFP and hPP10-E7-GFP proteins were expressed in *E. coli* as His-tagged proteins and purified by affinity chromatography. Then, protein delivery was evaluated by +36 GFP, hPP10 and TurboFect. The efficiency of wt GFP or E7-GFP protein delivery by hPP10 into the HEK-293T cells was ~ 77.77 and ~ 63.66%, into the TC-1 cells was ~ 26.01 and ~ 17.51%, and into the A549 was ~ 21.6 and ~ 16.36%, respectively. Flow cytometry analysis showed that the cellular uptake of wt GFP and E7-GFP proteins was ~ 57.96 and ~ 32.95% for HEK-293T, ~ 14.49 and ~ 14.09% for TC-1, and ~ 18.49 and ~ 14.54% for A549 at 24 h after transfection using TurboFect, respectively. Indeed, hPP10 and TurboFect were not effective for protein delivery in tumor cells. In contrast, the cellular uptake of the E7 DNA and E7 protein using +36 GFP into the HEK-293T cells was ~ 83.94 and ~ 77.01%, into the TC-1 cells

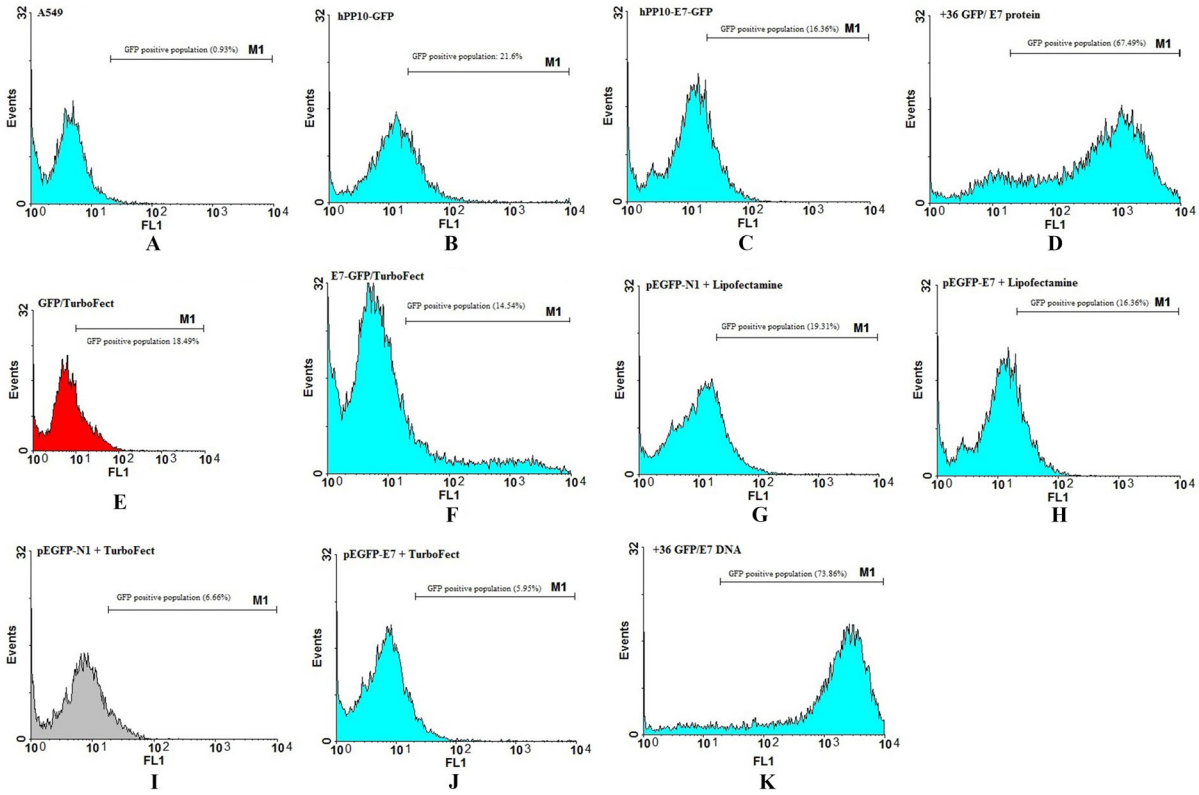

Fig. 4 Evaluation of supercharged +36 GFP (**d**, **k**), hPP10 (**b**, **c**), TurboFect (**e**, **f**; **i**, **j**) and Lipofectamine (**g**, **h**) for DNA and protein delivery into A549 tumor cells. Transfection efficiency was monitored by flow cytometry at 24 h post-transfection as compared to the negative control (**a**)

was ~ 71.69 and ~ 67.19%, and into the A549 was ~ 73.86 and ~ 67.49%, respectively indicating its high efficacy.

The studies demonstrated that the hPP10 was capable of penetrating into a variety of cancerous or immortalized cell lines (*e.g.,* B16, ECV304, HepG2, T24, L929, HSC-T6 and THP1) as well as primary cultured cells (*e.g.,* mouse spleen lymphocytes, human peripheral blood lymphocytes and mouse primary fibroblast cells). Moreover, the efficiency of hPP10-GFP fusion protein uptake was higher than Tat-GFP in ECV304 and Cos7 cells with same conditions (Wang et al. 2016). The findings of this study showed that human origin peptide hPP10 fused to GFP or E7-GFP could penetrate into HEK-293T normal cells significantly higher than A549 and TC-1 cancer cell lines indicating its low efficiency in these cells.

On the other hand, the studies showed that some proteins such as mCherry, ubiquitin and Cre recombinase can rapidly enter mammalian cells and access the cytosol using superpositively charged GFP with efficiency ~ 100-fold greater than known protein transduction domains (PTDs) including Tat, oligoarginine, and penetratin for protein transfection (Cronican et al. 2010; Thompson et al. 2012). Among the designed supercharged GFP protein, +36 GFP with 36 positive charges was highly aggregation-resistant and retained its fluorescence even after boiling or cooling (Wu et al. 2015). The studies indicated that +36 GFP was stable in murine serum and could significantly enhance the serum stability of siRNA and plasmid DNA (McNaughton et al. 2009). These results were confirmed in our experiments indicating high efficacy of +36 GFP for DNA and protein delivery in both normal and tumor cell lines. It was shown that +36 GFP can delay the maturation of early endosomes into mature endosomes, thus allowing more time for +36 GFP to escape. However, the exact mechanism of this endosomal disruption is unknown, but the delay in maturation could explain the efficiency of cytosolic

delivery of proteins by +36 GFP (Margie 2015). Our data showed that the potency of +36 GFP is higher than TurboFect cationic polymer and Lipofectamine cationic lipid as commercially available reagents as well as hPP10 cell penetrating peptide to transfect DNA and protein into the cells, and is stable even at 24 h post-transfection. Indeed, the effects of these reagents (TurboFect, Lipofectamine and hPP10) greatly varied by cell type.

Conclusion

Transfection tests in eukaryotic cells are important tools for evaluation of gene and protein function in vivo. Up to now, several gene or protein delivery vehicles were evaluated for their transfection efficiency. In summary, we compared the efficiency of DNA and protein delivery into HEK-293T normal cell line, and A549 and TC-1 tumor cell lines using two delivery systems such as supercharge GFP and hPP10 cell penetrating peptide, and two commercial transfection reagents including TurboFect and Lipofectamine. Our data indicated that the supercharge GFP is the most suitable delivery system for both DNA and proteins into TC-1 and A549 tumor cell lines compared to other carriers.

Acknowledgements Financial support of this work was provided by Virology Research Center, Shahid Beheshti University of Medical Sciences, and Pasteur Institute of Iran.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Supplementary data Physiochemical characterization and stability analysis of the +36GFP/DNA nanoparticles: A) Representative gel retardation assay of +36 GFP complexed with pcDNA-E7 at different N/P ratios (GFP: E7DNA); Lane 1: naked plasmid DNA as a control (pcDNA-E7), Lane 2: N/P = 1:1, Lane 3: N/P = 2:1, Lane 4: N/P = 5:1, Lane 5: N/P = 10:1, and Lane 6: N/P = 20:1. The DNA complexed with GFP that was not able to migrate into the gels was observed at an N/P ratio of 5:1; B) Stability analysis of GFP-based nanoparticles against DNase I; Lane 1: naked plasmid DNA with DNase, Lane 2: naked plasmid DNA without DNase, and Lane 3: N/P = 10:1.

References

Arukuusk P, Parnaste L, Oskolkov N, Copolovici DM, Margus H, Padari K et al (2013) New generation of efficient peptide-based vectors, NickFects, for the delivery of nucleic acids. Biochem Biophys Acta 1828:1365–1373. https://doi.org/10.1016/j.bbamem.2013.01.011

Bolhassani A, Zahedifard F, Taghikhani M, Rafati S (2008) Enhanced immunogenicity of HPV16 E7 accompanied by Gp96 as an adjuvant in two vaccination strategies. Vaccine 26(26):3362–3370. https://doi.org/10.1016/j.vaccine.2008.03.082

Bolhassani A, Zahedifard F, Taslimi Y, Taghikhani M, Nahavandian B, Rafati S (2009) Antibody detection against HPV16E7 and Gp96 fragments as biomarkers in cervical cancer patients. Indian J Med Res 130:533–541

Chugh A, Eudes F, Shim YS (2010) Cell-penetrating peptides: nanocarrier for macromolecule delivery in living cells. IUBMB Life 62(3):183–193. https://doi.org/10.1002/iub.297

Cronican JJ, Thompson DB, Beier KT, McNaughton BR, Cepko CL, Liu DR (2010) Potent delivery of functional proteins into mammalian cells in vitro and in vivo using a supercharged protein. ACS Chem Biol 5:747–752. https://doi.org/10.1021/cb1001153

Giard DJ, Aaronson SA, Todaro GJ, Arnstein P, Kersey JH, Dosik H, Parks WP (1973) *In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J Natl Cancer Inst 51(5):1417–1423

Horibe T, Torisawa A, Akiyoshi R, Hatta-Ohashi Y, Suzukib H, Kawakamia K (2014) Transfection efficiency of normal and cancer cell lines and monitoring of promoter activity by single-cell bioluminescence imaging. Luminescence 29:96–100. https://doi.org/10.1002/bio.2508

Huang YW, Lee HJ, Tolliver LM, Aronstam RS (2015) Delivery of nucleic acids and nanomaterials by cell-penetrating peptides: opportunities and challenges. Biomed Res Int 2015:1–16. https://doi.org/10.1155/2015/834079

Kadkhodayan S, Sadat SM, Irani S, Fotouhi F, Bolhassani A (2016) Generation of GFP native protein for detection of its intracellular uptake by cell-penetrating peptides. Folia Biol 62:103–109

Kim TK, Eberwine JH (2010) Mammalian cell transfection: the present and the future. Anal Bioanal Chem 397:3173–3178. https://doi.org/10.1007/s00216-010-3821-6

Kong Y, Zhang X, Zhao Y, Xue Y, Zhang Y (2017) Uptake of DNA by cancer cells without a transfection reagent. Biol Res 50:2. https://doi.org/10.1186/s40659-017-0107-x

Margie L (2015) The discovery and characterization of endosomal escape enhancing compounds to improve protein delivery efficacy. Doctoral dissertation, Harvard University, Graduate School of Arts & Sciences pp 1–115

McNaughton BR, Cronican JJ, Thompson DB, Liu DR (2009) Mammalian cell penetration, siRNA transfection, and DNA transfection by supercharged proteins. PNAS 106:6111–6116. https://doi.org/10.1073/pnas.0807883106

Motevalli F, Bolhassani A, Hesami S, Shahbazi S (2017) Supercharged green fluorescent protein delivers HPV16 E7 DNA and protein into mammalian cells in vitro and in vivo.

Immunol Lett 194:29–39. https://doi.org/10.1016/j.imlet.2017.12.005

Nikcevic G, Kovacevic-Grujicic N, Stevanovic M (2003) Improved transfection efficiency of cultured human cells. Cell Biol Int 27(9):735–737

Pipes BL, Vasanwala FH, Tsang TC, Zhang T, Luo P, Harris DT (2005) Brief heat shock increases stable integration of lipid-mediated DNA transfections. Biotechniques 38:48–52

Shahbazi S, Bolhassani A, Arashkia A, Sadroddiny E (2018) Generation of the fluorescent HPV16 E7 protein for detection of delivery in vitro. Protein Pept Lett. https://doi.org/10.2174/0929866525666180115123620 (in press)

Thompson DB, Cronican JJ, Liu DR (2012) Engineering and identifying supercharged proteins for macromolecule delivery into mammalian cells. Methods Enzymol 503:293–319. https://doi.org/10.1016/B978-0-12-396962-0.00012-4

Tong H, Liu H, Wang Y, Yang F, Shi Q, Fernandes JC et al (2014) A novel in vitro system for intracellular delivery of non-viral DNA. J Orthop Transl 2:157–164

Wang H, Ma JL, Yang YG, Song Y, Wu J, Qin YY et al (2016) Efficient therapeutic delivery by a novel cell-permeant peptide derived from KDM4A protein for anti-tumor and anti-fibrosis. Oncotarget 7(31):1–16

Wu Q, Cheng Q, Yuan S, Qian J, Zhong K, Qian Y, Liu Y (2015) A cell-penetrating protein designed for bimodal fluorescence and magnetic resonance imaging. Chem Sci 6:6607–6613. https://doi.org/10.1039/c5sc01925g