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Cell line-dependent internalization pathways determine DNA transfection efficiency of decaarginine-PEG-lipid

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ABSTRACT

Previously, we have reported that decaarginine-conjugated PEG-lipids (R10B) efficiently delivered plasmid DNA (pDNA) into human cervical carcinoma HeLa cells *via* macropinocytosis; however, the mechanism of cellular uptake by R10B was not evaluated in other cell lines. In this study, we investigated the internalization mechanism by R10B/pDNA complex (R10B-lipoplex) in human prostate tumor PC-3 and human nasopharyngeal tumor KB cells, and compared with that in HeLa cells. Although it was necessary for R10B-lipoplex to associate with heparan sulfate (HS) on the cell surface in all cell lines, the R10B-lipoplex was internalized primarily through clathrin-mediated endocytosis in PC-3 and KB cells, and macropinosytosis in HeLa cells. In HeLa cells, treatment with the R10B-lipoplex induced the formation of lamellipodia for macropinocytosis, but did not in KB and PC-3 cells. Furthermore, the highest transfection efficiency by R10B-lipoplex was observed in HeLa cells. These findings indicated that the R10B-lipoplex induced the formation of lamellipodia in HeLa cells after binding to HS on the cells and was then internalized by macropinocytosis, which could induce high gene expression because of escaping degradation in lysosomes. Cell physiology might be a critical factor in cellular internalization and efficient transfection by cell penetration peptide.

1. Introduction

Cell-penetrating peptides (CPPs), less than 30 amino acid residues in length, such as HIV-1 Tat fragments, are abundant in arginine residues and the number of arginine residues seems to be an important essential factor for cellular uptake (Futaki et al., 2001a; Kaplan et al., 2005; Mitchell et al., 2000; Wender et al., 2000; Zaro and Shen, 2003). CPPs were originally identified to have the ability to cross the plasma membrane (Derossi et al., 1994; Futaki et al., 2001b; Morris et al., 2001; Oehlke et al., 1998; Pooga et al., 1998; Vives et al., 1997); however, it has been recently reported that cellular uptake of CPPs was inhibited by the presence of endocytosis inhibitors (Drin et al., 2003; Fischer et al., 2004; Vives, 2003). In the process of cellular internalization by CPPs, the first step was attachment to the cell surface by electrostatic interaction with heparan sulfate proteoglycans (HSPGs). HSPGs have been reported to mediate gene transfer into cultured cells by CPPs, such as HIV-1 Tat (Console et al., 2003; Tyagi et al., 2001; Wadia et al., 2004) and oligoarginine (Fuchs and Raines, 2004; Kawamura et al., 2006; Kosuge et al., 2008; Suzuki et al., 2002). Nakase et al. have shown that the interaction of arginine-rich peptides with HSPGs activated intracellular signals to induce actin organization and promote macropinocytosis (Nakase et al., 2007). Macropinocytosis is one of the major endocytic pathways and accompanies membrane ruffling. Macropinosomes decrease their pH, but are not delivered to lysosomes, thus avoiding DNA degradation (Conner and Schmid, 2003), resulting in that transgene internalized by macropinocytosis could be effectively delivered to the nucleus and induce high gene expression.

Octaarginine peptide has been reported to be taken up by macropinocytosis in human cervical carcinoma (HeLa) and chinese hamster ovary (CHO) cells (Nakase et al., 2004, 2007), and T cells (Wadia et al., 2004). However, some researchers have reported that CPPs are taken up *via* clathrin-dependent or caveolae-dependent endocytosis even in the same cell lines (Richard et al., 2003, 2005; Potocky et al., 2003; Jones et al., 2005; Ferrari et al., 2003); Tat peptides were taken up by clathrin-mediated endocytosis in HeLa and CHO cells (Richard et al., 2003, 2005; Potocky et al., 2003), while Tat peptide and GST-Tat-GFP fusion peptide were taken up by caveolae-mediated endocytosis into HeLa and HeLa-derived cell line HL3T1 cells (Jones et al., 2005; Ferrari et al., 2003). In CPP-modified carriers, octaarginine-modified liposome was taken up *via* macropinocytosis into mouse fibroblast NIH3T3 cells (Khalil et al., 2006) and *via* both clathrin-mediated endocy-

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tosis and macropinocytosis in Madin-Darby canine kidney MDCK cells (Fujiwara et al., 2010). Therefore, the exact cellular uptake mechanism of CPPs remains to be elucidated, because the cellular uptake mechanism of CPPs was dependent on the cell line.

Previously, we synthesized decaarginine-PEG-lipid (R10B), conjugating decaarginine and 3,5-bis(dodecyloxy)benzamide (BDB) with poly(ethylene glycol) (PEG) spacer (Furuhata et al., 2006a), and demonstrated that the cellular uptake mechanism of R10B/plasmid DNA complex (R10B-lipoplex) was mainly macropinocytosis in HeLa cells (Furuhata et al., 2008, 2009); however, we did not examine the uptake mechanisms of R10B-lipoplex in other cell lines. In this study, we selected three cell lines, HeLa, human prostate tumor PC-3 and human nasopharyngeal tumor KB cells, and investigated the mechanism of cellular uptake and transfection efficiency by R10B-lipoplex.

2. Materials and methods

2.1. Plasmid DNA

Plasmid DNA (pDNA) encoding the luciferase gene under the control of cytomegalovirus promoter (pCMV-luc) was constructed as previously described (Igarashi et al., 2006). A protein-free preparation of pCMV-luc was purified using the EndoFree Plasmid Max Kit (Qiagen, Hilden, Germany). Fluorescein isothiocianate (FITC) and rhodamine-labeling for pDNA were performed using the protocol of the Label IT TM-FITC labeling kit and Label IT TM-Rhodamine labeling kit (Mirus, Madison, WI, USA), respectively, as previously described (Furuhata et al., 2009).

2.2. Cell culture

HeLa cells were obtained from the European Collection of Cell Culture (Wiltshire, UK). KB and PC-3 cells were supplied by the Cell Resource Center for Biomedical Research, Tohoku University (Miyagi, Japan). HeLa cells were grown in Eagle's Minimum Essential Medium, and KB and PC-3 cells in RPMI-1640 medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS) and kanamycin (100 μg/mL) at 37 °C in a 5% CO_2 humidified atmosphere.

2.3. Transfection

R10B was synthesized as previously reported (Furuhata et al., 2006a, 2006b). R10B solution was prepared at 5 mM by simply dispersing R10B in water. One microliter of micelle solution was added to 2 μg pDNA at a charge ratio (+/−) of 8.5/1 with gentle shaking and leaving at room temperature for 10–15 min. For transfection, the complex (R10B-lipoplex) was diluted with medium (1 mL) to 5 μM R10B and then gently added to the cells.

2.4. Luciferase assay

R10B-lipoplex of pCMV-luc was diluted with serum-free medium (5 μM at a concentration of R10B), and then gently added to the cells. After incubation for 3 h at 37 °C, the cells were added to culture medium (1 mL) containing 10% FBS and then incubated for another 21 h. Luciferase expression was measured as counts per sec (cps)/μg protein using the luciferase assay system (Pica gene; Toyo Ink Mfg. Co. Ltd., Tokyo, Japan) and BCA reagent (Pierce, Rockford, IL, USA) as previously reported (Furuhata et al., 2006a).

2.5. Measurement of the amount of heparan sulfate in the cells

To measure the amount of HS in the cell, the cells were lysed with sampling buffer containing 0.5% Triton X-100 in phosphate-buffered saline (PBS, pH 7.4). After they were centrifuged at 10,000 × *g* for 10 min, the protein concentration of the supernatant was quantitated with BCA protein assay reagent as described above. After 100 μg protein in the supernatants was digested by 1.8 mg/mL Actinase E (Kaken Pharmaceutical Co., Tokyo, Japan), the amount of HS was determined as μg HS/μg protein by a Heparan Sulfate ELISA Kit (Seikagaku Biobusiness Co., Tokyo, Japan).

2.6. Immunostaining for heparan sulfate

The cells were placed in a Lab-Tek Chamber slide glass (Nalge Nunc, Rochester, NY, USA), which was coated with human fibronectin on the surface as previously described (Hattori and Maitani, 2007). For immunostaining, the cells were fixed in 4% paraformaldehyde for 15 min at room temperature. After protein blocking with 5% goat serum for the cells, HS on the cell surface was identified using a rat anti-human heparan sulafate proteoglycan monoclonal antibody (Acris Antibodies GmbH, Herford, Germany) with Alexa 555-labeled goat anti-rat IgG (Invitrogen, Carlsbad, CA, USA) as the secondary antibody. The fluorescence was examined microscopically using an ECLIPSE TS100 microscope (Nikon, Tokyo, Japan).

2.7. Cellular uptake

To investigate the effect of HS on the cell surface on cellular uptake, the cells were digested with 1 unit/mL heparinase-III in medium at 37 °C for 30 min, and then transfected with the R10B-lipoplex of FITC-labeled pDNA in medium containing 10% FBS. To investigate the effect of HS in the medium on cellular uptake, the R10B-lipoplex was incubated with the medium containing 20 μg/mL heparin (Sigma Chemical Co.) for 15 min, and then incubated with the cells for 1 h. For investigation of the cellular uptake mechanism by endocytosis inhibitors, the cells were treated with medium containing 400 mM sucrose, 50 μM 5-(*N*-Ethyl-*N*-isopropyl) amiloride (EIPA; Sigma Chemical Co.) or 5 μg/mL filipin (Sigma Chemical Co.) for 30 min. After treatment, the cells were transfected with the R10B-lipoplex for 1 h in the presence of each inhibitor. After incubation, the amount of FITC-labeled pDNA in the cells was determined by examining fluorescence intensity on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) as previously described (Furuhata et al., 2006a, 2006b).

2.8. Confocal laser scanning microscopy

For observation of lamellipodia-like formations, the cells were incubated with R10B-lipoplex or 400 ng/mL EGF (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 5 min. After incubation, the cells were fixed with 4% formaldehyde and then cellular F-actin was stained with phalloidin-TRITC (Sigma Chemical Co.) after treatment with 0.1% Triton X-100 in PBS. For investigation of the cellular uptake mechanism, the cells were co-incubated for 3 h with R10B/rhodamine-labeled pDNA and 0.5 mg/mL Oregon green 488-labeled dextran (70 kDa, anionic, lysine fixable, Invitrogen). After incubation, the cells were fixed with 4% formaldehyde.

Examinations were performed with a LSM5 EXCITER confocal laser scanning microscope (Carl Zeiss, Thornwood, NY) as previously described (Furuhata et al., 2009). For phalloidin-TRITC and rhodamine-labeled pDNA, maximal excitation was performed with a 543-nm internal He–Ne laser, and fluorescence emission was observed with an LP560. Oregon green 488-labeled dextran was imaged using an argon laser at 488 nm excitation, and fluorescence emission was observed with a filter, BP505-530.

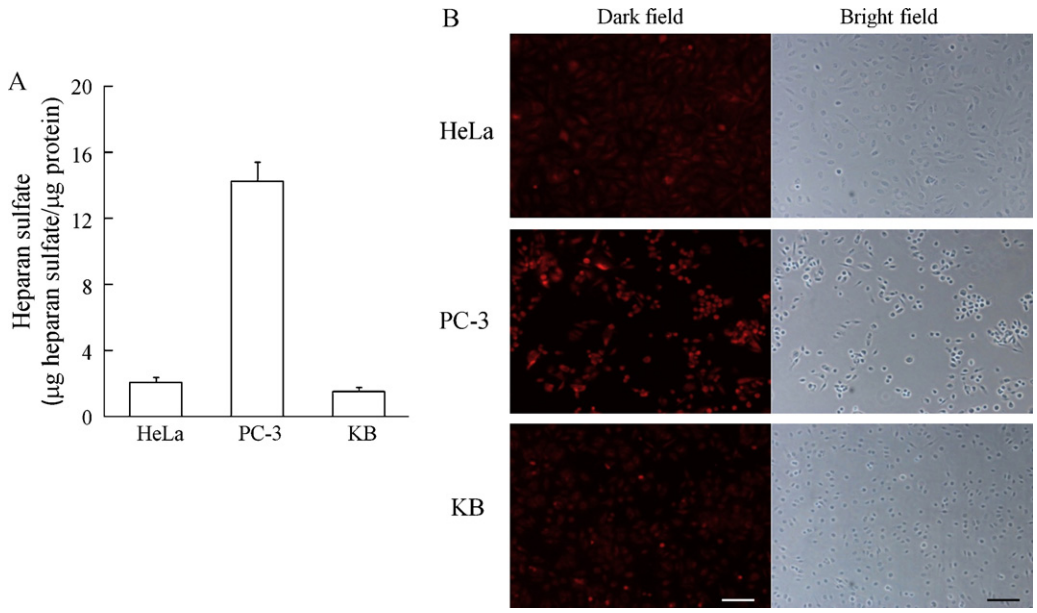

Fig. 1. Assessment of HS in HeLa, PC-3 and KB cells by ELISA (A) and immunostaining (B). In A, the amounts of HS were measured as μg HS/μg protein by the Heparan Sulfate ELISA-kit and BCA reagent. Each bar represents the mean ± S.D. of three experiments. In B, red indicates localization of heparan sulfate on the cell surface. Scale bar = 100 μm.

2.9. Statistical analysis

Significant differences in the mean values were evaluated by Student's unpaired *t*-test. A *p*-value of 0.05 or less was considered significant.

3. Results

3.1. Assessment of heparan sulfate amount in various cells

In the process of cellular internalization by CPPs, the first step was attachment to the cell surface by electrostatic interaction with HSPGs, which are transmembrane proteins conjugated to negatively charged sulfated glycan chains (heparan sulfate, HS); therefore, we first evaluated the amount of HS in three cell lines by ELISA and immunostaining (Fig. 1). The amounts of HS in homogeneous cell lysate of HeLa, PC-3 and KB cells by ELISA were 2.1 ± 0.2, 14.8 ± 1.3 and 1.6 ± 0.3 μg HS/μg protein, respectively (Fig. 1A). In immunostaining by anti-HS antibody, the presence of HS on the cell surface was strongly observed in PC-3 cells, and moderately in HeLa and KB cells (Fig. 1B), corresponding to the results by ELISA (Fig. 1A). From these findings, PC-3 cells had the highest amount of HS on the cell surface among the cell lines.

3.2. Effects of HS on cellular uptake

To investigate the effect of HS on cellular uptake by R10B-lipoplex, the cells were digested with heparinase-III before transfection. The digestion of HS significantly decreased the cellular amount of pDNA after transfection by the R10B-lipoplex to about 60% (Fig. 2). Heparin is closely related with heparan sulfate. The addition of heparin to the medium completely inhibited cellular uptake of the R10B lipoplex (Fig. 2). These results suggested that cell surface-expressed HSPGs played an important role in the cellular association with R10B-lipoplex.

3.3. Cellular uptake mechanism of R10B-lipoplex

To determine the uptake mechanism by R10B-lipoplex in three cell lines, we examined the effect of endocytosis inhibitors on the cellular uptake of R10B-lipoplex. EIPA is an inhibitor of macropinocytosis through the interaction with Na^+/H^+ exchanger, filipin of caveolae-mediated endocytosis through cholesterol depletion, and sucrose of clathrin-mediated endocytosis. EIPA strongly inhibited cellular uptake by the R10B-lipoplex in HeLa cells and weakly in KB cells (Fig. 3A); in contrast, sucrose strongly inhibited in PC-3 and KB cells (Fig. 3B and C), suggesting that the cellular uptake mechanism of the R10B-lipoplex in KB was mainly clathrin-mediated endocytosis and partly macropinocytosis, that in PC-3 cells was clathrin-mediated endocytosis and that in HeLa was macropinocytosis. Furthermore, to confirm the cellular uptake by macropinocytosis in HeLa cells, we observed the co-localization of R10B/rhodamine-labeled pDNA with Oregon green 488-labeled dextran, a marker of macropinocytosis. In HeLa cells, pDNA by transfection with R10B was co-localized with dextran (Fig. 4A), but not in PC-3 and KB cells (Fig. 4B and C), suggesting that the cellular uptake mechanism of R10B-lipoplex was macropinocytosis in

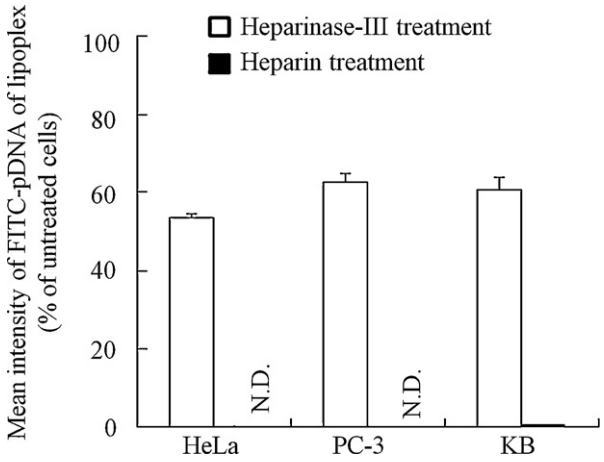

Fig. 2. Effects of HSPGs on cellular uptake of FITC-labeled pDNA by R10B-lipoplex. R10B-lipoplex was transfected into the cells for 1 h after treatment of the cells with heparinase-III (1 unit/mL) for 30 min (white bar) or after incubation of the R10B-lipoplex with heparin (20 μg/mL) for 15 min (black bar). Each bar represents the mean ± S.D. of three experiments.

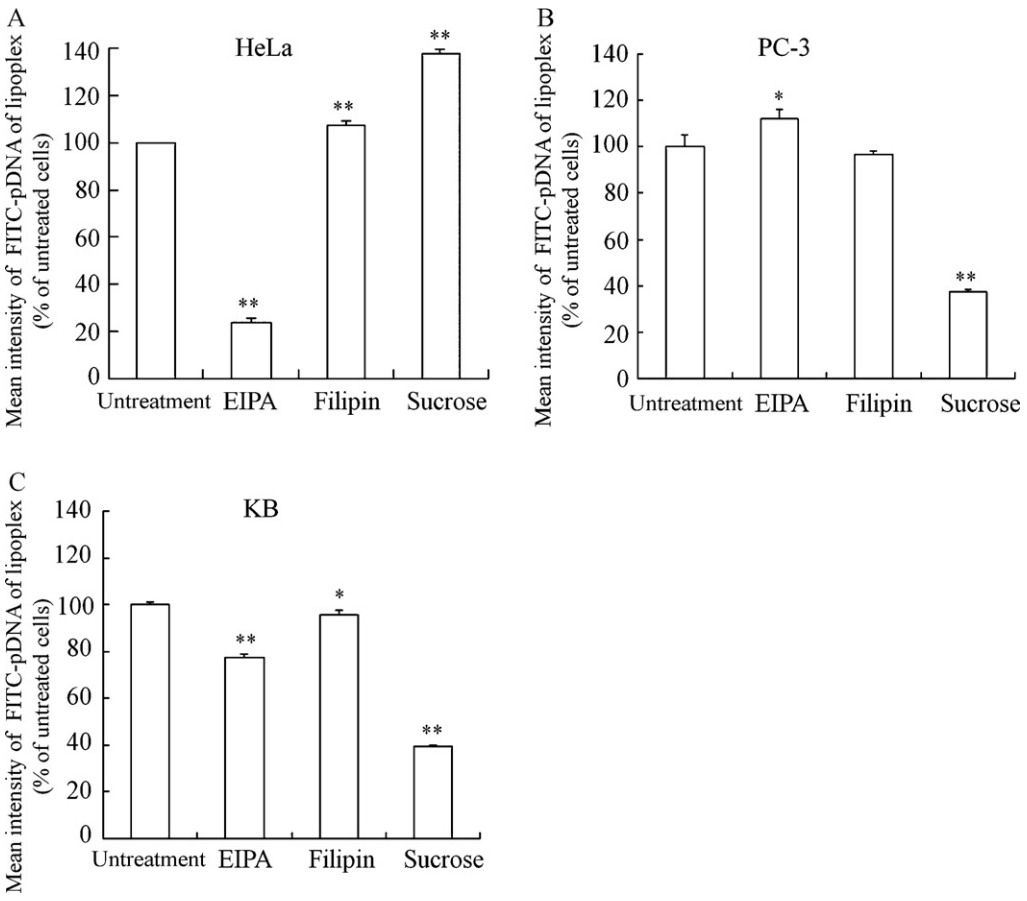

Fig. 3. Effect of endocytosis inhibitors on the cellular uptake of R10B-lipoplex in HeLa (A), PC-3 (B) and KB cells (C). The R10B-lipoplex of FITC-labeled pDNA was transfected into the cells after treatment with 50 μM EIPA, 5 μg/mL filipin or 400 mM sucrose for 30 min. Each bar represents the mean ± S.D. of three experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with untreated cells.

HeLa cells. The cellular uptake mechanism was not dependent on the amount of HS.

3.4. Induction of actin organization by R10B-lipoplex

Macropinocytosis accompanied the organization of F-actin and EGF induced the organization of F-actin, including lamellipodia-like formations (Nakase et al., 2004). To investigate why R10B-lipoplex was internalized by macropinocytosis in HeLa cells, we observed actin organization in the cells after treatment with R10B-lipoplex by confocal laser scanning microscopy (Fig. 5). EGF was used as a positive control for the induction of lamellipodia-like formations (Nakase et al., 2007). In R10B-lipoplex treatment, the induction of lamellipodia-like formations was observed in HeLa cells but not in PC-3 and KB cells. In contrast, the apparent organization of F-actin after the treatment with EGF was observed in all cells. These results suggested that R10B-lipoplex treatment could induce lamellipodia-like formations *via* the organization of F-actin only for HeLa cells.

3.5. Gene expression by R10B lipoplexes

Finally, we evaluated the transfection efficiency by R10B-lipoplex in the cells by assaying luciferase activity. Transfection activity by R10B-lipoplex was the highest in HeLa cells among the cell lines (Fig. 6). The transfection activity for HeLa cells was about 45-fold and 2500-fold higher than for PC-3 and KB cells, respectively. Although the amount of HS on the cell surface was highest in PC-3 cells (Fig. 1), the transfection activity in PC-3 cells was lower than in HeLa cells (Fig. 6).

4. Discussion

In this report, we investigated the cellular uptake mechanism and transfection activity by R10B-lipoplex in three cell lines, HeLa, PC-3 and KB cells. In the process of cellular internalization by CPPs, the first step was attachment to the cell surface by electrostatic interaction with HSPGs. The cellular uptake of R10B-lipoplex was inhibited in all the cell lines by heparinase-III treatment with the cells or heparin pretreatment with R10B-lipoplex (Fig. 2). Although the amount of HS was highest in PC-3 cells among the cell lines (Fig. 1), it was not correlated with the cellular uptake and transfection efficiency by R10B (Fig. 6). From these findings, HS was necessary for cellular association with R10B-lipoplex, but cellular uptake by R10B-lipoplex was independent of the amount of HS on the cell surface.

In effective transfection, elucidating the mechanism of R10B-lipoplex is a prerequisite to understanding and improving transfection. To investigate the cellular uptake mechanism of R10B-lipoplex, we examined the effect of endocytosis inhibitors on the cellular uptake of R10B-lipoplex. In HeLa cells, cellular association with the lipoplex decreased by EIPA, but did not decrease by sucrose (Fig. 3A); in contrast, in PC-3 and KB cells, it was mainly decreased by sucrose (Fig. 3B and C). These findings suggested that the internalization of R10B-lipoplex occurred mainly through macropinocytosis in HeLa cells, and clathrin-mediated endocytosis in PC-3 and KB cells. It has previously been reported that complex size can affect the mechanism of internalization, with clathrin-mediated endocytosis limited to particles under 200 nm, caveolae-mediated endocytosis for particles between 200 and

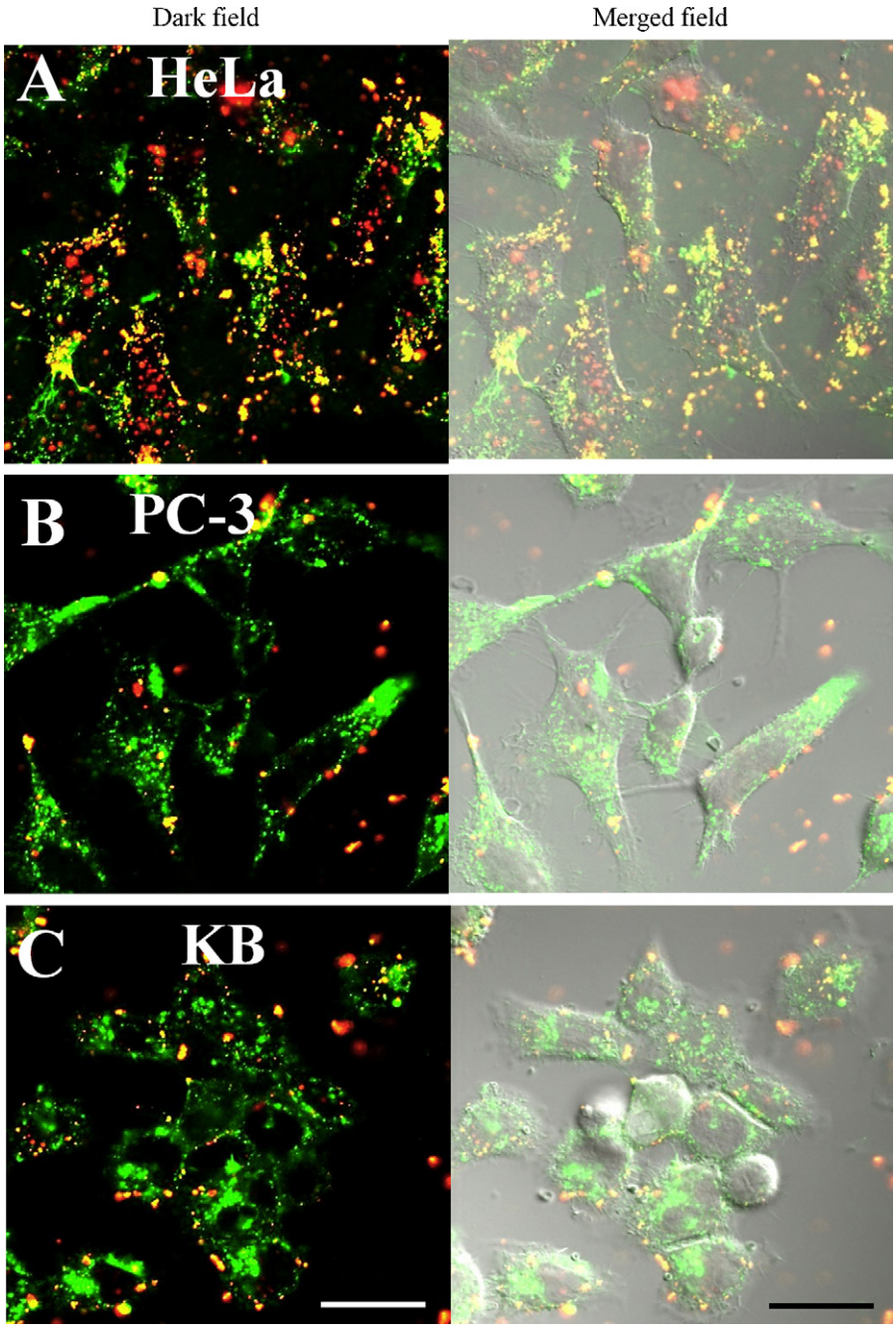

Fig. 4. Localization of R10B-lipoplex in cells by confocal microscopy. The cells were co-incubated with R10B/rhodamine-labeled pDNA and oregon green-labeled dextran for 3 h. Red shows the localization of pDNA and green that of dextran. Yellow indicates the co-localization of pDNA with dextran. Scale bar = 20 μm.

500 nm, and macropinocytosis for particles over 1 μm (Conner and Schmid, 2003; Medina-Kauwe et al., 2005; Rejman et al., 2004); however, the different pathways observed in our study cannot be explained by size, since all the cells were treated with similar-sized R10B-lipoplex (about 250 nm in medium, 0.28 in polydispersity index). It has been reported that the arginate-chitosan nanoparticle/pDNA complex was internalized through the clathrin-dependent pathway in 293T and COS7 cells, but through the caveolin-mediated pathway in CHO cells (Douglas et al., 2008). Moreover, CPPs have been reported to be taken up *via* macropinocytosis (Nakase et al., 2007), clathrin-dependent (Richard et al., 2003, 2005; Potocky et al., 2003) and caveolae-dependent endocytosis in HeLa and CHO cells (Jones et al., 2005; Ferrari et al., 2003). Although the exact cellular uptake mechanism of R10B-lipoplex remains to be elucidated, the cellular uptake mechanism of the lipoplex might be dependent on the cell line.

Macropinocytosis involves an actin-driven membrane protrusion (Conner and Schmid, 2003; Grimmer et al., 2002). Treatment with R10B-lipoplex induced actin-driven membrane ruffling in HeLa cells, but did not induce in PC-3 and KB cells (Fig. 5). It has been reported that the interaction of arginine-rich peptides with membrane-associated proteoglycans quickly activated the intracellular signals of Rac1 proteins and induced actin organization and lamellipodia following by macropinocytosis (Nakase et al., 2007; Schlunck et al., 2004; Nobes and Hall, 1995). It is not clear why R10B-lioplex could not induce lamellipodia in PC-3 and KB cells, but treatment with R10B-lipoplex might activate Rac1 protein in HeLa cells after binding to the cell surface but remain inactive in PC-3

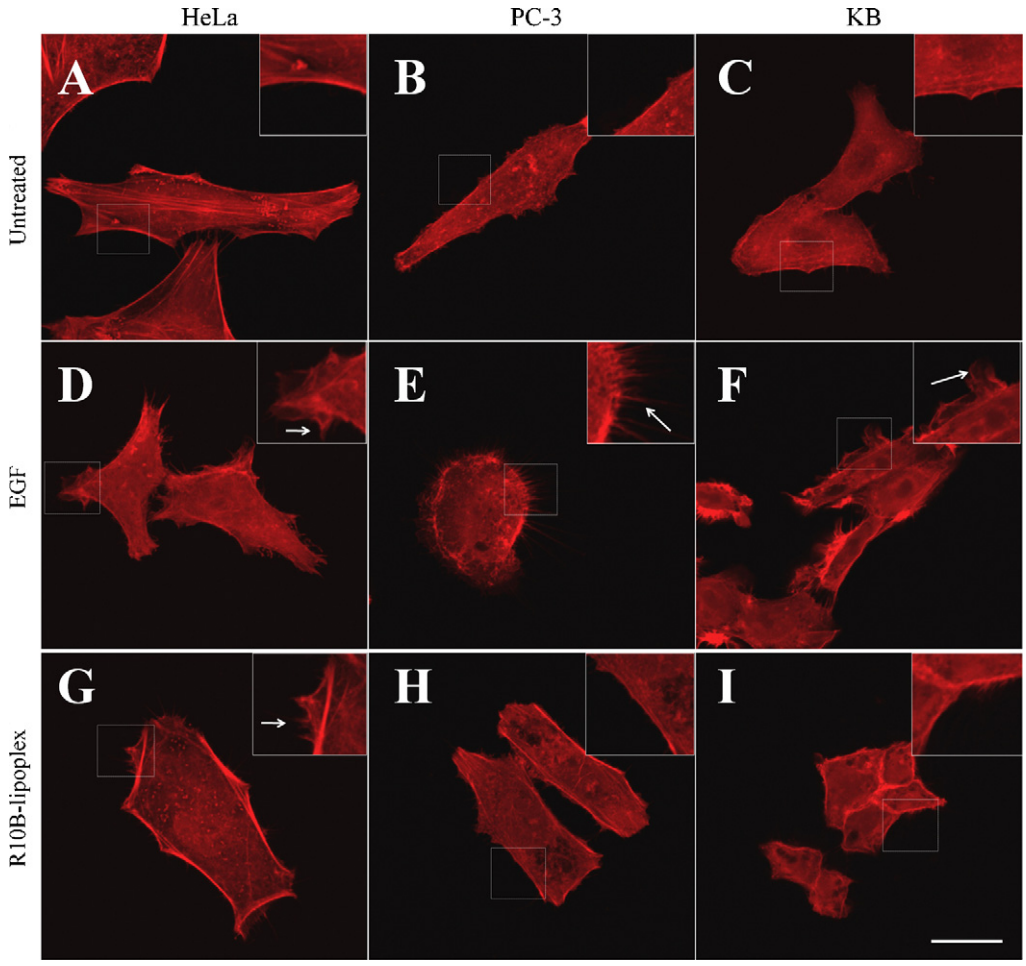

Fig. 5. Observation of lamellipodia formation by treatment with EGF (D–F) and R10B-lipoplex (G–I) in HeLa (A, D and G), PC-3 (B, E and H) and KB (C, F and I) cells. The cells were treated with 400 ng/mL EGF (D–F) or with R10B-lipoplex (G–I) in serum-free medium for 5 min and then stained with phalloidin-TRITC. Insets show images of boxed regions at 2× magnification. Arrows showed lamellipodia induced by EGF or R10B-lipoplex. Scale bar = 20 μm.

and KB cells. Macropinocytosis has the ability to avoid lysosomal degradation (Conner and Schmid, 2003; Medina-Kauwe et al., 2005; Meier et al., 2002); therefore, R10B-lipoplex might induce high gene expression in HeLa cells *via* macropinocytosis. DNA transfection efficiency with decaarginine-PEG-lipid was determined by cell line-dependent internalization pathways.

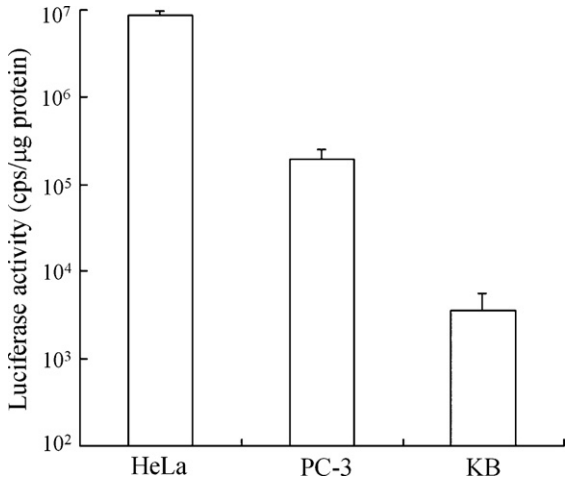

Fig. 6. Luciferase activities in HeLa (A), PC-3 (B) and KB cells (C) after transfection by R10B-lipoplex. The cells were incubated for 3 h in serum-free medium after transfection, and then incubated for another 21 h in medium containing 10% FBS. Each bar represents the mean ± S.D. of three experiments.

5. Conclusion

In this report, we demonstrated that the internalization of R10B-lipoplex occurred mainly through macropinocytosis in HeLa cells, and clathrin-mediated endocytosis in PC-3 and KB cells, not depending on HSPG amounts in each cell. Furthermore, R10B-lipoplex induced the highest gene expression in HeLa cells among their cell lines. Although the exact cellular uptake mechanism of R10B-lipoplex remains to be elucidated, the cellular uptake mechanism of the lipoplex might be dependent on the cell line and affect transfection activity.

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References

Conner, S.D., Schmid, S.L., 2003. Regulated portals of entry into the cell. Nature 422, 37–44.

Console, S., Marty, C., Garcia-Echeverria, C., Schwendener, R., Ballmer-Hofer, K., 2003. Antennapedia and HIV transactivator of transcription (TAT) "protein transduction domains" promote endocytosis of high molecular weight cargo upon binding to cell surface glycosaminoglycans. J. Biol. Chem. 278, 35109–35114.
Derossi, D., Joliot, A.H., Chassaing, G., Prochiantz, A., 1994. The third helix of the Antennapedia homeodomain translocates through biological membranes. J. Biol. Chem. 269, 10444–10450.
Douglas, K.L., Piccirillo, C.A., Tabrizian, M., 2008. Cell line-dependent internalization pathways and intracellular trafficking determine transfection efficiency of nanoparticle vectors. Eur. J. Pharm. Biopharm. 68, 676–687.
Drin, G., Cottin, S., Blanc, E., Rees, A.R., Temsamani, J., 2003. Studies on the internalization mechanism of cationic cell-penetrating peptides. J. Biol. Chem. 278, 31192–31201.
Ferrari, A., Pellegrini, V., Arcangeli, C., Fittipaldi, A., Giacca, M., Beltram, F., 2003. Caveolae-mediated internalization of extracellular HIV-1 tat fusion proteins visualized in real time. Mol. Ther. 8, 284–294.
Fischer, R., Kohler, K., Fotin-Mleczek, M., Brock, R., 2004. A stepwise dissection of the intracellular fate of cationic cell-penetrating peptides. J. Biol. Chem. 279, 12625–12635.
Fuchs, S.M., Raines, R.T., 2004. Pathway for polyarginine entry into mammalian cells. Biochemistry 43, 2438–2444.
Fujiwara, T., Akita, H., Harashima, H., 2010. Intracellular fate of octaarginine-modified liposomes in polarized MDCK cells. Int. J. Pharm. 386, 122–130.
Furuhata, M., Danev, R., Nagayama, K., Yamada, Y., Kawakami, H., Toma, K., Hattori, Y., Maitani, Y., 2008. Decaarginine-PEG-artificial lipid/DNA complex for gene delivery: nanostructure and transfection efficiency. J. Nanosci. Nanotechnol. 8, 2308–2315.
Furuhata, M., Izumisawa, T., Kawakami, H., Toma, K., Hattori, Y., Maitani, Y., 2009. Decaarginine-PEG-liposome enhanced transfection efficiency and function of arginine length and PEG. Int. J. Pharm. 371, 40–46.
Furuhata, M., Kawakami, H., Toma, K., Hattori, Y., Maitani, Y., 2006a. Design, synthesis and gene delivery efficiency of novel oligo-arginine-linked PEG-lipids: effect of oligo-arginine length. Int. J. Pharm. 316, 109–116.
Furuhata, M., Kawakami, H., Toma, K., Hattori, Y., Maitani, Y., 2006b. Intracellular delivery of proteins in complexes with oligoarginine-modified liposomes and the effect of oligoarginine length. Bioconjug. Chem. 17, 935–942.
Futaki, S., Ohashi, W., Suzuki, T., Niwa, M., Tanaka, S., Ueda, K., Harashima, H., Sugiura, Y., 2001a. Stearylated arginine-rich peptides: a new class of transfection systems. Bioconjug. Chem. 12, 1005–1011.
Futaki, S., Suzuki, T., Ohashi, W., Yagami, T., Tanaka, S., Ueda, K., Sugiura, Y., 2001b. Arginine-rich peptides. An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. J. Biol. Chem. 276, 5836–5840.
Grimmer, S., van, D.B., Sandvig, K., 2002. Membrane ruffling and macropinocytosis in A431 cells require cholesterol. J. Cell Sci. 115, 2953–2962.
Hattori, Y., Maitani, Y., 2007. DNA/Lipid complex incorporated with fibronectin to cell adhesion enhances transfection efficiency in prostate cancer cells and xenografts. Biol. Pharm. Bull. 30, 603–607.
Igarashi, S., Hattori, Y., Maitani, Y., 2006. Biosurfactant MEL-A enhances cellular association and gene transfection by cationic liposome. J. Control. Release 112, 362–368.
Jones, S.W., Christison, R., Bundell, K., Voyce, C.J., Brockbank, S.M., Newham, P., Lindsay, M.A., 2005. Characterisation of cell-penetrating peptide-mediated peptide delivery. Br. J. Pharmacol. 145, 1093–1102.
Kaplan, I.M., Wadia, J.S., Dowdy, S.F., 2005. Cationic TAT peptide transduction domain enters cells by macropinocytosis. J. Control. Release 102, 247–253.
Kawamura, K.S., Sung, M., Bolewska-Pedyczak, E., Gariepy, J., 2006. Probing the impact of valency on the routing of arginine-rich peptides into eukaryotic cells. Biochemistry 45, 1116–1127.
Khalil, I.A., Kogure, K., Futaki, S., Harashima, H., 2006. High density of octaarginine stimulates macropinocytosis leading to efficient intracellular trafficking for gene expression. J. Biol. Chem. 281, 3544–3551.
Kosuge, M., Takeuchi, T., Nakase, I., Jones, A.T., Futaki, S., 2008. Cellular internalization and distribution of arginine-rich peptides as a function of extracellular peptide concentration, serum, and plasma membrane associated proteoglycans. Bioconjug. Chem. 19, 656–664.
Medina-Kauwe, L.K., Xie, J., Hamm-Alvarez, S., 2005. Intracellular trafficking of non-viral vectors. Gene Ther. 12, 1734–1751.
Meier, O., Boucke, K., Hammer, S.V., Keller, S., Stidwill, R.P., Hemmi, S., Greber, U.F., 2002. Adenovirus triggers macropinocytosis and endosomal leakage together with its clathrin-mediated uptake. J. Cell Biol. 158, 1119–1131.
Mitchell, D.J., Kim, D.T., Steinman, L., Fathman, C.G., Rothbard, J.B., 2000. Polyarginine enters cells more efficiently than other polycationic homopolymers. J. Pept. Res. 56, 318–325.
Morris, M.C., Depollier, J., Mery, J., Heitz, F., Divita, G., 2001. A peptide carrier for the delivery of biologically active proteins into mammalian cells. Nat. Biotechnol. 19, 1173–1176.
Nakase, I., Niwa, M., Takeuchi, T., Sonomura, K., Kawabata, N., Koike, Y., Takehashi, M., Tanaka, S., Ueda, K., Simpson, J.C., Jones, A.T., Sugiura, Y., Futaki, S., 2004. Cellular uptake of arginine-rich peptides: roles for macropinocytosis and actin rearrangement. Mol. Ther. 10, 1011–1022.
Nakase, I., Tadokoro, A., Kawabata, N., Takeuchi, T., Katoh, H., Hiramoto, K., Negishi, M., Nomizu, M., Sugiura, Y., Futaki, S., 2007. Interaction of arginine-rich peptides with membrane-associated proteoglycans is crucial for induction of actin organization and macropinocytosis. Biochemistry 46, 492–501.
Nobes, C.D., Hall, A., 1995. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. Cell 81, 53–62.
Oehlke, J., Scheller, A., Wiesner, B., Krause, E., Beyermann, M., Klauschenz, E., Melzig, M., Bienert, M., 1998. Cellular uptake of an alpha-helical amphipathic model peptide with the potential to deliver polar compounds into the cell interior non-endocytically. Biochim. Biophys. Acta 1414, 127–139.
Pooga, M., Hallbrink, M., Zorko, M., Langel, U., 1998. Cell penetration by transportan. FASEB J. 12, 67–77.
Potocky, T.B., Menon, A.K., Gellman, S.H., 2003. Cytoplasmic and nuclear delivery of a TAT-derived peptide and a beta-peptide after endocytic uptake into HeLa cells. J. Biol. Chem. 278, 50188–50194.
Rejman, J., Oberle, V., Zuhorn, I.S., Hoekstra, D., 2004. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochem. J. 377, 159–169.
Richard, J.P., Melikov, K., Brooks, H., Prevot, P., Lebleu, B., Chernomordik, L.V., 2005. Cellular uptake of unconjugated TAT peptide involves clathrin-dependent endocytosis and heparan sulfate receptors. J. Biol. Chem. 280, 15300–15306.
Richard, J.P., Melikov, K., Vives, E., Ramos, C., Verbeure, B., Gait, M.J., Chernomordik, L.V., Lebleu, B., 2003. Cell-penetrating peptides. A reevaluation of the mechanism of cellular uptake. J. Biol. Chem. 278, 585–590.
Schlunck, G., Damke, H., Kiosses, W.B., Rusk, N., Symons, M.H., Waterman-Storer, C.M., Schmid, S.L., Schwartz, M.A., 2004. Modulation of Rac localization and function by dynamin. Mol. Biol. Cell 15, 256–267.
Suzuki, T., Futaki, S., Niwa, M., Tanaka, S., Ueda, K., Sugiura, Y., 2002. Possible existence of common internalization mechanisms among arginine-rich peptides. J. Biol. Chem. 277, 2437–2443.
Tyagi, M., Rusnati, M., Presta, M., Giacca, M., 2001. Internalization of HIV-1 tat requires cell surface heparan sulfate proteoglycans. J. Biol. Chem. 276, 3254–3261.
Vives, E., 2003. Cellular uptake of the Tat peptide: an endocytosis mechanism following ionic interactions. J. Mol. Recognit. 16, 265–271.
Vives, E., Brodin, P., Lebleu, B., 1997. A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. J. Biol. Chem. 272, 16010–16017.
Wadia, J.S., Stan, R.V., Dowdy, S.F., 2004. Transducible TAT-HA fusogenic peptide enhances escape of TAT-fusion proteins after lipid raft macropinocytosis. Nat. Med. 10, 310–315.
Wender, P.A., Mitchell, D.J., Pattabiraman, K., Pelkey, E.T., Steinman, L., Rothbard, J.B., 2000. The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. Proc. Natl. Acad. Sci. U.S.A. 97, 13003–13008.
Zaro, J.L., Shen, W.C., 2003. Quantitative comparison of membrane transduction and endocytosis of oligopeptides. Biochem. Biophys. Res. Commun. 307, 241–247.