FOCUS ISSUE ARTICLE

Enhanced cellular uptake of lactosomes using cell-penetrating peptides

Akiya Akahoshia, Eiji Matsuura, Eiichi Ozekic, Hayato Matsuic, Kazunori Watanabea and Takashi Ohtsukia

aDepartment of Medical Bioengineering, Okayama University, Okayama 700-8530, Japan
bCollaborative Research Center forOMIC, Okayama University, Okayama 700-8558, Japan
cTechnology Research Laboratory, Shimadzu Corporation, Kyoto, Japan

ABSTRACT
Polymeric micelles that are composed of synthetic polymers are generally size controllable and can be easily modified for various applications. Lactosomes (A3B-type) are biodegradable polymeric micelles composed of an amphipathic polymer, including three poly(sarcosine) blocks and a poly(l-lactic acid) block. Lactosomes accumulate in tumors in vivo through the enhanced permeability and retention (EPR) effect, even on frequently administering them. However, lactosomes cannot be efficiently internalized by cells. To improve cellular uptake of lactosomes, cell-penetrating peptide (CPP)-modified lactosomes were prepared. Seven CPPs (including EB1 and Pep1) were used, and most of them improved the cellular uptake efficiency of lactosomes. In particular, EB1- and Pep1-modified lactosomes were efficiently internalized by cells. In addition, by using CPP-modified and photosensitizer-loaded lactosomes, we demonstrated the photoinduced killing of mammalian cells, including human cancer cells. Accumulation of the EB1-modified lactosomes in NCI-N87 tumors was shown by in vivo imaging. Thus, this study demonstrated that the CPP-modified lactosome is a promising drug carrier.

1. Introduction
The use of nanoparticles as drug carriers for the selective delivery of pharmacological agents in various diseases such as cancer and viral infections has been studied. [1,2] Over the past few decades, many nanoparticles, including liposomes,[3] polymeric micelles,[4,5] metal nanoparticles,[6] and dendrimers,[7] have been developed for drug delivery. Among them, polymeric micelles are size-controllable and easily modified for adding new abilities, such as targeting and imaging. The polymers that comprise the micelles are size-controllable and can have various reactive groups. Several liposome drugs have been approved and are in clinical use, and polymeric micelles are in the clinical test phase.[5,8,9]

Lactosomes are polymeric micelles composed of an amphiphatic polymer with a hydrophilic poly(sarcosine) (PSar) block and a hydrophobic poly(l-lactic acid) (PLLA) block (AB-type).[10,11] The high density of PSar chains around the molecular assemblies contributes to their ability to escape from the reticuloendothelial system (RES) recognition, similarly to polyethylene glycol (PEG) modification.[12] Furthermore, PSar is considered to have an advantage over PEG in terms of biodegradability. AB-type lactosomes persist in the bloodstream for a long time.[13] However, AB-type lactosomes exhibit a drawback in multiple in vivo administrations,[14] in which they accumulate in solid tumors after the first administration, but accumulate only slightly after the second administration. To compensate for this drawback, a lactosome composed of (PSar),-block-PLLA (A3B-type) has recently been developed.[15] The AB-type and A3B-type lactosomes accumulate in tumor tissue through the enhanced
permeation and retention (EPR) effect.[16] However, the lactosomes are not efficiently internalized by cells even if they accumulate around them. Improving the cellular uptake of the lactosomes is important for the efficient delivery of drugs that act intracellularly.

In this study, we attempted to modify $\alpha_{\beta}$-type lactosomes with cell-penetrating peptides (CPPs) to increase efficiency of their cellular uptake. CPPs are short peptides often rich in cationic residues, which have the ability to internalize various cargo molecules such as nucleic acids, proteins, and nanoparticles.[17–19] We used seven CPPs, including those derived from natural proteins (Tat and DPV3) [20–22] and chimeric or artificial peptides (PTD4, MPGΔNLS, Pep1, and EB1).[23–26] Cellular uptake efficiency of various CPP-modified lactosomes was compared. In addition, the photosensitizer 5,10,15,20-tetraphenyl-21H,23H-porphyrin (TPP) [27] was delivered to mammalian cells (including cancer cell lines) by the CPP-modified lactosomes. The cells were treated with the TPP-encapsulated lactosome and photoirradiated to evaluate the photodynamic therapy (PDT) effect. Finally, in vivo localization of the CPP-modified lactosomes was investigated using tumor-bearing mice.

2. Experimental details

2.1. Materials

(PSAr$_{25}$)$_3$-block-PLLA$_{35}$ indocyanine green (ICG)-PLLA$_{34}$, and maleimide-PSar$_{56}$-PLLA$_{30}$ were synthesized as described.[11,15,28] Chinese hamster ovary (CHO) cells (FLIP-In cell line) were purchased from Invitrogen (Waltham, Massachusetts, USA). NCI-N87 human gastric cancer cells and PANC-1 human pancreatic carcinoma cells were purchased from the American Type Culture Collection. The CPPs listed in Table 1 were prepared by Fmoc-based solid-phase peptide synthesis and provided by the Central Research Laboratory at the Okayama University Medical School.

2.2. Preparation of lactosome complexes

A chloroform solution containing the (PSAr$_{25}$)$_3$-block-PLLA$_{35}$ (100 nmol, 7.7 µg) with 4 mol% of TPP (Wako Pure Chemical, Osaka, Japan), 2 mol% of ICG-PLLA$_{34}$, and 10 mol% maleimide-PSar$_{56}$-PLLA$_{30}$ was evaporated under reduced pressure to remove the solvent, forming a thin film on the surface of the glass test tube. Saline (Otsuka normal saline, Otsuka Pharmaceutical Factory, Tokushima, Japan) (50 µl) was added to the test tube, which was then placed at room temperature for 5 min. Then, 50 µl of the saline solution containing 50 nmol CPP and 10 nmol Tris(2-carboxyethyl)phosphine hydrochloride (Nacalai Tesque, Kyoto, Japan) was added to the solution (50 µl) in the test tube and stirred for 12 h at room temperature. In this procedure, lactosome formation and TPP encapsulation by the lactosome were expected. At the same time, maleimide-PSar$_{56}$-PLLA$_{30}$ in the lactosome is expected to react with CPP, which contains a cysteine residue at its C-terminus (Table 1). This lactosome mixture was diluted with 400 µl of saline, and passed through a 0.1 µm syringe filter (Membrane Solutions, Dallas, USA) to remove large aggregates. Low molecular weight molecules were excluded from the mixture using Amicon Ultra-0.5 (MWCO 50 kDa, Merck Millipore, Darmstadt, Germany). To analyze concentrations of CPP, TPP and ICG-PLLA$_{34}$, absorption spectra of the lactosome complexes were measured using a BioSpec spectrometer (Shimadzu, Kyoto, Japan). The particle size, size distribution, polydispersity index (PDI), and zeta potential were measured by Zetasizer Nano ZSP (Malvern Instruments, Malvern, UK).

2.3. Analysis of lactosome complexes using size exclusion chromatography

Size exclusion chromatography was performed using a Superdex 200 10/300 GL column (GE Healthcare, Little Chalfont, UK) on an HPLC system (Shimadzu). The mobile phase was 10 mM Tris-HCl (pH 7.4) at a flow rate of 0.5 ml min$^{-1}$. Absorbance was measured at 230, 280, 417, and 700 nm.

2.4. Detection of photogenerated singlet oxygen

Singlet oxygen ($^{1}$O$_2$) generation from the lactosome complexes (±TPP) was measured using Singlet Oxygen Sensor Green (SOSG) (Molecular Probes, Eugene, Oregon, USA). In a 96-well black plate with a clear bottom, 5 µl of the TPP-encapsulated lactosome and photoirradiated to evaluate the photodynamic therapy (PDT) effect. Finally, in vivo localization of the CPP-modified lactosomes was investigated using tumor-bearing mice.

Table 1. Cell-penetrating peptides used in this study.

| CPP        | Classification | Length | Sequence                  |
|------------|----------------|--------|---------------------------|
| Tat        | Cationic       | 11     | YGRKRRRQRRR-C             |
| PTD4       | Cationic       | 11     | YARRAAQQRA-C              |
| DPV3       | Cationic       | 16     | RKKRRRESRRRRRES-C         |
| MPGΔNLS    | Amphipathic    | 27     | GFLGFLGLGAAGSTMGAWSQPYSKRV-C |
| R9MPG      | Amphipathic    | 25     | RRRRRRRRRGFLALFARALALSLMG-C |
| Pep1       | Amphipathic    | 23     | KETWQETITWETKSOQPPIKRRR-C |
| EB1        | Amphipathic    | 23     | LIRLWSHLHIWFQNRRLKWWK-C  |
2.5. Evaluation of cellular uptake of lactosome complexes containing CPPs

CHO cells were cultured at 37°C under 5% CO2 in Ham's F12 medium (Wako Pure Chemical) supplemented with 10% fetal bovine serum (FBS, Nichirei Biosciences, Tokyo, Japan), 100 units ml–1 penicillin (Gibco; Thermo Fisher Scientific, Waltham, Massachusetts, USA), and 100 μg ml–1 streptomycin (Gibco; Thermo Fisher Scientific). The cells were seeded at a density of 2 × 10^4 cells/well in the 96-well plate, and incubated at 37°C under 5% CO2 overnight. The cells were then incubated at 37°C for 2 h with ICG-labeled lactosomes modified with CPPs (Tat, PTD4, DPV3, MPG^NLS, R9MPG, Pep1, or EB1) dissolved in 200 μl T buffer. The lactosome solution was exchanged for Ham's F12 medium before the fluorescence imaging. The cellular fluorescence images were obtained by fluorescence microscopy using an Olympus IX71 microscope (Olympus, Tokyo, Japan) with a 40 × objective lens and a U-DM-CY7–3 mirror unit.

LysoTracker Green (Thermo Fisher Scientific) was used for investigating cellular localization of CPP-modified lactosomes. After incubation of the cells with CPP-modified ICG-labeled lactosomes, the lactosome solution was exchanged with Ham's F12 medium containing 2 μM LysoTracker Green. The cells were further incubated for 3 h. Then, the cell medium was replaced with Ham's F12 medium, and fluorescence images were obtained.

To evaluate the cellular uptake of the lactosome complexes, the ICG fluorescence images were obtained using an IVIS spectrum system (Xenogen, Hopkinton, Massachusetts USA) with a filter set specific for ICG (excitation at 745 nm and emission at 840 ± 10 nm). The ICG fluorescence intensity was estimated from the photon counts of the images. Then, the cell medium was replaced with solution containing 10 μl of Cell Counting Kit-8 solution and treated with light on cell viability. The cell viability was measured by absorbance at 450 nm using the microplate reader SunriseR. Statistical analysis was performed with EZR (Saitama Medical Center, Saitama, Japan) using one-way ANOVA followed by Dunnett's test.

2.6. Measurement of cell viability after treatment with the TPP-loaded lactosomes and light

NCI-N87 cells were cultured at 37°C under 5% CO2 in RPMI1640 medium (Wako Pure Chemical) supplemented with 10% FBS, 100 units ml–1 penicillin, and 100 μg ml–1 streptomycin. NCI-N87 cells were seeded at a density of 4 × 10^4 cells/well in a 96-well plate, and incubated at 37°C for two days before treatment with the lactosome complex. CHO cells were cultured and seeded as described above. The effect of treatment with the TPP-loaded lactosomes and light on cell viability, which is related to PDT efficacy, was evaluated as follows: TPP-loaded lactosomes modified with CPPs (Pep1 or EB1) dissolved in 200 μl of T buffer (20 mM HEPES-KOH (pH 7.4), 115 mM NaCl, 5.4 mM KC1, 1.8 mM CaCl2, 0.8 mM MgCl2, and 13.8 mM glucose) were added to NCI-N87 cells (70% confluent) in the 96-well plate. The cells were incubated at 37°C for 1.5 h. After the lactosome solution was exchanged for T buffer, the cells were irradiated at 340–390 nm at 200 mW cm–2 for 100 s. After irradiation, the cells were further incubated for 24 h at 37°C under 5% CO2 in the medium. Then, the cell medium was exchanged for the solution containing 10 μl of Cell Counting Kit-8 solution and 90 μl of RPMI1640 medium, and the cells were incubated at 37°C for 2 h. The cell viability was measured by absorbance at 450 nm using a microplate reader SunriseR. Statistical analysis was performed with EZR (Saitama Medical Center, Saitama, Japan) using one-way ANOVA followed by Dunnett's test.

2.7. Tumor-bearing mice and in vivo imaging

Six-week-old male nude mice (BALB/c nu/nu) were purchased from Charles River. Five weeks before the imaging, PANC-1 cells (1 × 10^7) suspended in 100 μl of 50% Matrigel Matrix (Corning Life Sciences, Corning, New York USA) in phosphate buffered saline was subcutaneously inoculated into the front right leg of mice. Two weeks after the first transplantation, NCI-N87 cells (4 × 10^6) were suspended in the Matrigel solution and subcutaneously inoculated into the front right leg of mice. Three weeks after the second transplantation, CPP/TPP/ICG-lactosomes were injected into the tumor-bearing mouse via the tail vein. After 24 h, ICG fluorescence images were taken using the IVIS spectrum system (excitation at 745 nm and emission at 840 ± 10 nm, field of view = 12.5 cm (width and height), f/stop = 2, binning = 4, and exposure time at 3 s). During the imaging process, the mice were anesthetized using 3.0% isoflurane gas in oxygen flow (0.5 l min–1).

3. Results and discussion

3.1. Characterization of the CPP-modified lactosomes

The particle sizes of lactosome complexes were measured by dynamic light scattering (DLS). The average particle sizes of the CPP-modified lactosomes were 36–49 nm, which were slightly larger than the unmodified lactosomes (34.2 nm) (Table 2, Figure 1(b)). The zeta potential value of CPP-modified lactosomes (e.g. Pep1-modified lactosome: 1.7 mV, EB1-modified lactosome: 1.6 mV) was higher than that of the unmodified lactosomes (–4.6 mV) (Table 2). The increase in the zeta potential seems to be due to positively charged CPPs.
These results indicated that modification of lactosomes with CPPs increased their surface charge and particle size.

To confirm the incorporation of the CPP (EB1), TPP, and indocyanine green (ICG)-PLLA into the lactosomes, size exclusion chromatography was performed using a Superdex 200 10/300 GL column and the HPLC system (Figure 1(c)–(g)). The purified lactosome complex containing EB1, TPP, and ICG (EB1/TPP/ICG-lactosome) was eluted as a single peak from the column, and its retention time was the same as in the unmodified lactosomes (Figure 1(c) and 1(d)). Absorbance traces of the chromatography at 230, 280, 417, and 700 nm suggested that EB1, TPP, and ICG-PLLA were successfully incorporated into lactosome complexes (Figure 1(c)–(g)). EB1 is thought to bind to maleimide-PSar₅₆-PLLA₃₀ in the lactosome, and TPP is thought to be encapsulated in the hydrophobic core of the lactosome by hydrophobic interaction.

### 3.2. Cellular uptake of CPP-modified lactosomes

Internalization of CPP-modified lactosomes by CHO cells was observed by fluorescence microscopy (Figure 2(a)). ICG-labeled CPP-modified lactosomes were added to CHO cells and incubated at 37°C for 2 h. After the incubation and washing steps, significantly strong ICG fluorescence was observed in the CHO cells treated with the Pep1- and EB1-modified lactosomes, compared to the lactosomes without CPP. The CPP-modified lactosomes showed dotted localization. Pep1- and EB1-modified lactosomes co-localized with LysoTracker Green (Figure 2(b)), indicating that they localized mainly in acidic organelles, including endosomes and lysosomes. Furthermore, the lactosome uptake level of the cells was estimated from the ICG fluorescence intensity and cell viability of each culture well (Figure 2(c)). The EB1-modified lactosomes was the most abundantly internalized by the cells. Pep1-modified lactosomes were also highly internalized (their uptake level relative to the EB1-modified lactosomes was 0.62). These results indicated that cellular uptake of lactosomes was improved by the addition of CPPs, especially EB1 and Pep1. These results showed that cellular uptake efficiency of the lactosomes modified with amphipathic CPPs was higher than that of lactosomes modified with non-amphipathic CPPs,

### Table 2. Particle size, zeta potential and polydispersity index (PDI) of CPP-modified lactosomes.

| CPP   | Diameter (nm) | Zeta potential (mV) | PDI  |
|-------|----------------|---------------------|------|
| -cPP  | 34.2±0.5       | −4.6±0.2            | 0.13±0.01 |
| Tat   | 36.2±0.3       | −3.6±0.1            | 0.10±0.01 |
| PTD4  | 37.0±0.4       | −4.2±0.3            | 0.14±0.02 |
| DPTV  | 37.0±0.4       | −3.7±0.3            | 0.12±0.01 |
| MPG₅₆₅₅ | 49.1±0.5    | −2.9±0.6            | 0.29±0.02 |
| R9MPG | 43.9±1.5       | −2.7±0.9            | 0.22±0.01 |
| Pep1  | 37.7±0.6       | 1.7±0.1             | 0.13±0.02 |
| EB1   | 38.3±0.2       | 1.6±0.3             | 0.12±0.01 |

These values were measured by DLS and laser Doppler electrophoresis ($n = 3$, mean ± standard deviation).

![Figure 1. Characterization of lactosome complexes. (a) A diagram of the lactosome complex. (b) Size distribution of lactosomes modified with Pep1 or EB1 peptide. (c)–(g) Size exclusion chromatography of (c) unmodified and (d)–(g) EB1/TPP/ICG-lactosomes. Absorbance traces at (c), (d) 230 nm, (e) 280 nm, (f) 417 nm, and (g) 700 nm.](image-url)
both EB1- and Pep1-modified lactosomes had positive zeta potential (Table 2), while the others had negative potential. This effect seems logical since the first step in CPP uptake is usually interaction with negative surface of the plasma membrane. Which may be related to the binding efficiency of the CPPs to lactosomes. There was no relationship between the size of the CPP-modified lactosomes (Table 2) and the cellular uptake efficiency. Zeta potential seems to be related to the cellular uptake efficiency because both EB1- and Pep1-modified lactosomes had positive zeta potential (Table 2), while the others had negative potential.

Figure 2. Cellular uptake of the ICG-labeled lactosomes modified with CPPs (Tat, PTD4, DPV3, MPG^MNS, R9MPG, Pep1, or EB1). CHO cells were cultured with 10 nmol of the lactosome complexes for 2 h at 37°C. (a) Optical microscopy images of the cells cultured with the ICG-labeled and CPP-modified lactosomes. Scale bars represent 50 μm. (b) Images of Pep1- and EB1-modified ICG-lactosomes in the cells co-stained with LysoTracker Green. Scale bars indicate 20 μm. (c) Cellular uptake level calculated by dividing ICG fluorescence intensity by cell viability relative to that of the EB1-modified lactosomes. Values are expressed as mean ± standard error of the mean, n = 4.
after treatment with CPP/TPP-lactosomes followed by photoin irradiation, and 24 h culture in the medium. EB1/TPP-lactosomes and Pep1/TPP-lactosomes with photoin irradiation efficiently induced the killing of NCI-N87 cells (89% and 67%, respectively, compared to the experiment without CPP/TPP-lactosomes) (Figure 4(a)). Similar results were obtained in CHO cells (Figure 4(b)). The cell damage observed with these CPP/TPP-lactosomes is thought to be due to the photo-generated singlet oxygen (Figure 3). The efficiency of the cell killing was related to the cellular uptake efficiency of CPP-modified lactosomes (Figure 2) (EB1 > Pep1 > -CPP). These results indicated that the EB1 peptide was the most effective CPP for cellular internalization of TPP lactosomes.

3.5. In vivo imaging

To confirm the accumulation of EB1/TPP/ICG-lactosomes in tumor sites, lactosomes were administered to mice bearing NCI-N87 and PANC-1 tumors, and ICG fluorescence images were obtained. Reproducibility was confirmed using three mice, and the representative image is shown in Figure 5. Twenty-four h after injection, ICG fluorescence was detected in the NCI-N87 tumor, indicating that the EB1-modified lactosome accumulated in NCI-N87 tumors and liver (Figure 5(a)), though it accumulated in NCI-N87 tumors less than the lactosome lacking the CPP (Figure 5(b)). This accumulation in liver indicates that the escape ability (stealth property) of EB1-modified lactosome from RES is slightly lower than the lactosome lacking the CPP. In contrast to the NCI-N87 tumor, only slight ICG fluorescence was detected in the PANC-1 tumor (Figure 5(a)). These observations are consistent with those of the previous reports.[28,29] The accumulation of lactosomes in NCI-N87 tumors has also been reported in a study that used HER2-modified AB-type lactosomes.[28] Pancreatic tumors are known to be poorly permeable,
Acknowledgements

We thank Dr Kazuko Kobayashi (Okayama University) for technical assistance in the animal experiments.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Grants-in-Aid for Scientific Research on Innovative Areas “Nanomedicine Molecular Science” to T.O. [26107711]; and the Project for Development of Innovative Research on Cancer Therapeutics (P-DERECT) [7211500107].

References

[1] Durymanov MO, Rosenkranz AA, Sobolev AS. Current Approaches for Improving Intratumoral Accumulation and Distribution of Nanomedicines, Theranostics. 2015;5:1007.
[2] Ndeboko B, Lemamy GJ, Nielsen PE, et al. Therapeutic Potential of Cell penetrating Peptides (CPPs) and Cationic Polymers for Chronic Hepatitis B, Int J Mol Sci. 2015;16:28230–28241.
[3] Allen TM, Cullis PR. Liposomal drug delivery systems: From concept to clinical applications, Adv Drug Deliv Rev. 2013;65:36.
[4] Deng C, Jiang Y, Cheng R, et al. Biodegradable polymeric micelles for targeted and controlled anticancer drug delivery: Promises, progress and prospects, Nano Today. 2012;7:467.
[5] Cabral H, Kataoka K. Progress of drug-loaded polymeric micelles into clinical studies, J Control Release. 2014;190:465.
[6] Arvizo RR, Bhattacharyya S, Kudgus RA, et al. Intrinsic Therapeutic Applications of Noble Metal

and among polymeric micelles with diameters of 30, 50, 70, and 100 nm, only the 30 nm micelles accumulated in pancreatic tumors.[29] Since the size of the EB1-modified lactosomes used in this study was ~38 nm, reducing the size of the CPP-modified lactosomes may increase their accumulation in tumors.

4. Conclusions

The A,B-type lactosome, a biocompatible and biodegradable polymeric nanomicelle, was modified with CPPs to improve its cellular uptake. Among the seven kinds of CPPs (Tat, PTD4, DPV3, MPG\textsuperscript{ΔNLS}, R9MPG, Pep1, and EB1), amphipathic EB1 and Pep1 peptides greatly improved the uptake efficiency of the lactosomes. The CPP-modified lactosomes internalized by cells were localized mainly in endosomes or acidic organelles. We also conducted PDT experiments using the CPP-modified and photosensitizer-loaded lactosomes. Cell killing was efficiently photoinduced using the EB1/TPP and Pep1/TPP lactosomes. \textit{In vivo} imaging of the EB1/TPP/ICG-lactosomes showed that they accumulated in NCI-N87 tumors in mice. More efficient tumor accumulation may be accomplished through the size-control of the CPP-modified lactosomes. The CPP-modified lactosome is promising as an efficient drug carrier. This study demonstrated that CPP-modified lactosomes encapsulated the hydrophobic agent TPP and delivered it into cells. Thus, CPP-modified lactosomes can deliver hydrophobic agents into cells. These lactosomes may also be able to deliver hydrophilic drugs such as proteins and nucleic acids into cells by attaching hydrophobic modifications to them.

Figure 5. \textit{In vivo} imaging of EB1/TPP/ICG-lactosomes (a) and TPP/ICG-lactosome (b). Ten-week-old male BALB/c nu/nu mice, grafting NCI-N87 and PANC-1 at front legs (right; NCI-N87, left; PANC-1) were used. The ICG image was obtained 24 h after administration of lactosomes to the tumor-bearing mouse. Arrows indicate tumor sites (white; NCI-N87, and yellow; PANC-1). The color bar indicates the fluorescence radiant efficiency (photons s\textsuperscript{-1} cm\textsuperscript{-2} steradian\textsuperscript{-1}).
intercellular cargo delivery, J Control Release. 2014;174:126.

[18] Copolovici DM, Langel K, Eriste E, et al. Cell-Penetrating Peptides: Design, Synthesis, and Applications, ACS Nano. 2014;8:1972.

[19] Endoh T, Ohtsuki T. Cellular siRNA delivery using cell-penetrating peptides modified for endosomal escape, Adv Drug Deliv Rev. 2009;61:704.

[20] Frankel AD, Pabo CO. Cellular uptake of the tat protein from human immunodeficiency virus, Cell. 1988;55:1189.

[21] Vives E, Brodin P, Lebleu B. A Truncated HIV-1 Tat Protein Basic Domain Rapidly Translocates through the Plasma Membrane and Accumulates in he Cell Nucleus, J Biol Chem. 1997;272:16010.

[22] De Coupade C, Fittipaldi, A., Changas, V., et al. Novel human-derived cell-penetrating peptides for specific subcellular delivery of therapeutic biomolecules, Biochem J. 2005;390:407.

[23] Ho A, Schwarze SR, Mermelstein SJ, et al. Synthetic Protein Transduction Domains: Enhanced Transduction Potential in Vitro and in Vivo, Cancer Res. 2001;61:474.

[24] Simeoni F, Morris MC, Heitz F, et al. Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells, Nucleic Acids Res. 2003;31:2717.

[25] Morris MC, Depollier J, Mery J, et al. A peptide carrier for the delivery of biologically active proteins into mammalian cells, Nat Biotechnol. 2001;19:1173.

[26] Lundberg P, El-Andaloussi S, Sutlu T, et al. Delivery of short interfering RNA using endosomolytic cell-penetrating peptides, FASEB J. 2007;21:2664.

[27] Canete M, Villanueva A, Dominguez V, et al. Mesotetraphenylporphyrin: photosensitizing properties and cytotoxic effects on cultured tumor cells, Int J Oncol. 1998;13:497.

[28] Shimizu Y, Temma T, Hara I, et al. Micelle-based activatable probe for in vivo near-infrared optical imaging of cancer biomolecules, Nanomedicine. 2014;10:187.

[29] Cabral H, Matsumoto, Y., Mizuno, K., et al. Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size, Nat Nanotechnol. 2011;6:815.