A42
Cytosolic delivery of macromolecules using pH-dependent fusogenic peptide

Ikuhiko Nakase*, Sachiko Kobayashi, Shiroh Futaki
Institute for Chemical Research, Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan

*Corresponding author.
E-mail: nakase@scl.kyoto-u.ac.jp (I. Nakase).

The plasma membrane plays critical roles in maintaining cellular homeostasis. It serves as a barrier against unfavorable attack on cells from an unpredictable external world. However, the membranes are also barriers to intracellular delivery of various therapeutic molecules. For improving their translocation, we developed a novel method using GALA peptide/cationic lipid complexes. The GALA peptide (amino acid sequence: WEAALAEALAEALAEHLAEALAEALEALAA) is a 30-residue amphipathic peptide with a repeat sequence of glutamic acid–alanine–leucine–alanine, and designed to mimic the function of viral fusion protein sequences that mediate escape of virus genes from acidic endosomes into cytosol [1]. The GALA peptide converts its structure from random to helical when the pH is reduced from 7.0 to 5.0, and this leads to destabilization of the membranes. When attached with bioactive cargoes, the GALA peptide may thus serve as intracellular vector bearing efficient endosomal escape function. However, the negative charges from glutamic acids (seven residues) in the GALA sequences reduce the efficiency of binding to a negatively charged cell surface. To overcome this problem, a cationic lipid was employed as an 'adhesive' for pasting the GALA peptide onto cell surface to accelerate its cellular uptake. We examined the ability of GALA peptide as a delivery vector using FITC as a model of membrane-impermeable low-molecular weight drugs. When FITC–GALA (1 μM) was administrated to HeLa cells, co-addition of cationic lipid, Lipofectamine 2000 (LF2000), significantly increased uptake efficiency. In a time-dependent manner, FITC–GALA escaped from endosomes, and diffuse fluorescent signals were observed in both cytosol and nucleus, suggesting that the cytosolic translocation proceeds along with endosomal acidification. The GALA/cationic lipid system was also applied for the intracellular delivery of FITC–avidin protein (68 kDa). When FITC–avidin (250 nM) was mixed with biotinylated-GALA (1 μM)/LF2000 complexes, FITC–avidin effectively internalized into cells, and diffuse signals of the FITC–avidin in cytosol were observed. In the absence of these complexes, efficiency of cytosolic diffusion of the FITC–avidin was quite low. These results suggest the usefulness of our approach for intracellular delivery of macromolecules using GALA peptide and cationic lipid [2].

Reference

1. Subbarao, et al. *Biochemistry* 1987;**26**:2964–72.
2. Kobayashi, et al. *Bioconjug Chem* 2009;**20**:953–9.

doi:10.1016/j.drudis.2010.09.392

A44
A dual uptake mechanism for the peptide Tat–LK15

Myasar Alkotaji[1,2,*], Alain Pluen[1,2]
[1] *School of Pharmacy, University of Manchester, UK*
[2] *Stopford Building, Oxford Road, Manchester, UK*

*Corresponding author.
E-mail: Myasar.alkotaji@postgrad.manchester.ac.uk (M. Alkotaji).

Knowing the mechanism of uptake is fundamental in developing new and refining existing drug delivery systems. Recently, Tat–LK15 peptide resulting from the fusion of Tat peptide and the synthetic amphipathic LK15 has shown greater transfection efficiency than Tat alone [1]. However, its uptake mechanism is ambiguous as the influence of the amphipathic peptide (LK15) and Tat peptide upon binding remains unclear. To elucidate this issue, the present study investigates the effect of temperature and peptide concentration on the cellular uptake mechanism of TAMRA–Tat–LK15 peptide. HeLa and HT29 cell lines were incubated with 1, 2.5 or 5 μM TAMRA–Tat–LK15 and TAMRA–Tat peptide solutions at different temperatures (4°C, 20°C, and 37°C) or in the presence of sodium azide (a metabolic inhibitor). A Zeiss LSM510 microscope was used to monitor cellular uptake in using a thermoelectric controlled temperature chamber. Our data indicate clearly TAMRA–Tat–LK15 peptide uptake (diffuse distribution in the cells) at 4°C for a 5 μM bulk solution while images do not suggest uptake in these conditions at lower concentrations (1 and 2.5 μM). At higher temperatures (20°C and 37°C) TAMRA–Tat–LK15 was observed in cells at all concentrations (a mixture of diffuse and punctuated fluorescence in cells). Interestingly, pre-incubation with 10 mM sodium azide did not completely block peptide uptake in cells for 1 and 2.5 μM bulk peptide solutions, and, had no effect at 5 μM and above. Overall, the results suggest the presence of concentration dependent uptake mechanisms of the Tat–LK15 peptide in cells.

Reference

1. Saleh AF, et al. Improved Tat-mediated plasmid DNA transfer by fusion to LK15 peptide. *J Control Release* 2010;**143**:233–42.

doi:10.1016/j.drudis.2010.09.393

A45
Visualizing the effect of integrin targeting and surface shielding on gene vector uptake by live cell imaging

F.M. Koenig*, N. Ruthardt, Y. Vachutinsky, M. Oba, K. Miyata, K. Kataoka, C. Bräuchle
Ludwigs-Maximilian Universität München, Physikalische Chemie I, Butenandt Str 11, Haus E, 81377 München, Germany

*Corresponding author.
E-mail: Frauke.Koenig@cup.uni-muenchen.de (F.M. Koenig).

Nonviral vectors enable the safe delivery of transgenes into target tissues but are still less efficient than viral gene vectors. To develop novel artificial systems with enhanced efficiencies a detailed understanding of the cellular uptake and intracellular trafficking is essential. By visualizing the entire pathway of a single nanoparticle, from its first contact with the cell surface to the delivery of the DNA to the cell nucleus, detailed information about the mechanisms of uptake, intracellular trafficking and DNA release can be gained. Here we study the effect of integrin targeting and surface shielding on the internalization of gene vectors by live cell imaging with highly sensitive fluorescent microscopy. αvβ3 and αvβ5 integrin receptors are attractive targets for antiangiogenic cancer gene therapy as they play a pivotal role in angiogenesis and proliferation of malignant tumors. A cyclic RGD peptide specifically binds to those receptors and thus can be used for specific targeting of gene vectors such as polyplex micelles. In this study the analyzed micelles consisted of a thiolated PEG-block-poly(lysine) copolymer complexed with fluorescently labeled DNA [1]. To analyze the influence of shielding, two types of micelles containing a differently sized PEG shell were compared. To directly compare the internalization of targeted and untargeted micelles without knowing the details of their internalization pathway, we simultaneously added both micelle types with different fluorescent labels onto cells and evaluated their

colocalization degree over time. Additionally the internalization kinetics of integrin targeted micelles was compared to EGF targeted polyplexes that are well-known for their fast uptake kinetics [2]. The internalization pathway was then studied with inhibitor experiments and by colocalization with specific marker proteins. Our results reveal a strong competition between unspecific electrostatic interactions and specific receptor–ligand interactions that determines successful targeting of the micelles. Enhanced PEG shielding of the micelles leads to the reduction of electrostatic interactions resulting in a specific and faster internalization of the targeted micelles. Additionally we observed a considerable effect of the applied micelle concentration as well as the micelle size on their internalization properties. Our data lead to a more detailed understanding of the targeting effect than can be observed by conventional bulk instruments. The gained knowledge enables to maximize the therapeutic benefit of future gene vectors for clinical application.

Reference

1. Oba M, et al. Cyclic RGD peptide-conjugated polyplex micelles as a targetable gene delivery system directed to cells possessing alphavbeta3 and alphavbeta5 integrins. *Bioconjug Chem* 2007;**18**:1415–23.
2. de Bruin K, et al. Cellular dynamics of EGF receptor-targeted synthetic viruses. *Mol Ther* 2007;**15**:1297–305.

doi:10.1016/j.drudis.2010.09.394

A46
A designer biomimetic vector for breast cancer gene therapy

H.O. McCarthy[1,2,*], A.V. Zholobenko[1], J.A. Coulter[1], H.D. McKeen[1], D.G. Hirst[1], T. Robson[1], A. Hatefi[2]

[1] *School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, UK*

[2] *Department of Pharmaceutical Sciences, Centre for Integrated Biotechnology, Washington State University, Pullman, WA, USA*

*Corresponding author.

E-mail: h.mccarthy@qub.ac.uk (H.O. McCarthy).

Introduction: Gene therapy holds the potential to cure many diseases, provided that the genetic or molecular basis is understood. In cancer, the delivery of therapeutic genes via viral vectors has proven more effective than the current alternative non-viral methods. However tissue specificity, high costs of production and safety remain major concerns with viral delivery. This study examines the use of a designer biomimetic vector (DBV), that is essentially a recombinant fusion protein, to deliver the therapeutic inducible nitric oxide synthase (iNOS) gene to breast cancer. The DBV is composed of several discrete motifs each designed with single function architecture including: (a) a DNA condensing motif (DCM) obtained from the adenovirus mu peptide, (b) a ZR-75-1 breast cancer cyclic targeting peptide (TP) for specific delivery of the nanoparticles, (c) an endosomal disruption motif (EDM) that mimics the influenza virus fusogenic peptide and (d) a nuclear localization signal (NLS), rev, obtained from the human immune-deficiency virus type-1. We now use this DBV to deliver the cytotoxic iNOS gene *in vitro* and the GFP reporter gene *in vivo* to ZR-75-1 tumours. *Methods*: The DBV was expressed in *Escherichia coli*, extracted with affinity chromatography and purified using size exclusion chromatography. The DBV was complexed to piNOS to form nanoparticles which were used either for characterisation via electrophoretic mobility shift assays, serum stability assays or dynamic light scattering analysis. ZR-75-1 breast cancer cells were transfected with DBV/piNOS nanoparticles and toxicity was quantified using the WST-1 cell toxicity and clonogenic assays. Over expression of iNOS was also confirmed via western blotting and greiss test. Finally ZR-75-1 intradermal tumours were grown using SCID models and the DBV/pEGFP-N1 nanoparticles were delivered both intratumourally and intravenously. Tumours and organs were excised and the GFP distribution was determined. *Results*: The DBV was effectively expressed in *E. coli* at approximately 3 mg/l yield. The DBV condenses piNOS into spherical nanoparticles between N:P ratios of 4–10. At a N:P ratio of 9, piNOS was fully condensed with an average size of 75.1 nm. Transfection with the DBV/piNOS nanoparticles resulted in a maximum of 62% cell kill. INOS overexpression was confirmed and total nitrite levels were in the range of 18 μM and comparable with lipofectamine/piNOS. Finally 48 h after i.v. injection of the DBV/pEGFP-N1 nanoparticles GFP protein was detected in all the organs. The addition of chloroquine (30 mg/kg I.P.) did not enhance the expression of GFP indicating functionality of the EDM. Furthermore the addition of nocodazole (3 mg/kg I.P.) resulted in a reduction in GFP expression again indicating NLS functionality *in vivo*. *Conclusions*: The DBV/piNOS nanoparticles gave significant cytotoxicity in ZR-75-1 breast cancer cells *in vitro* and with less than 20% transfection this indicates a bystander effect. Despite a lack of tumour targeting by the DBV vector *in vivo*, the data indicates that the DBV/pEGFP-N1 nanoparticles do not aggregate and can travel through the bloodstream with confirmation of gene expression in all the organs. Future studies will concentrate on using the human osteocalcin promoter (hOC) to transcriptionally target the iNOS plasmid to ZR-75-1 breast tumours.

doi:10.1016/j.drudis.2010.09.395

A47
Cellular delivery and biological activity of metall complex-peptide conjugates

Katrin Splith*, Jan Hoyer, Ines Neundorf

Leipzig University, Faculty of Biosciences, Pharmacy and Psychology, Department of Biochemistry, Brüderstr. 34, 04103 Leipzig, Germany

*Corresponding author.

E-mail: splith@uni-leipzig.de (K. Splith).

Bioorganometallic chemistry has become more and more important in several fields, especially in the development of new drugs for cancer treatment. A number of metal-based building blocks have promising features for applications in therapy and diagnosis. Introduction of a metal centre could add new features that may help to overcome some problems in cancer treatment. However the low water solubility and bioavailability of these organometallic compounds inhibits their therapeutic use in medicine. Therefore intracellular delivery of therapeutics is the challenging task in medicinal chemistry research. Recently, so-called cell-penetrating peptides (CPP) have emerged as potent tools to introduce substances into cells. CPP are an inhomogenic group of peptides that share the ability to translocate in a large number of different cell-lines without the need of any receptor or transporter molecule. Thereby they are capable to transport various cargos inside cells, like proteins, oligonucleotides, nanoparticles or small organic drugs. This work describes the coupling of metal-based building blocks to cell-penetrating peptides based on an antimicrobial peptide cathelicidin CAP18 or on the human peptide hormone calcitonin (hCT). Synthesis was achieved by solid phase peptide synthesis using standard Fmoc chemistry and activation by HOBt/DIC. Several different metal complexes have been investigated, for example, half-sandwich-complexes of different metals as iridium, manganese, rhodium or iron. To introduce the potential metal-specific activity to the bioconjugate, up to two organometal moieties were coupled either N-terminally, to a