Fusion of HIV-1 Tat protein transduction domain to poly-lysine as a new DNA delivery tool

H Hashida1,3, M Miyamoto*,1,3, Y Cho1, Y Hida1, K Kato1, T Kurokawa1, S Okushiba1, S Kondo1, H Dosaka-Akita2 and H Katoh1

1Department of Surgical Oncology, Division of Cancer Medicine, Hokkaido University Graduate School of Medicine, N-15, W-7, Kita-ku, Sapporo, Hokkaido 060-8638, Japan; 2Department of Medical Oncology, Division of Cancer Medicine, Hokkaido University Graduate School of Medicine, Hokkaido, Japan

Effective gene therapy depends on the efficient transfer of therapeutic genes to target cells. None of the current technologies, however, satisfy all of the requirements necessary for gene therapy, because the plasma and nuclear membranes of mammalian cells are tight barriers against gene transfer using synthetic delivery systems. The protein transduction domain (PTD) of human immunodeficiency virus type 1 (HIV-1) Tat protein greatly facilitates protein transfer via membrane destabilisation. We synthesised polyllysine peptides containing Tat PTD (TAT-pK), or other sequences, and investigated their potential as agents for gene transfer. The synthesised polypeptide TAT-pK retains DNA binding function and mediates delivery of a reporter gene to cultured cells. RGD motif binds with low affinity to alpha integrins which induce cell activation. Two control polypeptides, GGG-pK and RGD-pK, were synthesised and tested, but their gene transfer abilities were weaker than those of TAT-pK. TAT-pK-mediated gene transfer was enhanced in the presence of chloroquine or ammonium chloride, to a greater extent than that of cationic lipid-mediated gene transfer in most cancer cell lines tested. These data suggest that TAT-pK may be a potent candidate delivery vehicle that promotes gene transfer, dependent on the endocytic pathway. We conclude that the TAT-pK/DNA complex is useful as a minimal unit to package therapeutic genes and to transduce them into mammalian cells.

Gene therapy for cancer has been developed and a number of clinical therapeutic protocols are now being investigated. Vectors based on various viruses are useful for delivering therapeutic genes into primary cells in vitro and have also been applied in a number of gene therapy trials with humans. Viruses have some disadvantages as tools for medical application, however, with many elements of their biology yet to be elucidated. The utility of viral vectors for gene therapy is limited by DNA carrying capacity, difficulty in reliable and cost-effective manufacturing, and by immunogenicity and other safety concerns. One goal of cancer gene therapy is the development of gene delivery tools with lowered immunogenicity. While the construction of some viral vectors with reduced immunogenicity have been reported (Fisher et al., 1996; Haecker et al., 1996; Kochanek et al., 1996; Kumar-Singh and Chamberlain, 1996; Morrall et al., 1999), preparation of these vectors is difficult because the virus is composed of several kinds of large molecules.

Two elements are necessary to efficiently express foreign genes in cells: passage of DNA across the cell membrane and transport into the nucleus (Colin et al., 2000). From this point of view, recombinant viral vectors have a great advantage by depending on their intrinsic machinery for infection. Basic peptides derived from human immunodeficiency virus type 1 (HIV-1) Tat protein and Drosophila Antennapedia are protein transduction domain (PTD) of Tat (YGRKKRRQRRR) possess a high net positive charge at physiological pH, with nine of its 11 amino acids being either arginine or lysine. Fusion of several proteins and this 11 aa region of Tat protein enables the delivery of proteins into cells. Thus, this 11 aa region is considered a protein transduction domain (PTD). A 119-kDa protein, β-galactosidase, genetically fused to HIV-1 Tat PTD, was successfully carried into various mouse tissues, including the brain, following intraperitoneal injection (Nagahara et al., 1998).

Molecular conjugates of polyllysine with natural or artificial ligands utilise the DNA-binding and -condensing properties of polyllysine to mediate interaction with DNA (Wagner et al., 1991; Perales et al., 1994). Upon formation of a DNA-poly-lysine-ligand complex (polyplex (Felgner et al., 1997)), gene transfer is facilitated via receptor-mediated endocytosis.

In this study, we investigated the potential of a polyllysine fused Tat PTD (TAT-pK) as a gene delivery agent. We demonstrate that TAT-pK combines with DNAs and efficiently transports them into several human cell lines.
MATERIALS AND METHODS

Reagents

Reagents were obtained from the following sources: chloroquine and ammonium chloride from WAKO Pure Chemical Industries, Ltd (Osaka, Japan); 2,3-dioleyloxy-N-[t-(sperminecarboxamido)ethyl]-N′N′N-dimethyl-1-propanaminium trifluoroacetate (DOSPA)/dioleoyl phosphatidylethanolamine (DOPE) (LipofectAMINE™) from Life Technologies, a division of Invitrogen (Rockville, MD, USA).

Cell lines

The human embryonic kidney cell line HEK 293 was obtained from CLONTECH Laboratories. Human pancreatic carcinoma cell lines PCI10, PCI19, PCI35, and PCI43 were generously provided by Dr Yoshiki (Hokkaido University, Japan). Human oesophageal squamous cell carcinoma cell lines TE2, TE5, TE8, and TE13 were provided by Dr Nishihira (University of Tohoku, Japan). These cell lines were cultured in Dulbecco’s modified Eagle’s medium (DMEM; GIBCO, Rockville, MD, USA) with 2 mM l-glutamine, supplemented with 10% heat-inactivated foetal calf serum (FCS), at 5% CO2. Human lung carcinoma cell lines A549, RERF-LC-MS, PCI10, PCI19, PCI35, and PCI43 were generously provided by Dr Nishihira (University of Tohoku, Japan). These cell lines were cultured in RPMI medium (GIBCO) with 10% FCS.

Peptide synthesis

Three pKs (TAT-pK, RGD-pK, GGG-pK) and TAT were supplied by Hokkaido System Science Co., Ltd. (Sapporo, Japan). They were chemically synthesised by solid-phase methods, using Fmoc (9-fluorenylmethyloxycarbonyl) with a Pioneer Peptide Synthesis System (Applied Biosystems, CA, USA). Primary structures of the polypeptides were shown in Table 1.

Plasmid DNAs

The expression plasmid for enhanced green fluorescent protein (EGFP) (CLONTECH Laboratories, CA, USA) was constructed by inserting the cDNA into pcDNA3.1+ to produce pcDNA-EGFP. The expression vector for firefly luciferase pGL3 was obtained from Promega Corp (Madison, WI, USA).

Agarose gel electrophoresis of DNA-polypeptides complex

For the agarose gel electrophoresis assay, 0.5 μg of DNA (lambda DNA/HindIII digest) and peptides were mixed and incubated for 10 min at room temperature. The samples were loaded on a 1% agarose gel containing 0.5 μg ml−1 of ethidium bromide and run for 30 min at 100 V in 1× TBE buffer.

Transfection conditions

A standard protocol for gene transfer into cultured cells was followed. Cells were seeded at 2×104–1×105 cells well−1 in tissue culture plates, and cultured for 6 h. The cells were washed once with serum-free medium and incubated with medium containing DNA, DNA-polypeptides complex, or DNA complexed with cationic lipids (DOSPA/DOPE) for 8 h at 37°C. The cells were cultured for 48 h in medium with 10% FCS before assaying for the expression of reporter genes. DNAs were complexed with cationic lipids, according to the procedures recommended by the suppliers.

Luciferase assay and UV microscopy

Luciferase activity was evaluated using the Luciferase Assay System (Promega) and relative light units (RLU) were measured with Mini Lumat LB 9506 (BERTHOLD, Germany). RLUs are shown as averages with standard deviations. GFP and FITC were detected with fluorescence microscopy (Olympus Optical Co. Ltd., Japan) using a GFP cube. The cell nucleus was localised with fluorescence microscopy, using the fluorescent DNA binding dye, Hoechst 33342, and a WU cube (Olympus Optical Co. Ltd.). FITC labelled DNA fragments were prepared by phosphorimidate synthesis and purified by RP-HPLC purification.

WST-8 assay

Cytotoxicity of peptides was investigated using WST-8 assay. HEK293 cells were seeded in 96-well tissue culture plate at a density of 2×104 cells per well and incubated at 37°C for 72 h in fresh medium containing various peptides at a concentration of 10−320 μg ml−1. After incubation, 10 μl of 2-(2-methoxy-4-nitro-phenyl)-3-(4-nitrophenyl)-5-(2,4-disulphophenyl)-2H-tetrazolium (WST-8; Wako Pure Chemical Industries, Ltd, Japan) were added to each well. After 4 h of incubation, the optical density was read on a microplate autoreader (SPECTRAMax™ 190; Molecular Devices Corp, Sunnyvale, CA, USA) using a test wavelength of 490 nm and a reference wavelength of 620 nm.

FACS analysis

Where indicated, 10,000 events were counted on a Becton Dickinson FACSscan analyzer (Becton Dickinson, Franklin Lakes, NJ, USA) using a 15 mW air-cooled argon laser set at 488 nm, and recorded with a 530 nm emission filter in the FL1 emission channel. Cell populations are represented on a FACS histogram plotting FITC intensity on a logarithmic scale against cell number. Fluorescence intensity of cell populations is indicated by a shift to the right of the histogram plots of treated cells. Fluorescence enhancement was determined by obtaining the number of gated fluorescent events for untreated and treated cells.

RESULTS

DNA mobility shift analyses of pKs using agarose gel electrophorase

The pK tracts should impart DNA binding function to the fusion protein, by interacting with the negatively charged phosphate backbone of nucleic acids. To determine DNA binding ability, increasing concentrations of peptides were incubated with constant amounts of DNA marker (λ/HindIII), and the resulting effects on DNA mobility were analysed on agarose gels (Figure 1). At concentrations where peptides completely bind and then neutralise the DNA, it appears immobilised on the gel. The DNA was immobilised with peptides at a protein-to-DNA (w/w−1) ratio of 1. These results show that peptides can bind DNA. Furthermore, excess amounts of peptides did not induce the DNA to migrate in the opposite direction from the positive electrode.

| Peptides | Sequences |
|----------|-----------|
| TAT-pK   | NH2—YGRKRRQRRR-GGG-K000000000000000K—COOH |
| RGD-pK   | NH2—ARGDFTATGAS-GGG-K000000000000000K—COOH |
| GGG-pK   | NH2—GGG-K000000000000000K—COOH |
| TAT      | NH2—YGRKRRQRRR-GGG—COOH |

Table 1 Primary structures of synthesised polypeptides
Induction of EGFP and luciferase gene expressions by various peptides

We examined the efficiency of peptide-mediated gene transfer by evaluating the expression of the complexed EGFP gene. Under standard transfection conditions, we detected strong EGFP expression in approximately 5% of HEK 293 cells treated with the TAT-pK/pcDNA-EGFP complex. In cells treated with the GGG-pK or RGD-pK/pcDNA-EGFP complex, however, EGFP expression was detected in less than 1% of cells (Figure 2A). The number of cells expressing EGFP increased in proportion to the dose of pKs/DNA complex (figure not shown). In this study, the RGD-pK/DNA complex was also introduced into cells in a dose-dependent manner, but there was no difference in the transduction efficiency of RGD-pK and of GGG-pK. On the other hand, DNA complexed with TAT-pK was efficiently introduced, even at lower doses. The transduction efficiency of TAT peptide without a sequence of poly-lysine was lower than those of pKs (Figure 2B).

Cell-binding activities evaluated by FACScan

To investigate the reason for the high efficiency of TAT-pK-mediated gene transfer, cell-binding activity of pK/DNA complexes using FITC-labelled DNA was assessed (Figure 3A-H). There was scarcely any complex bound to cells with GGG-pK (Figure 3A) and RGD-pK (Figure 3B), and the amount of complex bound to cells did not increase, even 6 h after incubation (data not shown). TAT-pK/DNA complex, however, bound to almost all cells 60 min after incubation (Figure 3C). The difference in binding activity between TAT-pK and other pKs should be reflected in the DNA transduction efficiency. Interestingly, treatment with a small quantity of TAT-pK/DNA complex rarely led to cell binding (Figure 3D), and less than 0.1% of cells expressed EGFP when exposed to the equivalent quantity of TAT-pK/pcDNA-EGFP (data not shown). Most of the TAT-pK/pcDNA complex binds to the cells 10–30 min after incubation (Figure 3E and F). Tat-(48–60) has been reported to enter cells extremely rapidly, reaching the nucleus within 5 min (Futaki et al., 2001). Similarly, in our analysis, binding activity was observed immediately after incubation, and 1 h after incubation, FITC emission was detected in the nucleus (Figure 3I). No FITC emission was observed in cells exposed to the other pK/DNA complexes (data not shown). On the other hand, a four-fold excess of peptides, for neutralisation of the electrical charge, was required for high affinity (Figure 3G and H). A large number of peptides appear to require the complex to remain stably on the cell membrane or in the cytoplasm.
functions are similar to those of viral vectors that actively bind to the cell surface via high-affinity ligands, and transport their DNA into the nucleus by endocytosis. Thus, TAT-pK/DNA complexes possess the necessary elements for transfection as a small particle, although the efficiency is lower than that of adenoviral vectors. When we compared the efficiency of TAT-pK-mediated gene transfer with that mediated by cationic lipids (DOSPA/DOPA), we found that TAT-pK can induce higher levels of luciferase activity in the presence of chloroquine, but not in its absence (Figure 5B). The cytotoxicity of ammonium chloride or chloroquine was investigated (Figure 5C). Both of ammonium chloride and chloroquine showed cytotoxicity at a higher concentration. There were no cytotoxic effects with TAT-pK.

TAT-pK-mediated transduction efficiency in various human cancer cell lines

To utilise TAT-pK for various purposes, we investigated the efficiency of TAT-pK-mediated luciferase gene transfer using several human cancer cell lines that tend to accept gene transfer with low efficiency (Figure 6). The TAT-pK complex was successfully introduced into almost all cell lines at superior levels to DOSPA/DOPA, in the presence of chloroquine.

DISCUSSION

In order to be utilised for gene therapy, gene delivery systems require convenience and safety. Even transporting a single DNA encoding a small protein needs a vector construct. Moreover, the vector has longer DNA and 'the shell' that wraps it. Because large vectors may have adverse effects, our aim was to construct the smallest possible unit permitting efficient and safe transfection of DNA into mammalian cells. Cationic polypeptides, such as poly-arginine and poly-lysine, have been reported to bind DNA, form complexes with DNA, and introduce themselves into cells (Wagner et al., 1991; Perales et al., 1994; Felgner et al., 1997; Futaki et al., 2001; Suzuki et al., 2002). Their efficiency for practical applications to gene therapy, however, remains untested. In the present study, we fused the Tat PTD to poly-lysine in order to improve the efficiency of DNA delivery.

As the pancreatic cancer cell line PCI355 has poor DNA transfection efficiency with cationic liposomes, we investigated...
Initially, a recombinant Histidine6-tagged TAT-pK (H6-TAT-pK) construct was produced in *E. coli* strain BL21(DE3), carrying the pLysS plasmid (One Shot; Invitrogen Corp., Carlsbad, CA, USA) to control leak-through expression and to allow subsequent cell lysis by freeze thawing. This construct was purified using a HiTrap column (Amersham Pharmacia Biotech, Buckinghamshire, UK) and the AKTA prime system (Amersham Pharmacia Biotech), and a standard protocol for protein production was followed. The H6-TAT-pK/DNA complex was successfully introduced into PCI35 cells and EGFP expression was detected. The efficiency of transfection, however, was not better than with lipofection (data not shown). Subsequently, in order to elucidate the mechanism for TAT-pK-mediated gene transfer and to

---

**Figure 5** Characterisation of TAT-pK-mediated gene transfer in HEK 293 cells. (A) Enhancement of TAT-pK-mediated GFP gene expression in HEK 293 cells. Cells were seeded in 24-well tissue culture plates at a density of 2 x 10⁴ cells/well⁻¹. The cells were treated with 1 ml fresh medium containing TAT-pK/pDNA-EGFP complex (4 µg of peptide and 1 µg of DNA) in the absence (left) or the presence of 100 µM chloroquine (right). EGFP expression was detected with fluorescent microscopy as described under Materials and Methods. (B) Comparison of transfection activity of DOSPA/DOPE/DNA complex with TAT-pK/DNA complex and effects of ammonium chloride or chloroquine on TAT-pK-mediated gene transfer. HEK 293 cells (5 x 10⁴/well⁻¹) were seeded into 12-well tissue culture plates. The cells were treated with DOSPA/DOPE/DNA complex (2 µl of DOSPA/DOPA and 1 µg of DNA, open bar), according to the procedures recommended by the suppliers, or with TAT-pK/DNA complex (4 µg of peptide and 1 µg of DNA) as described under Materials and Methods. The cells with TAT-pK/DNA complex were incubated for 48 h in the absence (filled bar) or presence (grey bars) of ammonium chloride (25, 50, 100, and 200 mM) or chloroquine (25, 50, 100, and 200 µM). After incubation, cells were harvested and luciferase activity was evaluated. The luciferase activities were averaged from the results of duplicate experiments and are presented relative to the control value, indicated with the filled bar. (C) Cytotoxicity of ammonium chloride or chloroquine on HEK293 cells. Cells were seeded into 96-well plates and incubated at 37°C for 48 h in fresh medium containing a given concentration of ammonium chloride (left) or chloroquine (right) with (filled) or without (open) 20 µg/ml⁻¹ TAT-pK. After incubation, absorbance was measured by the WST-8 assay, as described under Materials and Methods. Each end point represents the mean ± s.d.

---

**Figure 6** Transduction efficiency of DOSPA/DOPE or TAT-pK with chloroquine in various human cancer cell lines. Cells (5 x 10⁴) were incubated with 1 ml medium containing pGL3-promoter DNA (1 µg) complexed with DOSPA/DOPA (open bars) or with TAT-pK in the presence of 50 µM chloroquine (grey bars) or 100 µM chloroquine (filled bars) and grown as described under Materials and Methods. After 48 h incubation, cells were harvested and luciferase activity was measured. Each end point represents the mean ± s.d. RLU, relative light units.
improve the efficiency, we synthesised TAT-pK without the His6 tag, as well as two control polypeptides GGG-pK and RGD-pK. The adenooviral RGD (Arg-Gly-Asp) motif AIRDFTFATGAS was fused to pK to compare the transfection efficiency with that of TAT-pK, because interaction of the RGD motif in the adenooviral penton base with cell membrane integrins is required to induce or trigger endocytosis (Wickham et al., 1993).

We anticipated that the cytotoxicity of TAT-pK would be stronger than that of RGD-pK, but interestingly, this was not the case. Actually, as cells would not be exposed to high concentrations for a prolonged time, toxicity is not likely to be a serious concern. No toxicity was observed, even at the highest doses examined for DNA transduction.

Most nonviral vehicles deliver their genes passively, relying on uptake into vesicular compartments by endocytosis, thus we examined the effects of ammonium chloride or chloroquine on transduction. Ammonium chloride is a weak acidotropic base. Chloroquine elevates the pH of vesicular compartments (Cotten et al., 1990), and either stimulates or inhibits the efficiency of endocytosis-mediated gene transfer, depending on the delivery vehicle. We found that TAT-pK-mediated gene transfer is affected by either of these agents, suggesting that transduction relies on the endocytic pathway. These results are similar to past reports showing that gene transfer via receptor-mediated endocytosis (Cotten et al., 1990) or mediated by DEAE-dextran (Luthman and Magnusson, 1983) is markedly enhanced with endosomotropic agents, such as chloroquine. However, our data contradict other studies on TAT-peptide-mediated protein transduction (Mann and Frankel, 1991; Derossi et al., 1996; Vives et al., 1997; Elliott and O’Hare, 1997) and TAT-phage-mediated gene transfer (Eguchi et al., 2001) that do not depend on endosomotropic reagents. The mechanism of action of Tat-(48–60) peptide and the full-length Tat protein may not be the same (Liu et al., 2000). Rather, TAT-pK-mediated gene transfer seems to share features of both systems, operating by both an energy-dependent endocytic pathway and an independent pathway. These dual mechanisms may account for the high efficiency of DNA transduction. In short, the Tat PTD anchors the TAT-pK/DNA complex to the cell surface within a few minutes by membrane destabilisation, and then the complex crosses the cell membrane by endocytosis.

The conditions suitable for gene transfer differed for each cell line. As PBS or RPMI medium reduce the efficiency of TAT-pK-mediated gene transfer (data not shown), we used sterillised water for diluting pKs and only used DMEM in transfection. Transduction efficiency of TAT-pK was easily influenced by several factors, such as pH or temperature of medium, preincubation period, and quantity of DNA (data not shown). Moreover, the fold absorptions were decreased at high concentrations of chloroquine because of its cytotoxicity.

Several Tat PTD fused proteins have been reported as potential therapeutic strategies for cancer (Mann and Frankel, 1991; Derossi et al., 1996; Elliott and O’Hare, 1997; Vives et al., 1997), but the quantity of protein transduced into tissues would be lower than that from administration of vector DNA. Moreover, selectivity and transduction efficiency are very important factors in order to apply gene therapy for cancer patients. Although we have not achieved targeting transduction for cancer cells by using TAT-pK, it may be easily modified to target cancerous, but not normal cells, since TAT-pK is much smaller than the capsid proteins of viral vectors. Also immunogenicity by TAT-pK should be investigated, but we do not think it higher than that of viral vectors because of its size. We have started in vivo experiments to assess these factors and to improve them.

In conclusion, although there is need for further improvement, TAT-pK is a candidate for a new DNA transfection system. Many problems still exist in clinical trials using viral vector-mediated gene therapy, therefore the development of artificial viral vector systems is urgently needed. TAT-pK is likely a minimal unit to efficiently package therapeutic genes and transduce them into mammalian cells.

ACKNOWLEDGEMENTS

This work was supported in part by a Grant-in Aid for Scientific Research from the Japan Society for the Promotion of Science, and from the Uehara Foundation.

REFERENCES

Colin M, Maurice M, Trunghan G, Kornprobst M, Harbottle RP, Knight A, Cooper RG, Miller AD, Capeau J, Coutelle C, Brahimi-Horn MC (2000) Cell delivery, intracellular trafficking and expression of an integrin-mediated gene transfer vector in tracheal epithelial cells. Gene Therapy 7: 139 – 152

Cotten M, langle-Rouault F, Kirlappos H, Wagner E, Mechtl K, Zenke M, Beug H, Birnstiel ML (1990) Transferin-polycond médiated introduction of DNA into human leukemic cells: stimulation by agents that affect the survival of transfected DNA or modulate transferrin receptor levels. Proc Natl Acad Sci USA 87: 4033 – 4037

Derossi D, Calvet S, Trembleau A, Brunissen A, Chassaing G, Prochiantz A (1996) Cell internalization of the third helix of the Antennapedia homeodomain is receptor-independent. J Biol Chem 271: 18188 – 18193

Derossi D, Joliot AH, Chassaing G, Prochiantz A (1994) The third helix of the Antennapedia homeodomain translocates through biological membranes. J Biol Chem 269: 10444 – 10450

Dostman WRG, Tayor MS, Nickl CR, Brayden JE, Frank R, Tegege WJ (2000) Antisense inhibition of G-polyplex expression using peptide-diolonucleotide conjugates. Proc Natl Acad Sci USA 97: 14772 – 14777

Eguchi A, Akuta T, Okuyama H, Senda T, Yokoi H, Inokuchi H, Fujita S, Hayakawa T, Takeda K, Hasegawa M, Nakanishi M (2001) Protein transduction domain of HIV-1 Tat protein promotes efficient delivery of DNA into mammalian cells. J Biol Chem 276: 26204 – 26210

Elliott G, O’Hare P (1997) Intercellular trafficking and protein delivery by a herpesvirus structural protein. Cell 88: 223 – 233

Felgner PL, Barenholz Y, Behr JP, Cheng SH, Cullis P, Huang L, Jesse JA, Seymour L, Szkoka F, Thierry AR, Wagner E, Wu G (1997) Nomenclature for synthetic gene delivery systems. Hum Gene Ther 8: 511 – 512

Fishre KJ, Choi H, Burda J, Chen S-J, Wilson JM (1996) Recombinant adenovirus deleted of all viral genes for gene therapy of cystic fibrosis. Virology 217: 11 – 22

Futaki S, Suzuki T, Ohashi W, Yagami T, Tanaka S, Ueda K, Sugiuira Y (2001) Arginine-rich peptides. An abundant source of membrane-permeable peptides having potential as carriers for intracellular protein delivery. J Biol Chem 276: 5836 – 5840

Haecker SE, Stedman HH, Balice-Gordon RJ, Smith DB, Grealish JP, Mitchell MA, Wells A, Sweeney HL, Wilson JM (1996) In vivo expression of full-length human dystrophin from adenoviral vectors deleted of all viral genes. Hum Gene Ther 7: 1907 – 1914

Kochanek S, Clemens PR, Mitani K, Chen H-H, Chan S, Caskey CT (1996) A new adenoviral vector: replacement of all viral coding sequences with 28 kb of DNA independently expressing both full-length dystrophin and beta-galactosidase. Proc Natl Acad Sci USA 93: 5731 – 5736

Kumar-Singh R, Chamberlain JS (1996) Encapsidated adenovirus mini-chromosomes allow delivery and expression of a 14 kb dystrophin cDNA to muscle cells. Hum Mol Genet 5: 913 – 921

Liu Y, Jones M, Hingtgen CM, Bu G, Laribee N, Tanzi RE, Moir RD, Nath A, He JJ (2000) Uptake of HIV-1 tat protein mediated by low-density lipoprotein receptor-related protein disrupts the neuronal metabolic balance of the receptor ligands. Nat Med 6: 1380 – 1387

© 2004 Cancer Research UK

British Journal of Cancer (2004) 90(6), 1252 – 1258
Luthman H, Magnusson G (1983) High efficiency polyoma DNA transfection of chloroquine treated cells. *Nucleic Acids Res* 11: 1295 – 1308

Mann DA, Frankel AD (1991) Endocytosis and targeting of exogenous HIV-1 Tat protein. *EMBO J* 10: 1733 – 1739

Morral N, O’Neal W, Rice K, Leland M, Kaplan J, Piedra PA, Zhou H, Parks RJ, Velji R, Aguilar-Cordova E, Wadsworth S, Graham FL, Kochanek S, Carey KD, Beaudet AL (1999) Administration of helper-dependent adenoviral vectors and sequential delivery of different vector serotype for long-term liver-directed gene transfer in baboons. *Proc Natl Acad Sci USA* 96: 12816 – 12821

Nagahara H, Vocero-Akbani AM, Snyder EL, Ho A, Latham DG, Lissy NA, Becker-Hapak M, Ezhevsky SA, Dowdy SF (1998) Transduction of full-length TAT fusion proteins into mammalian cells: TAT-p27Kip1 induces cell migration. *Nat Med* 4: 1449 – 1452

Perales JC, Ferkol T, Molas M, Hanson RW (1994) An evaluation of receptor-mediated gene transfer using synthetic DNA–ligand complexes. *Eur J Biochem* 226: 255 – 266

Schwartz SR, Ho A, Vocero-Akbani A, Dowdy SF (1999) *In vivo* protein transduction: delivery of a biologically active protein into the mouse. *Science* 285: 1569 – 1572

Suzuki T, Futaki S, Niwa M, Tanaka S, Ueda K, Sugiyama Y (2002) Possible existence of common internalization mechanisms among arginine-rich peptides. *J Biol Chem* 277: 2437 – 2443

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

Wagner E, Cotton M, Foisner R, Birnstiel ML (1991) Transferrin–polycation–DNA complexes: the effect of polycations on the structure of the complex and DNA delivery to cells. *Proc Natl Acad Sci USA* 88: 4255 – 4259

Wickham TJ, Mathias P, Cheresh DA, Nemerow GR (1993) Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell* 73: 309 – 319