Protein kinase Cα-responsive polymeric carrier: its application for gene delivery into human cancers

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(Received March 9, 2009/Revised April 9, 2009/Accepted April 19, 2009/Online publication May 13, 2009)

For cancer-targeting gene delivery, we applied a protein kinase C (PKC)α-responsive polymeric carrier to human cancers (U-87 MG [human glioblastoma-astrocytoma, epithelial-like cell line] and A549 [human lung adenocarcinoma epithelial cell line]). Two polymers, one a PKCα-responsive polymer (PPC[S]) containing the phosphorylation site serine, and the other a negative control polymer (PPC[A]), in which the serine was substituted with alanine, were synthesized. No cytotoxicity of the polymer was identified. When the complexes were transfected into cancer cells or tissues in which PKCα was hyper-activated, the luciferase expression from the PPC(S)/plasmid (pDNA) complex was higher than that from the PPC(A)/pDNA complex. These results show that the phosphorylation of complex by PKCα in cancer cells leads to high gene expression and that our system can be used as a human cancer cell–targeting gene delivery system. (*Cancer Sci* 2009; 100: 1532–1536)

In recent years there has been an increasing interest in gene therapy as a new medical approach for the treatment of diseases that are difficult to cure, such as cancers. For efficient gene therapy, therapeutic genes need to be delivered efficiently into cells. Several viral or non-viral carriers for gene transfer have been developed for either *in vivo* or *ex vivo*/*in vitro* use. To ensure the safety of gene therapy, genes have to be transferred to the targeted disease cells without any adverse effects on normal cells. Several systems have been developed to resolve the issue of target-selective gene delivery. They generally involve the use of various ligands that selectively bind to a cell-surface molecule of the target cell.[1–4]

Previously, we have proposed a novel drug or gene delivery system that responds to intracellular signals that are specifically and abnormally activated in the target diseased cells. This system uses a new class of polymer-based gene regulator consisting of a neutral main chain and a disease-targeting peptide in the side chain. The polymer forms a complex with DNA through an electrostatic interaction that prevents gene expression from the DNA. However, when polymer/DNA complexes are transferred into target disease cells, cleavage or phosphorylation of the side-chain peptide by intracellular target enzymes leads to dissociation of the polymer/DNA complex enabling the DNA to bind transcription factors and polymerases.[5–7]

Among intracellular signals, protein kinase C (PKC)α is one of 11 PKC isozymes and is broadly expressed in tissues. Because PKCα is hyper-activated in many cancer cell lines and tissues, it has attracted much attention as a potential target of anticancer therapies.[8–10]

Recently, we found a PKCα-specific peptide (Alphatomega) from a peptide library designed using the Scansite database. This peptide was highly phosphorylated by cancer cell or tissue lysates, whereas only a low level of phosphorylation was produced by normal tissue lysates.[11] Alphatomega was previously used for the development of a gene delivery system responding to PKCα. The complex of polymer, containing Alphatomega, with plasmid DNA (pDNA) showed gene expression in B16 melanoma tissues, but no expression in normal subcutaneous tissues.[12] From these results, we anticipate that the PKCα-responsive gene delivery system can be applied to several cancers in which PKCα is hyper-activated.

In the present study, we applied the gene delivery system to U-87 MG (human glioblastoma-astrocytoma, epithelial-like cell line) and A549 (human lung adenocarcinoma epithelial cell line). After transfection of the system into the cells or/and tissues, gene expression in cancer cells or/and tissues was identified. Our study suggests that the PKCα-responsive gene delivery system can be used for general cancer cell–targeting gene therapy.

Materials and Methods

Synthesis of the peptide substrate. Two peptide substrates, FKKQGSFAKKK and FKKQGAFAKKK, each with a methacryloyl group at the amino-terminus, were synthesized using an automatic peptide synthesizer, according to standard 9-fluorenylmethyloxycarbonyl (Fmoc)-chemistry procedures and were purified as described previously.[6,7,12]

Phosphorylation of peptide substrate by cell or tissue lysate. Cells (U-87 MG [human glioblastoma-astrocytoma, epithelial-like cell line] and A549 [human lung adenocarcinoma epithelial cell line]) were obtained from the American Type Culture Collection (Manassas, VA, USA). The cells (1×10^7 cells) were homogenized in 0.2 mL of buffer (20 mM Tris-HCl [pH 7.5] containing 250 mM sucrose and Complete protease inhibitor cocktail [EDTA-Free] [Roche, Tokyo, Japan]). After sonication for 10 s, the samples were centrifuged at 5000*g* at 4°C for 15 min, and the resulting supernatant was used for the phosphorylation of the peptide. The total protein concentration of the lysate was assessed using the Bio-Rad Protein Assay Dye reagent (Bio-Rad Laboratories, CA, USA) with bovine serum albumin as the standard. Phosphorylation was carried out in 30 μL buffer (20 mM Tris-HCl [pH 7.5] containing 10 mM $MgCl_2$ and 100 μM ATP) containing 30 μM peptide and lysate at a protein concentration of 200 μg/mL. After phosphorylation for 60 min at 37°C, the sample was analyzed by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) to determine the phosphorylation ratio, as described previously.[12,13]

Synthesis of peptide-pendant polymer. Polymers were synthesized as described previously.[6,7,9] Briefly, acrylamide (41.2 mg, 581 μmol) and *N*-methacryloylpeptide (8.0 mg, 5.9 μmol), from which the methacryloyl group attached to the amino terminus of

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doi: 10.1111/j.1349-7006.2009.01198.x

the peptide, were dissolved in water, degassed with nitrogen for 5 min, and then polymerized using ammonium persulfate (3.9 mg, 17 mmol) and *N*,*N*,*N*′,*N*′,-tetramethylethylenediamine (5.1 μL, 34 mmol) as a redox couple at room temperature for 90 min. The synthesized sample was dialyzed against water overnight in a semipermeable membrane bag with a molecular weight cut-off of 50 000. The dialyzed sample was lyophilized and a final sample was obtained as a white powder, which was used as the polymer for polymer-peptide conjugate (PPC). The concentration of the peptide was estimated by elemental analysis.

Measurements of ζ-potential and diameter of complexes. Complexes of polymer and pDNA (pCMV-luciferase) were prepared simply by incubation of both the pDNA and the PPC in buffer solution for 15 min. The final concentration of pDNA was adjusted to 2.5 μg/mL using 10 mM HEPES buffer (pH 7.3). The ζ-potential and diameter of the complexes were determined using a Zetasizer (Malvern Instruments, Worcestershire, UK) with the He/Ne laser at a detection angle of 173° and a temperature of 25°C.

Monitoring complex phosphorylation using a coupled enzyme assay. The determination of complex pendant peptide phosphorylation was carried out using a coupled enzyme assay.[12] The complex dispersions were diluted to obtain a measurement solution (100 μL): 32 μM peptide, 1 mM phosphoenolepyruvate, 0.3 mM nicotinamide adenine dinucleotide (NADH), 10 U/μL lactate dehydrogenase (LDH), pyruvate kinase 4 U/μL, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, 100 μM ATP, 2.0 mg/mL diacylglycerol (DAG), 2.5 μg/mL phosphatidylserine (PS), 1 ng/mL PKCα, and 20 mM HEPES (pH 7.3). The reaction was initiated by adding ATP and was performed at 25°C. The consumption of NADH was detected with a UV/Vis spectrophotometer (UV-2550; Shimadzu, Tokyo, Japan) equipped with an SPR-8 temperature controller (Shimadzu) at 340 nm.

Cytotoxicity of the polymer. Cell viability was determined using a cell counting kit containing 4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate sodium salt (WST-8) (Dojindo Laboratories, Kumamoto, Japan). U-87 MG cells were incubated in the absence or presence of the polymer (10–30 μg/mL) for 24 h in a 96-well plate. The conditioned medium in each well was replaced with 100 μL of fresh medium containing WST-8, and the cells were incubated for a further 2 h, before measurement of the absorbance at 440 nm. The cell viability (%) was calculated by normalizing the absorbance of treated cells to that of the untreated control cells.

Transfection of complexes into cells. Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Gibco Invitrogen, Grand Island, NY, USA) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.25 μg/mL amphotericin B (all Gibco). The cells were incubated in a humidified atmosphere containing 5% CO_2 and 95% air at 37°C for 24 h. After 24 h the medium was changed to 500 μL of Opti-MEM (Gibco) and the complexes at cation/anion (C/A) ratios of 1.0 or 2.0 (pDNA, 5 μg/mL) were added to wells. The cells were incubated at 37°C for 6 h. After 6 h, the medium was changed to DMEM, and cells were further incubated for 18 h. The cultured cells were then scraped and lyzed in 100 μL of lysis buffer (20 mM Tris-HCl, pH 7.4, 0.05% Triton-X 100, and 2 mM EDTA). After centrifuging the sample at 12 000*g* at 4°C for 10 min, a 10 μL aliquot of the supernatant was used for measuring chemiluminescence in a MiniLumat LB 9506 (EG & G Berthold, Wildbad, Germany) directly after adding 40 μL of the luciferin substrate. The results are presented as relative luminescence units (RLU)/mg total protein.

Animal experiments. Animal studies were performed in accordance with the Guidelines for Animal Experiments of Kyushu University. Male 5-week-old BALB/c nude mice were used in this study. Mice were inoculated with a dorsal, subcutaneous injection of 1×10^7 cells in 100 μL of Matrigel (BD Biosciences, Bedford, MA, USA) per animal. Tumors were allowed to grow to a mean diameter of approximately 8 mm. Introduction of complexes (100 μL) (pDNA, 100 μg/mL) into cancers or normal subcutaneous tissue was performed by a direct injection. After 24 h, mice were anesthetized and injected intraperitoneally with 0.2 mL of 15 mg/mL D-luciferin (potassium salt) (Promega, Madison, WI, USA) in Ringer's solution. Images were obtained using a cooled IVIS CCD camera (Xenogen, Alameda, CA, USA) and analyzed with Living Image software.

Results

Phosphorylation of substrate peptide by cell lysates. We identified whether the substrate peptide can be phosphorylated by A549 or U-87 MG cell lysates. Twenty-four h after the phosphorylation reaction, the sample was analyzed by MALDI-TOF MS. Phosphorylation of the substrate by each lysate was identified by an increase in the m/z-value of + 80 Da (Fig. S1).[13] The phosphorylation ratio for A549 and U-87 MG cell lysates was 74.4 ± 9.3 and 63.5 ± 8.5%, respectively.

Synthesis of polymer. Two polymers, a PKCα-responsive polymer (PPC[S]) containing the phosphorylation site serine and a negative control polymer (PPC[A]), in which the serine was substituted with alanine, were synthesized. The polymer consisted of polyacrylamide as the main chain and the peptide substrate as side chains. The content of the peptide substrate for PPC(S) and PPC(A) was estimated using elemental analysis to be 3.2 and 3.0 mol%, respectively. Since the peptide substrate has five cationic amino acids (lysine), it can bind to anionic DNA sequences (Fig. S2).

Diameter and ζ-potential of polymer/pDNA complexes. The diameter and ζ-potential of polymer/pDNA complexes, which were prepared simply by incubation of the pDNA and the polymer in water for 15 min, were detected using a Zetasizer. The diameter of the polymer/pDNA complex was below 170 nm at C/A ratios of 1.0 and 2.0. The ζ-potential of complex was negative at a C/A ratio of 1.0, but changed to positive (>10 mV) at a C/A ratio of 2.0 (Table S1).

Phosphorylation and dissociation of the polymer/pDNA complex. Our strategy requires the polymer to make a stable complex with DNA, however, the polymer/pDNA complex must be able to be phosphorylated in the presence of PKCα. Phosphorylation of the complex was identified using a coupled-enzyme assay, which detects the decrease of absorbance (340 nm) by NADH reduction after phosphoryation. The time course of the decrease of absorbance for Alphatomega and for the PPC(S)/pDNA complex were similar in the presence of PKCα, but no change of absorbance was observed from the PPC(A)/pDNA complex. These results mean that the peptide grafted in PPC(S) can be phosphorylated to a similar level as Alphatomega itself (Fig. 1a).

Dissociation of polymer/pDNA complex after phosphorylation was examined using scattering intensity. After adding PKCα, a decrease of scattering intensity was found from the PPC(S)/pDNA complex, indicating that the complex is weakened by the net cationic charge decrease. However, we failed to identify a change of scattering intensity in the PPC(A)/pDNA complex (Fig. 1b).

Cytotoxicity of the polymer. Staining with WST-8 was used to identify the cytotoxicity of the developed polymer. Twenty-four h after incubation with polymers, at several concentrations (10–30 μg/mL), the percentage of viable cells was calculated. No cytotoxicity of the polymer was found (Fig. 2).

Transfection of the polymer/pDNA complex into cells. To examine whether the PPC/pDNA complex can be phosphorylated by PKCα in cells, thereby activating gene expression, the complex was transfected into U-87 MG cells at C/A ratios of 1.0 and 2.0. When the PPC(S)/luciferase-gene complex was transfected, significant luciferase expression was observed ($P < 0.01$), compared

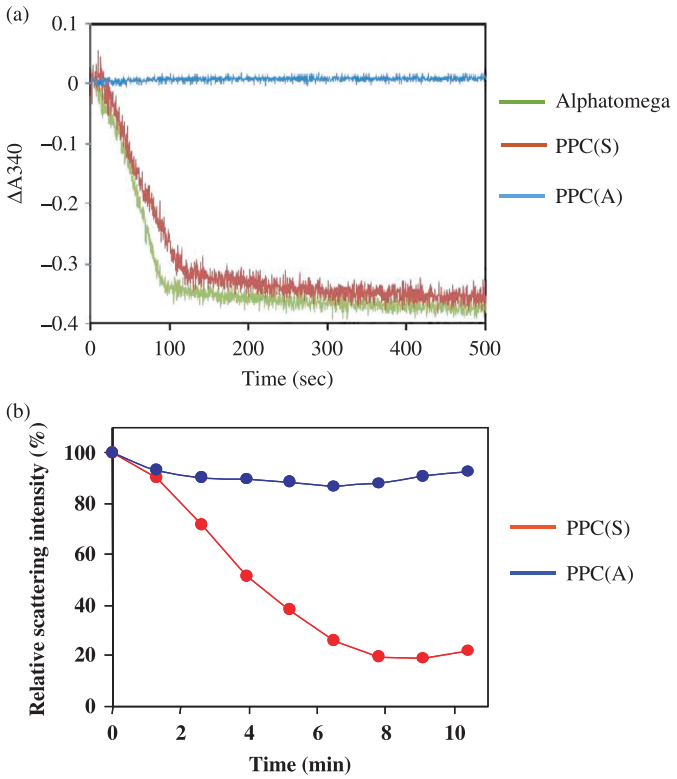

Fig. 1. (a) Monitoring of phosphorylation of grafted peptide in the form of a complex using a coupled-enzyme assay. The enzymatic reaction was started at 0 min by adding protein kinase C (PKC)α to the reaction solution containing complexes (cation/anion [C/A] = 2.0) or Alphatomega only. (b) Change of relative scattering intensity of complex dispersions after starting enzymatic reaction of PKCα. The charge ratio (C/A) of complexes was 2.0. PPC(S), PKCα-responsive polymer containing the phosphorylation site serine; PPC(A), negative control polymer in which the serine was substituted with alanine.

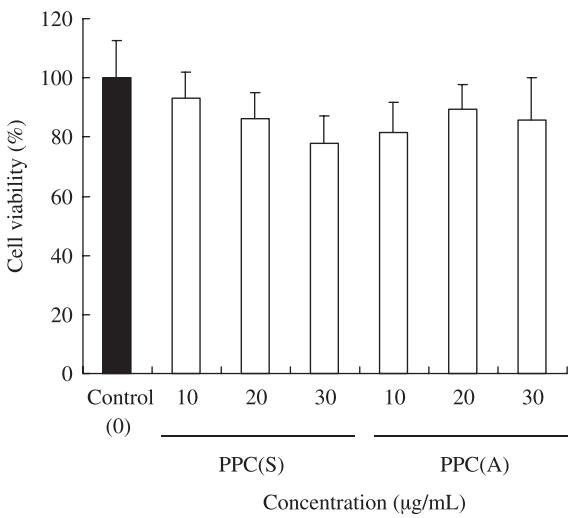

Fig. 2. Toxicity of the polymer toward U-87 MG (human glioblastoma-astrocytoma, epithelial-like cell line) cells. Cells were incubated in the presence or absence of the polymer (0–30 μg/mL) for 24 h. The cell viability (%) was calculated by normalizing the absorbance of treated cells to that of the untreated control cells. PPC(S), protein kinase C (PKC)α-responsive polymer containing the phosphorylation site serine; PPC(A), negative control polymer in which the serine was substituted with alanine.

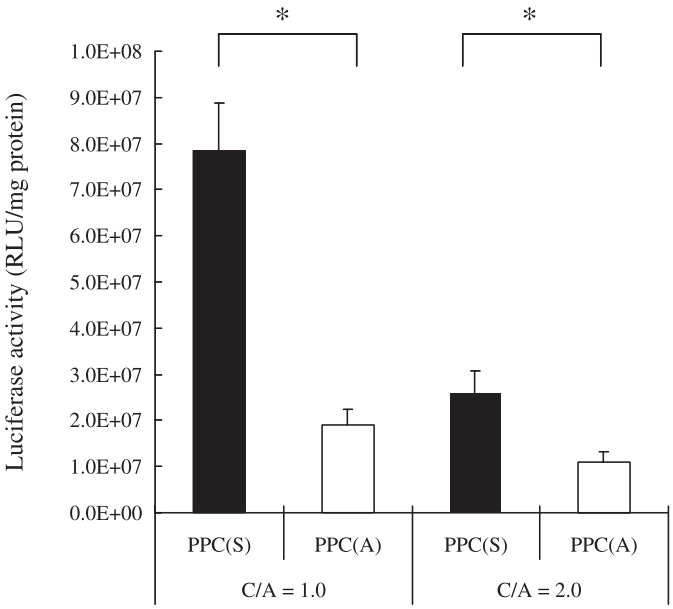

Fig. 3. Luciferase expression in U-87 MG (human glioblastoma-astrocytoma, epithelial-like cell line) cells 24 h after transfection of complexes (cation/anion [C/A] = 1.0 and 2.0). Error bars represent standard deviation of three experiments. *, $P < 0.01$. PPC(S), protein kinase C (PKC)α-responsive polymer containing the phosphorylation site serine; PPC(A), negative control polymer in which the serine was substituted with alanine; RLU, relative luminescence units.

with that from the negative control PPC(A)/pDNA complex. This indicates that the higher gene expression seen from the PPC(S)/pDNA complex compared to the PPC(A)/pDNA complex is caused by the phosphorylation of complex by PKCα in cells. In the case of a C/A ratio of 1.0, however, high luciferase expression was identified from cells transfected with the PPC(S)/pDNA complex, but comparably high luciferase expression was also observed from the PPC(A)/pDNA cells (Fig. 3). These results show that the control polymer, at a C/A ratio of 1.0, can make weak complexes with pDNA, resulting in undesired high luciferase expression.

Transfection of polymer/pDNA complex into tumor tissues. To evaluate the possible application of our system to gene therapy, transfection of the polymer/pDNA complex into tumor tissues was examined via intratumor delivery of a luciferase-encoding pDNA/polymer complex. From the results of transfection in cells, we used a PPC/pDNA complex *in vivo* with a C/A ratio of 2.0. Luciferase expression was identified in the PPC(S)/pDNA complex-transfected cancers. The luminescence intensity from the PPC(S)/pDNA complex was over 4-fold higher than that from the PPC(A)/pDNA complex. On the other hand, no expression was observed from both PPC(S)/pDNA and PPC(A)/pDNA complexes injected into normal subcutaneous tissues (Fig. 4). A similar result was also observed in A549 cancers, into which the PPC/pDNA complexes were transfected (Fig. S3). These results show that our polymer could successfully regulate transgene expression, specifically in cancer cells, and that phosphorylation of the polymer/pDNA complex by PKCα leads to release of the pDNA from the complex *in vivo*.

Discussion

The efficient targeting of therapies to cancer cells is an important issue for effective treatment and to avoid undesired side effects. Differentiation, proliferation, and apoptosis of cancer cells are controlled by several intracellular protein kinase signals, such as PKCα, c-Src, and Akt (also known as protein kinase B).

doi: 10.1111/j.1349-7006.2009.01198.x

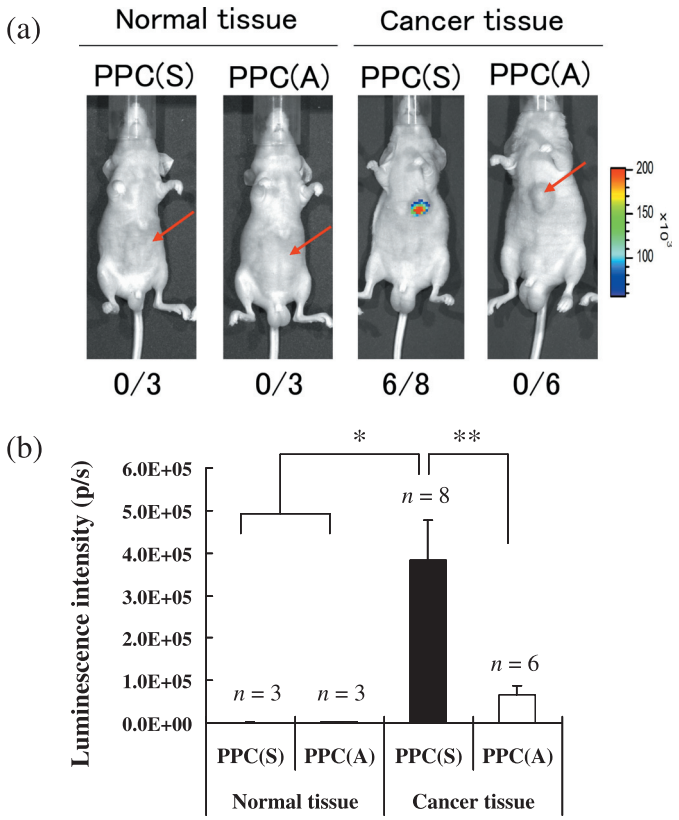

Fig. 4. (a) Images of luciferase activity in normal subcutaneous and xenografted U-87 MG (human glioblastoma-astrocytoma, epithelial-like cell line) cancer 24 h after direct injection of polymer/pDNA complexes (cation/anion [C/A] = 2.0). Numbers of luciferase expressing mice per total mice are stated below. Arrows indicate the injection site of complexes. (b) Luminescence intensity of the injection site. p/s, photons/s; *, $P < 0.01$; **, $P < 0.05$. PPC(S), protein kinase C (PKC)α-responsive polymer containing the phosphorylation site serine; PPC(A), negative control polymer in which the serine was substituted with alanine.

The signals relating to the survival of cancer cells are expected to be important therapeutic targets for cancers. One therapeutic strategy is to design specific inhibitors to each survival signal. However, such inhibitors are generally ATP-competitive inhibitors of protein kinases and the close homology of ATP pockets is a serious obstacle for developing kinase-specific inhibitors. For example, ATP-competitive inhibitors (e.g. staurosporine, tamoxifen, and UCN-01) for targeting PKCα have been developed, but these inhibitors showed no specificity to PKCα.[8–10]

Gene therapy is regarded as one of the most promising approaches to treat cancers. Two main methods to target cancer cells using non-viral vectors are passive targeting and active targeting. Passive targeting is based on enhanced permeability and retention (EPR) effects, which can accumulate macromolecules in cancers, especially solid cancers.[1–4] On the other hand, active targeting uses ligands capable of recognizing cancer-specific molecular markers on the cell surface, such as antibodies and growth factors or the use of cancer-homing peptides selected from phage display libraries. The incorporation of these cancer cell-binding ligands into polycationic carriers, which are broadly used in the field of non-viral gene delivery because of their ability to bind with pDNA, can increase gene transfer efficiency and therapeutic value.[14,15] Moreover, stimuli-responsive gene carriers, such as thermo-responsive gene carrier,[16] photo responsive gene carrier,[17] and pH-responsive carrier,[18] have been reported to increase targeting activity.

For cancer-targeting gene delivery, we previously developed a novel system responding to the PKCα intracellular signal. This system is designed to act on cancer cells in which PKCα is hyper-activated, but not on normal cells that contain very little PKCα. The system can therefore distinguish cancer cells from normal ones. Our earlier study used B16 melanoma-bearing mice to demonstrate the cancer-targeting gene delivery of our system.[12] In the present study, we applied this system to human cancers (A549 and U-87 MG), in which over-expression of PKCα has been reported in several studies. Higher gene expression was identified in cancer cells and tissues transfected by the complex of a PKCα-responsive polymer/pDNA complex compared with that of a negative control polymer/pDNA complex. These results show that the phosphorylation of complex by PKCα in cells leads to high pDNA gene expression.

Suicide genes or other therapeutic genes suppressing cell proliferation or leading to apoptosis must express specifically in a target cancer cells to avoid any side effects. In spite of research efforts to secure the cell specificity, practical strategies satisfying cellular specificity have not yet been established because most strategies don't prohibit delivery to other normal cells.

On the other hand, our cancer-targeted gene regulation system responding to PKCα can distinguish between normal and tumor cells. Moreover, in the present study, we found that the PKCα-responsive gene delivery system can be applied to several cancers in which PKCα is hyper-activated. Thus, we anticipate our concept to be a new strategy for solving the problem of side effects in gene therapy for the treatment of cancers. In addition, our intracellular signal-responsive gene regulation can be designed for other protein kinases simply by changing the peptide moiety to another substrate, so that the system will be applicable to wide range of diseases.

Acknowledgment

This work was supported, in part, by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

References

1 Ferber D. Gene therapy: safer and virus-free? *Science* 2001; **294**: 1638–42.
2 Kaneda Y, Tabata Y. Non-viral vectors for cancer therapy. *Cancer Sci* 2006; **97**: 348–54.
3 Eliyahu H, Barenholz Y, Domb AJ. Polymers for DNA delivery. *Moleculs* 2005; **10**: 34–64.
4 Nishikawa M, Takakura Y, Hashida M. Pharmacokinetic considerations regarding non-viral cancer gene therapy. *Cancer Sci* 2008; **99**: 856–62.
5 Katayama Y, Fujii K, Ito E *et al*. Intracellular signal-responsive artificial gene regulation for novel gene delivery. *Biomacromolecules* 2002; **3**: 905–9.
6 Kawamura K, Oishi J, Kang JH *et al*. Intracellular signal-responsive gene carrier for cell-specific gene expression. *Biomacromolecules* 2005; **6**: 908–13.
7 Oishi J, Kawamura K, Kang JH *et al*. An intracellular kinase signal-responsible gene-carrier for cell specific gene therapy. *J Control Release* 2006; **110**: 431–6.
8 Hofmann J. Protein kinase C isozymes as potential targets for anticancer therapy. *Curr Cancer Drug Targets* 2004; **4**: 125–46.
9 Goekjian PG, Jirousek MR. Protein kinase C inhibitors as novel anticancer drugs. *Expert Opin Invest Drugs* 2001; **10**: 2117–40.
10 O'Brian CA, Chu F, Bornmann WG, Maxwell DS. Protein kinase Cα and ε small-molecule targeted therapeutics: a new roadmap to two holy grails in drug discovery? *Expert Rev Anticancer Ther* 2006; **6**: 175–86.
11 Kang JH, Asai D, Yamada S *et al*. A short peptide is a protein kinase C (PKC)α-specific substrate. *Proteomics* 2008; **8**: 2006–11.
12 Kang JH, Asai D, Kim JH *et al*. Design of polymer carriers for cancer-specific gene targeting: Utilization of abnormal protein kinase Cα activation in cancer cells. *J Am Chem Soc* 2008; **130**: 14906–7.

13 Kang JH, Katayama Y, Han A *et al*. Mass-tag technology responding to intracellular signals as a novel assay system for the diagnosis of tumor. *J Am Soc Mass Spectrom* 2007; **18**: 106–12.
14 Kumar TR, Soppimath K, Nachaegari SK. Novel delivery technologies for protein and peptide therapeutics. *Curr Pharm Biotechnol* 2006; **7**: 261–76.
15 Khandare JJ, Minko T. Antibodies and peptides in cancer therapy. *Crit Rev Ther Drug Crrier Syst* 2006; **23**: 401–35.
16 Kurisawa M, Yokoyama M, Okano T. Gene expression control by temperature with thermo-responsive polymeric gene carriers. *J Control Release* 2000; **69**: 127–37.
17 Nagasaki T, Taniguchi A, Tamagaki S. Photoenhancement of transfection efficiency using novel cationic lipids having a photocleavable spacer. *Bioconjug Chem* 2003; **14**: 513–6.
18 Xiong MP, Bae Y, Fukushima S *et al*. Ph-responsive multi-pegylated dual cationic nanoparticles enable charge modulations for safe gene delivery. *Chem Med Chem* 2007; **2**: 1321–7.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Diameter and ζ-potential of complexes

Fig. S1 Typical matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) spectra obtained from the phosphorylation reaction of peptide substrate with U-87 MG (human glioblastoma-astrocytoma, epithelial-like cell line) cell lysates. An increase of 80 Da identified the phosphorylated peptide.

Fig. S2 Synthetic scheme and chemical structure of the polymer. The polymer was synthesized by polymerization of acrylamide and *N*-methacryloylpeptide using ammonium persulfate (APS) and *N,N,N′,N′*,-tetramethylenediamine (TEMED).

Fig. S3 Images of luciferase activity in normal subcutaneous and xenografted A549 (human lung adenocarcinoma epithelial cell line) cancer 24 h after direct injection of polymer/pDNA complexes (cation/anion [C/A] = 2.0). Numbers of luciferase expressing mice per total mice are described below. Arrows indicate the injection site of complexes.

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doi: 10.1111/j.1349-7006.2009.01198.x