Antitumor Activity of Small Interfering RNA/Cationic Liposome Complex in Mouse Models of Cancer

**Junichi Yano,[1] Kazuko Hirabayashi,[1]
Shin-ichiro Nakagawa,[1] Tohru Yamaguchi,[1]
Masaki Nogawa,[1] Isao Kashimori,[1]
Haruna Naito,[1] Hidetoshi Kitagawa,[1]
Kouichi Ishiyama,[1] Tadaaki Ohgi,[1] and
Tatsuro Irimura[2]**

[1]Discovery Research Laboratories, Nippon Shinyaku Co, Ltd., Kyoto, Japan; and [2]Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

ABSTRACT

Purpose: The RNA interference effect is an alternative to antisense DNA as an experimental method of down-regulating a specific target protein. Although the RNA interference effect, which is mediated by small interfering RNA (siRNA) or micro-RNA, has potential application to human therapy, the hydrodynamic method usually used for rapid administration of oligonucleotides is unsuitable for use in humans. In this study, we have investigated the antitumor activity of a synthetic siRNA, B717, which is sequence specific for the human *bcl-2* oncogene, complexed with a novel cationic liposome, LIC-101.

Experimental Design: In a mouse model of liver metastasis, we administered B717/LIC-101 by bolus intravenous injection, adjusting the rate and volume of administration to what is feasible in human therapy. In a mouse model bearing prostate cancer in which the cells were inoculated under the skin, B717/LIC-101 was administered subcutaneously around the tumor.

Results: The B717/LIC-101 complex inhibited the expression of bcl-2 protein and the growth of tumor cell lines *in vitro* in a sequence-specific manner in the concentration range of 3 to 100 nmol/L. Furthermore, the complex had a strong antitumor activity when administered intravenously in the mouse model of liver metastasis. B717 (siRNA) was shown to be delivered to tumor cells in the mouse liver, but only when complexed with LIC-101. The complex also inhibited tumor cell growth in the mouse model bearing prostate cancer.

©2004 American Association for Cancer Research.

Conclusions: By combining siRNA with our cationic liposome, we overcame the difficulty of administering siRNA to animals in ways that can be applied in human therapy. Although our siRNA/liposome complex is not yet in clinical trials, it is expected to provide a novel siRNA therapy for cancer patients.

INTRODUCTION

To knock down the function of a target protein, transfection with antisense DNA has often been used. The type of antisense DNA most commonly used is phosphorothioate-type DNA. In cell culture experiments, antisense DNA is mixed with a cationic liposome for transfection into cells, whereas in *in vivo* experiments, it is administered by intravenous injection or continuous subcutaneous infusion without any vehicle. Phosphorothioate-type antisense DNA binds to serum proteins, is delivered to organs, and is taken up by various types of receptor or by endocytosis (1–4). Antisense DNA binds to the target mRNA and forms a DNA/RNA duplex, after which either the mRNA of the duplex is cleaved by RNase H or the duplex prevents translation or RNA splicing (5–9). A New Drug Application has been submitted to the United States Food and Drug Administration for G3139, a phosphorothioate-type DNA designed to specifically bind to the first six codons of the human bcl-2 mRNA sequence and intended to treat several types of cancer (10).

More recently, efforts have focused on the RNA interference effect as a tool to knock down the expression of a target protein. Small interfering RNAs (siRNAs), which are small double-stranded RNA (dsRNA) oligonucleotides with or without overhangs, are substrates for the RNA-induced silencing complex. Synthetic siRNAs strongly inhibit expression of the target protein in mammalian cells when they are transfected into the cells by cationic liposomes (11). Although the maximum effects of antisense DNA and siRNA in the inhibition of RNA or protein expression are comparable, the effect of siRNA lasts longer than that of antisense DNA (12–14). The application of siRNA to human therapy has attracted the attention of many researchers, but the delivery of siRNA to the appropriate cells, tissues, or organs remains a major problem. Small interfering RNAs or siRNA expression plasmid vectors have been administered intravenously to mice by hydrodynamic injection, which accomplishes a rapid infusion of siRNA solution in a volume of 1 mL per 10 g of body weight (15–18) but is unsuitable for use in humans. Virus-mediated delivery of siRNA has also been reported, but there is concern over the safety of viral vectors (19).

The bcl-2 protein regulates the mitochondria-mediated apoptosis pathway, and various cell death stimuli, including chemotherapeutic agents, activate caspases by this pathway, thereby promoting apoptosis. A high level of expression of bcl-2 is associated with resistance to radiation and chemotherapeutic

agents in a number of tumor types, so that a drug to reduce the levels of this protein would be expected to promote apoptosis and would therefore be considered a promising chemotherapeutic agent (20). We have already reported that poly(I):poly(C), a long dsRNA, when mixed with a cationic liposome, has strong antiproliferative activity against various tumor cell lines through the induction of apoptosis (21, 22), and we have developed a novel cationic liposome, LIC-101, which can be safely administered to animals, including monkeys. In the present study, we applied LIC-101 to the administration of siRNAs to animals and found that a siRNA that is sequence specific for human bcl-2 mRNA showed strong antitumor activity in a mouse model of liver metastasis when administered by bolus intravenous injection and in a mouse model bearing prostate cancer when administered by subcutaneous injection near the tumor.

MATERIALS AND METHODS

Human Cell Lines. A549 (lung carcinoma), A431 (epidermoid carcinoma), MDA-MB-231 (breast carcinoma), HT-1080 (fibrosarcoma), and PC-3 (prostate carcinoma) cells were obtained from American Type Culture Collection (Manassas, VA). The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (w/v) fetal bovine serum, 1 mmol/L sodium pyruvate, and 4 mmol/L L-glutamine at 37°C in 5% CO_2.

Oligonucleotides. Small interfering RNA strands and phosphorothioate-type DNAs were synthesized on an Expedite 8909 DNA synthesizer (Applied Biosystems, Foster City, CA). The purity was >90%, as determined by capillary electrophoresis. B717 and GL3 are siRNAs that are sequence specific for human bcl-2 mRNA and *Photinus pyralis* (firefly) luciferase mRNA, respectively; AS-sDNA is a phosphorothioate-type DNA that is sequence specific for human bcl-2 mRNA and has the same sequence as G3139 (10); and S-sDNA is a phosphorothioate-type DNA that is complementary to AS-sDNA. The sequences of the oligonucleotides used are as follows: B717 sense strand, 5′-GUGAAGUCAACAUGCCUGC-dTdT-3′; B717 antisense strand, 5′-GCAGGCAUGUUGACUUCAC-dTdT-3′; GL3 sense strand, 5′-CUUACGCUGAGUACUUCGAdTdT-3′; GL3 antisense strand, 5′-UCGAAGUACUCAGCGUAAGdTd-T-3′; AS-sDNA, 5′-TCTCCCAGCGTGCGCCAT-3′; and S-sDNA, 5′-ATGGCGCACGCTGGGAGA-3′.

Preparation of LIC-101 and Oligonucleotide/LIC-101 Complexes. The cationic liposome LIC-101 contains 2-*O*-(2-diethylaminoethyl)-carbamoyl-1,3-*O*-dioleoylglycerol and egg phosphatidylcholine (Nippon Yushi, Tokyo, Japan). LIC-101 was prepared, lyophilized, and formulated in house. To prepare oligonucleotide/LIC-101 complexes, each solution of phosphorothioate-type DNA or annealed siRNA was added to the same volume of LIC-101 solution with stirring. The ratio of oligonucleotide to LIC-101 was 1:16 (w/w).

Detection of Protein Expression by Western Blotting. Cells were seeded 15 to 24 hours before transfection at a density of 0.5 to 2 × 10^5 cells per 6-cm dish. At various times after transfection, the cells were harvested, and the cell pellets were resuspended in lysis buffer [20 mmol/L Tris-HCl (pH 7.5) containing 150 mmol/L NaCl and 1% (v/v) Nonidet P-40] supplemented with protease inhibitor mixture (Sigma, St. Louis,

MO). The cell debris was removed, and the protein concentration was determined with the bicinchoninic acid protein assay (Pierce, Rockford, IL). Samples of cell extracts containing 10 to 20 μg of protein were analyzed by SDS-PAGE and transferred to polyvinylidene fluoride membrane filters (Millipore, Billerica, MA). Bcl-2 protein was detected with anti–bcl-2 antibody (DakoCytomation, Glostrup, Denmark) and peroxidase-conjugated secondary antibody (Sigma). Actin was detected with antiactin (Santa Cruz Biotechnology, Santa Cruz, CA) and peroxidase-conjugated secondary antibody. The antigen-antibody complexes on the filters were visualized with the Enhanced Chemiluminescence Plus Western Blotting Detection System (Amersham Biosciences, Buckinghamshire, United Kingdom).

Antiproliferative Activity *In vitro*. The antiproliferative activity of oligonucleotide/LIC-101 complexes was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Nacalai Tesque, Kyoto, Japan). Cells were seeded in 96-well plates at a density of 1 × 10^3 (A549 and MDA-MB-231) or 5 × 10^2 (A431) cells per well, and oligonucleotide/LIC-101 complexes were added to each well. After 6 days, the MTT assay was performed in triplicate.

Localization of Fluorescein-Labeled siRNA/LIC-101 *In vitro* and *In vivo*. B717 labeled with 5′-fluorescein was made to order by Dharmacon (Lafayette, CO). For *in vitro* experiments, A549 cells were seeded on a Nunc chamber slide (Nalge Nunc International, Rochester, NY). The next day, the cells were treated with 100 nmol/L fluorescein-labeled B717/LIC-101. After 6 hours, the cells were fixed with 4% (v/v) formaldehyde in PBS, mounted with glycerol containing propidium iodide (1 μg/mL; Sigma), and observed under a fluorescence microscope. For *in vivo* experiments, mice bearing A549 cell liver metastases were given fluorescein-labeled B717/LIC-101 (5 mg/kg) or naked fluorescein-labeled B717 (5 mg/kg). After 5 minutes, tumors were collected and fixed with 4% (v/v) formaldehyde in PBS. Paraffin-embedded tissues were sectioned and processed for hematoxylin and eosin and immunohistochemical staining. The siRNA B717 was detected with anti-fluorescein isothiocyanate (FITC) antibody conjugated with alkaline phosphatase (DakoCytomation). 5-Bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium chloride (DakoCytomation) was used as substrate for alkaline phosphatase.

Mouse Models of Cancer. Male BALB/c nu$^{+/+}$ mice (5 weeks old) were purchased from Clea Japan (Tokyo, Japan). For a model of liver metastasis, mice were anesthetized with pentobarbital, and the spleen was exposed to allow direct intrasplenic injection of 10^6 A549 cells in 50 μL of PBS on day 0. Ten minutes after the injection of tumor cells, the spleen was removed. For a mouse model of prostate cancer, mice were inoculated with 2.5 × 10^6 PC-3 cells in 100 μL of PBS under the dorsal skin on day 0. The animal procedures were approved by the committee for the institutional care and use of animals at Nippon Shinyaku Co., in accordance with the guidelines for animal experimentation prepared by the Japanese Association for Laboratory Animal Science.

Antitumor Activity of B717/LIC-101 in Mouse Models of Cancer. In the mouse model of liver metastasis, siRNA/LIC-101 (10 mg/kg) or 10% (w/v) maltose solution was administered intravenously from day 6 to day 10 and from day 13 to

day 17 (two 5-day cycles of daily injections). Each group contained 10 mice, except for the sham-operated group, which contained 8 mice. The mice were euthanized on day 38, and the inhibitory effect of the treatments on the increase in liver weight was evaluated. The average liver weights were analyzed by Dunnett's test. In the mouse model of prostate cancer, 0.1 mg of B717/LIC-101 was administered subcutaneously near the tumor five times a week from day 7 to day 18. Control mice were treated with 10% (w/v) maltose solution. Each group contained seven mice. The tumor volume was monitored until day 36, and the average tumor volumes were analyzed by Dunnett's test.

RESULTS

A431, A549, MDA-MB-231, and HT-1080 cells were treated with B717/LIC-101 or GL3/LIC-101 for 3 days, and the expression of bcl-2 protein was assessed by Western blotting. GL3, a siRNA that is sequence specific for firefly luciferase, was used as a negative control. B717/LIC-101 inhibited the expression of bcl-2 protein in a dose-dependent manner from 3 to 300 nmol/L, whereas GL3/LIC-101 did not inhibit the expression of bcl-2, even at 300 nmol/L (Fig. 1). Small interfering RNA/LIC-101 and AS-sDNA/LIC-101 were tested on A549 cells, and bcl-2 levels were assessed at the indicated times (Fig. 2). B717/LIC-101 (100 nmol/L) almost completely inhibited the expression of bcl-2 after 2 or 3 days, and the effect continued for 6 days. AS-sDNA/LIC-101 (1 μmol/L) partially inhibited bcl-2 expression after 2 days, but the effect weakened rapidly. AS-sDNA is a phosphorothioate-type DNA that is sequence specific for human bcl-2 mRNA and has the same sequence as G3139.

A431, A549, and MDA-MB-231 cells were treated with oligonucleotide/LIC-101 complexes. B717/LIC-101 showed dose-dependent antiproliferative activity from 3 to 300 nmol/L in all three tumor cell lines, whereas GL3/LIC-101 showed no significant activity (Fig. 3A). In contrast, both AS-sDNA/LIC-101 and S-sDNA/LIC-101 inhibited cell growth in the same manner (Fig. 3B). S-sDNA is a phosphorothioate-type DNA that is complementary to AS-sDNA. The antiproliferative activity of

Fig. 2 Inhibition of bcl-2 protein expression by B717/LIC-101 or AS-sDNA/LIC-101 (time course). A549 cells were treated with B717/LIC-101, GL3/LIC-101, or AS-sDNA/LIC-101. Saline was added to control cells. The levels of bcl-2 protein or actin 2 to 8 days after transfection were assessed by Western blotting.

siRNA was therefore sequence specific, whereas that of phosphorothioate-type DNA was not.

A549 cells were treated with fluorescein-labeled B717/LIC-101. After incubation for 6 hours, the cells were observed by fluorescence microscopy. Fluorescence was observed in the cytosol but not in the nucleus (Fig. 4A).

Fluorescein-labeled B717/LIC-101 (5 mg per 10 mL/kg) was administered by intravenous bolus injection to mice with A549 liver metastasis. The staining of alkaline phosphatase conjugated to the anti-FITC antibody was detected in tumor cells of the liver when fluorescein-labeled B717/LIC-101 complex was administered (Fig. 4B and C), but not when naked fluorescein-labeled B717 (5 mg per 10 mL/kg) was administered (Fig. 4D and E).

B717/LIC-101 (10 mg per 10 mL/kg) was administered by intravenous bolus injection to mice bearing A549 liver metastasis in two 5-day cycles of daily injections (a total of 10 times). Control mice were treated with 10% (w/v) maltose solution. The average liver weight of the control mice was 2.35 ± 0.28 g, that of sham-operated mice was 1.82 ± 0.11 g, and that of the B717/LIC-101 group was 1.77 ± 0.12 g. These data indicate that B717/LIC-101 had a strong antitumor activity in the liver metastasis model. The average liver weight of the GL3/LIC-101 group was 2.17 ± 0.27 g; thus, GL3/LIC-101 did not show any significant antitumor activity (Fig. 5A). Photographs of the liver tumor nodules in each group are shown in Fig. 5B–E.

B717/LIC-101 (0.1 mg per 0.1 mL/mouse) was administered subcutaneously close to the tumor five times a week for 2 weeks to mice bearing PC-3 xenografts. The tumor volume was measured until day 36, and the average tumor volume of each group was calculated. B717/LIC-101 suppressed the growth of the prostate tumor cells throughout the experimental period (Fig. 6). The average tumor volume on day 36 was 1,300 mm³ in the control group and 487 mm³ in the B717/LIC-101 group. GL3/LIC-101 did not show antitumor activity (data not shown).

DISCUSSION

The field of RNA-based gene regulation has been attracting increasing interest over the past couple of years, and the regulation of gene expression by small dsRNAs is being studied intensively. Such interference can be mediated by siRNAs,
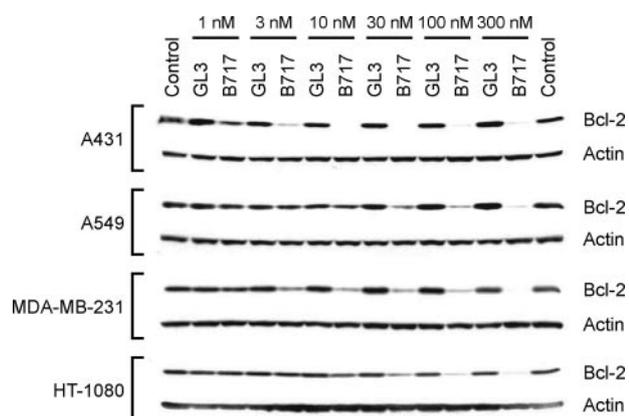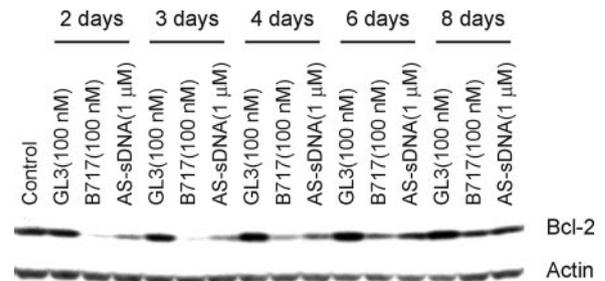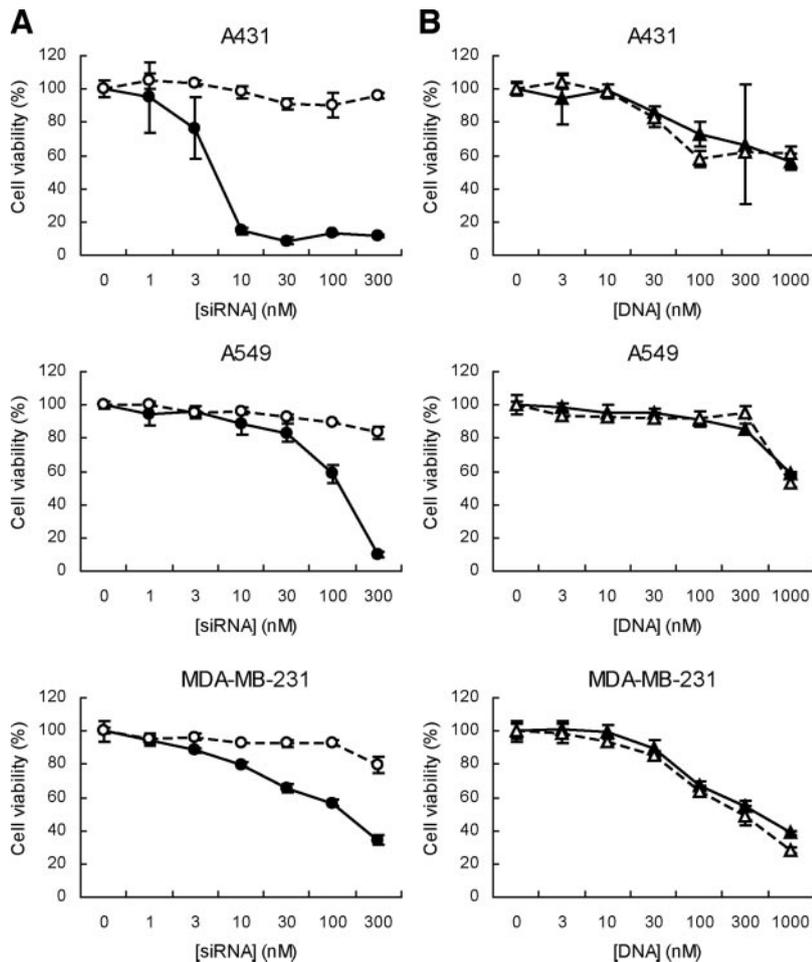

Fig. 1 Inhibition of bcl-2 protein expression by B717/LIC-101 (dose dependency). A431, A549, MDA-MB-231, and HT-1080 cells were treated with B717/LIC-101 or GL3/LIC-101. Saline was added to control cells. The levels of bcl-2 protein or actin 3 days after transfection were assessed by Western blotting.

Fig. 3 Antiproliferative activity of oligonucleo-tide/LIC-101. A431, A549, and MDA-MB-231 cells were treated with oligonucleotide/LIC-101. The antiproliferative activity of oligonucleotide/LIC-101 after 6 days was assessed by the MTT assay. *A*. Cells were treated with B717/LIC-101 (●) or GL3/LIC-101 (○). *B*. Cells were treated with AS-sDNA/LIC-101 (▲) or S-sDNA/LIC-101 (△). The *error bars* represent the SD.

which cleave a sequence-specific target mRNA, or by micro-RNAs, which inhibit translation of a target mRNA. Noncoding RNAs have also been found to play important roles in the regulation of gene expression, for example, in gene silencing by methylation of DNA or histones. Small interfering RNAs are expected to have medical application in human therapy as drugs with high specificity for their molecular targets.

A number of studies on synthetic siRNAs or DNA vector-derived small hairpin RNAs (shRNAs) in cell culture systems have been published, and there are also several animal studies (15–19). McCaffrey *et al.* (15) cotransfected the firefly lucifer-ase gene along with synthetic siRNAs or a shRNA expression vector into mice by hydrodynamic injection and showed that sequence-specific siRNA or shRNA reduces luciferase expres-sion in the liver. Lewis *et al.* (16) administered siRNA by hydrodynamic injection to transgenic mice expressing enhanced green fluorescent protein (EGFP) and found that siRNA specific for EGFP reduces the expression of EGFP in the liver, mainly in hepatocytes. Xia *et al.* (17) demonstrated that virus-mediated delivery of siRNA specifically reduces the expression of tar-geted genes in EGFP transgenic mice, both in the striatum after direct injection into the brain and in the liver after intravenous injection through the tail vein. Song *et al.* (18) found that

siRNA, targeted to the Fas receptor, protects mice from liver failure and fibrosis when administered by hydrodynamic injec-tion in two models of autoimmune hepatitis. Furthermore, McCaffrey *et al.* (19) also used hydrodynamic transfection to administer plasmids encoding the hepatitis B virus (HBV) ge-nome together with HBV-specific shRNAs into mice and dem-onstrated that treatment with shRNAs inhibits HBV replication in the liver. Various *in vivo* effects of siRNA and shRNA are therefore well established. However, the methods of delivering RNA in these studies (hydrodynamic injection, viral vectors, and direct injection into the brain) are not suitable for use in humans, and the safe delivery of nucleic acid drugs in human therapy is still an unresolved issue.

We used our novel cationic liposome, LIC-101, both to deliver siRNA to cells and to administer siRNA to mice by intravenous injection. We compared the inhibitory activity in several tumor cell lines of a siRNA oligonucleotide, B717, with that of a phosphorothioate-type antisense DNA oligonucleotide, AS-sDNA, both complexed with LIC-101. Both are sequence specific for the bcl-2 gene. B717/LIC-101 suppressed the expression of bcl-2 protein in a sequence-specific and dose-dependent manner, and the suppression continued for between 6 and 8 days. AS-sDNA/LIC-101 suppressed bcl-2 at higher oli-

much more toxic than LIC-101, as judged by their hemolysis activity on human erythrocytes (data not shown).

B717/LIC-101 also showed antitumor activity in a mouse model of liver metastasis. B717/LIC-101 completely suppressed
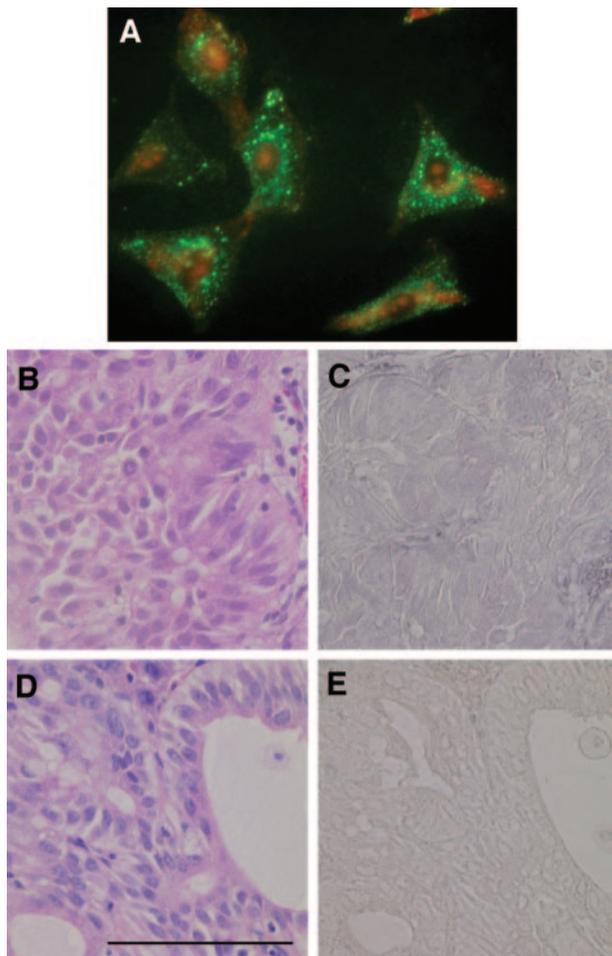

Fig. 4 Localization of fluorescein-labeled B717 *in vitro* and *in vivo*. *A*, A549 cultured cells treated with fluorescein-labeled B717/LIC-101. After incubation for 6 hours, the cells were observed under a fluorescence microscope. The nuclei are stained with propidium iodide. *B–E*. Mice bearing A549 liver metastasis were treated with fluorescein-labeled B717/LIC-101 or fluorescein-labeled B717. *B*, hematoxylin and eosin staining of tumor nodules in the liver of a mouse treated with fluorescein-labeled B717/LIC-101. *C*, anti-FITC antibody staining of the same field as shown in *B*. *D*, hematoxylin and eosin staining of tumor nodules in the liver of a mouse treated with fluorescein-labeled B717. *E*, anti-FITC antibody staining of the same field as shown in *D*.
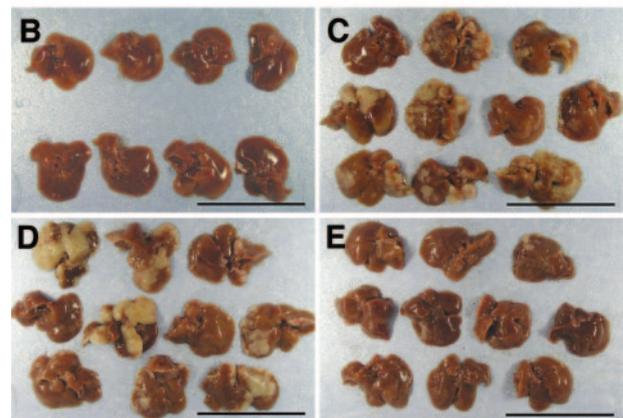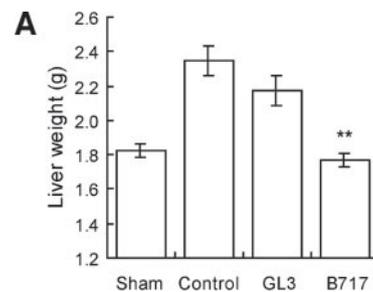

Fig. 5 Antitumor activity of B717/LIC-101 in a mouse model of liver metastasis. B717/LIC-101 was administered intravenously in a mouse model of liver metastasis (A549 cells). *A*, average liver weight for each group. The *error bars* represent the SE. The average liver weights were analyzed by Dunnett's test. **, $P < 0.001$. Photographs of mouse livers of sham-operated ($n = 8$; *B*), control ($n = 10$; *C*), GL3/LIC-101 ($n = 10$; *D*), and B717/LIC-101 ($n = 10$; *E*) groups on day 38. *Scale bar*, 5 cm.

gonucleotide concentrations than did B717/LIC-101, and the duration of its effect was shorter. B717/LIC-101 also inhibited tumor cell growth in a sequence-specific manner, whereas AS-sDNA/LIC-101 and S-sDNA/LIC-101 showed a non–sequence-specific cytotoxicity that may result from the physicochemical properties of phosphorothioate-type DNA. After treatment of tumor cells with fluorescein-labeled B717/LIC-101, fluorescence was observed in the cytosol but not in the nuclei, so that B717 was delivered only to the cytosol when it was added to cells as a complex with LIC-101. Small interfering RNA functions by binding to RNA-induced silencing complex in the cytosol, so that the delivery of siRNA specifically to the cytosol by LIC-101 may have the advantage of avoiding toxicity to the nucleus. As transfection reagents, other cationic liposomes are
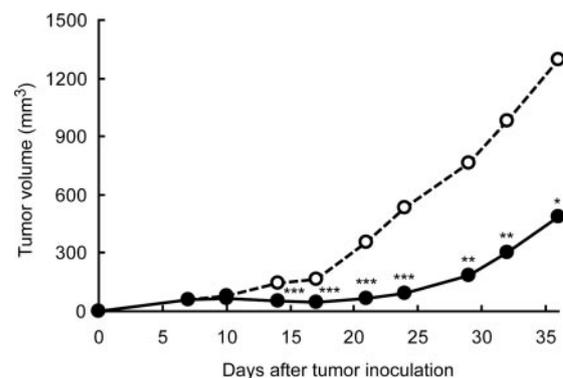

Fig. 6 Antitumor activity of B717/LIC-101 in a mouse model of prostate cancer. B717/LIC-101 was administered subcutaneously in a mouse model bearing prostate cancer (PC-3) cells xenografted under the dorsal skin. ○, control ($n = 7$); ●, B717/LIC-101 ($n = 7$). The average tumor volumes were analyzed by Dunnett's test. ***, $P < 0.0001$, **, $P < 0.001$, *, $P < 0.01$.

the growth of liver tumors, whereas GL3/LIC-101 had no effect. Our method of drug administration was bolus intravenous injection via the tail vein. Furthermore, the injection volume was 0.1 mL per 10 g of body weight, which is normally acceptable in human therapy. After administration of fluorescein-labeled B717/LIC-101, B717 was observed in tumor cells in the mouse liver; hence B717 was delivered to metastatic liver cancer cells when it was injected as a complex with LIC-101 (it was not delivered when it was injected as naked B717). Finally, the complex inhibited the growth of prostate cancer when injected subcutaneously in the vicinity of the xenografted tumor.

In conclusion, our siRNA/cationic liposome complex could be safely administered to animals and showed strong antitumor activity in mouse models of liver metastasis and xenografted prostate cancer. Furthermore, our cationic liposome, LIC-101, is shown to be a promising tool for the safe and effective delivery of oligonucleotides to tissues and organs with potential for application to human therapy. Our results take cancer therapy that includes siRNA one step closer to the clinic.

ACKNOWLEDGMENTS

REFERENCES

1. Raynaud FI, Orr RM, Goddard PM, et al. Pharmacokinetics of G3139, a phosphorothioate oligonucleotide antisense to bcl-2, after intravenous administration or continuous subcutaneous infusion to mice. J Pharmacol Exp Ther 1997;281:420–7.

2. Cotter FE. Antisense therapy for B cell lymphomas. Cancer Surv 1997;30:311–25.

3. Loke SL, Stein CA, Zhang XH, et al. Characterization of oligonucleotide transport into living cells. Proc Natl Acad Sci USA 1989;86: 3474–8.

4. Zhao Q, Zhou R, Temsamani J, et al. Cellular distribution of phosphorothioate oligonucleotide following intravenous administration in mice. Antisense Nucleic Acid Drug Dev 1998;8:451–8.

5. Baker BF, Lot SS, Condon TP, et al. 2′-O-(2-methoxy)ethyl-modified anti-intercellular adhesion molecule 1 (ICAM-1) oligonucleotides selectively increase the ICAM-1 mRNA level and inhibit formation of the ICAM-1 translation initiation complex in human umbilical vein endothelial cells. J Biol Chem 1997;272:11994–2000.

6. Condon TP, Bennet CF. Altered mRNA splicing and inhibition of human E-selectin expression by antisense oligonucleotide in human umbilical vein endothelial cells. J Biol Chem 1996;271:30398–403.

7. Hanecak R, Brown-Driver V, Fox MC, et al. Antisense oligonucleotide inhibition of hepatitis C virus gene expression in transformed hepatocytes. J Virol 1996;70:5203–12.

8. Monia BP, Sasmor H, Johnston JF, et al. Sequence-specific antitumor activity of a phosphorothioate oligonucleotide targeted to human C-*raf* kinase supports an antisense mechanism of action *in vivo*. Proc Natl Acad Sci USA 1996;93:15481–4.

9. Vidalin O, Major ME, Rayner B, et al. In vitro inhibition of hepatitis C virus gene expression by chemically modified antisense oligonucleotides. Antimicrob Agents Chemother 1996;40:2337–44.

10. Banerjee D. Genasense (Genta Inc). Curr Opin Investig Drugs 2001;2:574–80.

11. Elbashir SM, Harborth J, Lendeckel W, et al. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature (Lond) 2001;411:494–8.

12. Bertrand J-R, Pottier M, Vekris A, et al. Comparison of antisense oligonucleotides and siRNAs in cell culture and in vivo. Biochem Biophys Res Commun 2002;296:1000–4.

13. Vickers TA, Koo S, Bennet CF, et al. Efficient reduction of target RNAs by small interfering RNA and RNase H-dependent antisense agents. J Biol Chem 2003;278:7108–18.

14. Hough SR, Wiederholt KA, Burrier AC, Woolf TM, Taylor MF. Why RNAi makes sense. Nat Biotechnol 2003;21:731–2.

15. McCaffrey AP, Meuse L, Pham TT, et al. RNA interference in adult mice. Nature (Lond) 2002;418:38–9.

16. Lewis DL, Hagstrom JE, Loomis AG, Wolff JA, Herweijer H. Efficient delivery of siRNA for inhibition of gene expression in postnatal mice. Nat Genet 2002;32:107–8.

17. Xia H, Mao Q, Paulson HL, Davidson BL. siRNA-mediated gene silencing in vitro and in vivo. Nat Biotechnol 2002;20:1006–10.

18. Song E, Lee SK, Wang J, et al. RNA interference targeting Fas protects mice from fulminant hepatitis. Nat Med 2003;9:347–51.

19. McCaffrey AP, Nakai H, Pandey K, et al. Inhibition of hepatitis B virus in mice by RNA interference. Nat Biotechnol 2003;21:639–44.

20. Korsmeyer SJ, Zinkel SS. Molecular biology of cancer: apoptosis. In: DeVita VT Jr, Hellman S, Rosenberg, SA, editors. Cancer: principles and practice of oncology, 6th ed. Philadelphia: Lippincott, Williams & Wilkins; 2001. p. 111–22.

21. Hirabayashi K, Yano J, Inoue T, et al. Inhibition of cancer cell growth by polyinosinic-polycytidylic acid/cationic liposome complex: a new biological activity. Cancer Res 1999;59:4325–33.

22. Hirabayashi K, Yano J, Takesue H, Fujiwara N, Irimura T. Inhibition of metastatic cancer cell growth in livers by poly(I):poly(C)/cationic liposome complex (LIC). Oncol Res 1999;11:497–504.

Clinical Cancer Research

Antitumor Activity of Small Interfering RNA/Cationic Liposome Complex in Mouse Models of Cancer

Junichi Yano, Kazuko Hirabayashi, Shin-ichiro Nakagawa, et al.

Updated version	Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/10/22/7721

Cited articles	This article cites 21 articles, 9 of which you can access for free at: http://clincancerres.aacrjournals.org/content/10/22/7721.full#ref-list-1
Citing articles	This article has been cited by 5 HighWire-hosted articles. Access the articles at: http://clincancerres.aacrjournals.org/content/10/22/7721.full#related-urls

E-mail alerts	Sign up to receive free email-alerts related to this article or journal.
Reprints and Subscriptions	To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.
Permissions	To request permission to re-use all or part of this article, use this link http://clincancerres.aacrjournals.org/content/10/22/7721. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.