Tumor Targeting and Imaging of Intraperitoneal Tumors by Use of Antisense Oligo-DNA Complexed with Dendrimers and/or Avidin in Mice

Noriko Sato, Hisatake Kobayashi, Tsuneo Saga, Yuji Nakamoto, Takayoshi Ishimori, Kaori Togashi, Yasuhisa Fujibayashi, Junji Konishi, and Martin W. Brechbiel

Department of Nuclear Medicine and Diagnostic Imaging [N. S., T. S., Y. N., T. I., J. K.], and Hitachi Medical Co. chaired Department of Diagnostic and Interventional Imagiology [H. K., K. T.]. Kyoto University, Kyoto 606-8507, Japan; Molecular Imaging Division, Biomedical Imaging Research Center, Fukui Medical University, Fukui 910-1193, Japan [Y. F.]; and Chemistry Section, Radiation Oncology Branch, National Cancer Institute, NIH, Bethesda, MD 20892 [M. W. B.]

ABSTRACT

To establish an effective nonviral gene delivery and a corresponding imaging method for i.p.-disseminated tumors, various oligonucleotide-carrier complexes were synthesized, and their in vitro and in vivo properties were examined.

The 20-mer multiamino-linked oligonucleotide (oligo), synthesized as antisense against the c-erbB-2 sequence, and the 3'-biotinylated form of the same oligonucleotide (oligo-Bt) were In labeled through a diethylenetriaminepentaacetic acid chelate. 111In-oligo was mixed with generation 4 polyamidoamine dendrimer (G4) or with biotinylated G4 (G4-Bt), which are positively charged to form electrostatic complexes. 111In-oligo/G4-Bt and 111In-oligo-Bt were conjugated to avidin (111In-oligo/G4-Av and 111In-oligo-Av, respectively). 111In-oligo/G4, 111In-oligo/G4-Av, 111In-oligo-Av, and carrier-free 111In-oligo (2.96 kBq/22.4–45.9 ng of oligo) were examined for internalization in vitro in human ovarian cancer cells (SHIN3). Biodistribution of 111In-oligo-carrier complexes or 111In-oligo was examined in normal (n = 4–7) or i.p. SHIN3 tumor-bearing (n = 6–10) mice 2–24 h after i.p. injection (74 kBq/125–300 ng). Scintigraphy of i.p. tumor-bearing and normal mice was performed at various times postinjection of 111In-oligo-carrier complex or 111In-oligo (1.85 MBq/2.2 ng).

111In-oligo-carrier complexes bound to the tumor cells were internalized at a rate of 34–56% at 24 h. In vivo, G4, G4-Av, and Av significantly enhanced tumor delivery of 111In-oligo [9.1, 14.5, and 24.4% of injected dose per g of tissue (ID/g) at 24 h; P < 0.05, < 0.01, and < 0.0001, respectively] compared with delivery without carrier (0.8% ID/g). Scintigrams of 111In-oligo delivered to the i.p.-disseminated tumors by the carriers were successfully obtained.

In conclusion, G4, G4-Av, and Av can effectively deliver 111In-oligo to i.p.-disseminated tumors. 111In-oligo-carrier complexes also have potential as tracers for imaging and monitoring of gene delivery.

INTRODUCTION

Nonviral gene transfection systems, which can carry both oligonucleotides and plasmid DNA (1), have an advantage of lower immunogenicity when compared with viral systems (2). A number of nonviral carriers for gene transfer have been synthesized, including polylysines (3), cationic liposomes (4), polypeptides (5, 6), recombinant histones (7), and other reagents (8–10). However, the transfection/expression efficiency of these nonviral systems remains insufficient, especially in the presence of serum (11).

Recently, starburst polyamidoamine dendrimers have emerged as a novel synthetic gene carrier (12, 13). Dendrimers are highly branched spherical polymers that have a unique surface of primary amino groups with high solubility in water (13). Because of the high number of positive charges on their surfaces, dendrimers form stable electrostatic complexes with negatively charged nucleic acids in buffer at physiological pH. Dendrimers facilitate gene transfer to various cell lines with a higher efficiency than polylysines and are also less cytotoxic (12, 14, 15). Moreover, dendrimers are efficient in delivering oligonucleotides even in the presence of high concentrations of serum proteins by protecting the oligonucleotides from the degradation by exonuclease (15). Studies of gene/antisense therapy using dendrimers as a vector have been actively carried out, and expression of reporter genes was reported (2, 12, 14–20). However, the behavior of the dendrimer-nucleotide complexes in a biological environment and the mechanism of dendrimer-mediated cellular uptake of oligonucleotides have not been clarified. A mechanism similar to that proposed for cationic lipid complexes (2, 21) has been suggested. The DNA-dendrimer complex that possesses a net positive charge (17) binds to negatively charged receptors such as sialylated glycoproteins on the cell surface. After internalization, dendrimers buffer the endosome and inhibit pH-dependent lysosomal nucleases; at the same time, the electrostatic bonding between oligo-
nucleotide and dendrimer is weakened by the low pH environment in the lysosome, which allows migration of intact DNA to the nucleus (2).

Av, a natural polycationic protein of egg white, has been used as a nonviral carrier of DNA through the biotin-Av system and was reported to introduce a high internalization (transfection) rate in vitro by absorption-mediated endocytosis (22). Endocytosis of Av into the liver and tumor cells was accelerated by its highly glycosylated side chains (23, 24). Although the mechanism of this Av-mediated cellular uptake of oligonucleotides is also not fully understood, Av is known to show a high accumulation in tumors when injected i.p. into mice bearing i.p.-disseminated adenocarcinoma tumors (25).

Peritonitis carcinomatosa is a late manifestation of gastrointestinal and gynecological malignancies, and therapeutic methods for the widely spread tumors in the peritoneal cavity are limited to i.v. or i.p. chemotherapy, which produce unsatisfactory results. Gene/antisense therapy is one of the anticipated therapies for peritonitis carcinomatosa, and experimental studies have been carried out by several investigators (26–28).

In the present study, to establish effective nonviral gene transfer carriers for i.p.-disseminated tumors, three antisense oligonucleotide-carrier complexes were synthesized, using a dendrimer and/or the biotin-Av system, and their tumor targeting abilities were examined in vitro and in vivo. The word “targeting” has been used with two different meanings among researchers in different fields: gene targeting in molecular biology and organ/tissue targeting in pharmacology. In the present study, we focused on developing new gene carriers that deliver the gene to the tumor in vivo. The properties of these synthesized oligonucleotide-carrier complexes were examined in terms of tumor targeting, not gene targeting. Furthermore, to examine the potential of the carriers themselves, we used an antisense oligonucleotide and tumor cells that are not specific to each other in our experiments. Thus, we chose an antisense oligonucleotide for the primer of c-erbB-2, which is well known to be related to carcinogenesis of various cancers, and for tumor cells, we chose an ovarian adenocarcinoma cell line that often causes peritonitis carcinomatosa and is also c-erbB-2 negative. In addition, scintigraphic imaging of the i.p. tumor-bearing mice that received injections of the synthesized complexes was also performed. Imaging of the gene/antisense delivery to the targeted tumors or organs may be a promising method for monitoring a gene/antisense therapy.

MATERIALS AND METHODS

Antisense Oligonucleotides. The 20-mer antisense oligonucleotide 5′-CCAGGUCCAUGGGUCCACT-3′ [where U indicates the substitution of dT with amino-linked dU (M, 5418); hereafter designated “oligo”) synthesized based on the c-erbB-2 sequence, and the 3′-biotinylated version of the same internally amino-linked oligonucleotide (oligo-Bt) were pur-...
Intraperitoneal Tumor Targeting of Antisense DNA regarding animal care and handling. with the regulations of the Kyoto University animal facility.

Plexes or carrier-free $^{111}$In-oligo were injected i.p. into i.p. n oligo were injected i.p. into groups of mice bearing i.p. tumors and on the mesentery. Seventy-four kBq ($2 \times 10^5$ Ci) of $^{111}$In-oligo/Av in 1 ml of RPMI 1640 with 10% FCS (as oligo) of $^{111}$In-oligo/G4, $^{111}$In-oligo/G4-Av, or carrier-free $^{111}$In-oligo or with 2.96 kBq/45.9 ng (as oligo) of $^{111}$In-oligo-Av in 1 ml of RPMI 1640 with 10% FCS for 1 h at 37°C or $4^\circ$C as a noninternalized control. The medium containing the tracers was then replaced with 1 ml of standard RPMI 1640. The cells were further cultured at 37°C or $4^\circ$C to examine internalization of radioactivity. At 0, 1, 3, 10, and 24 h ($n = 4$), the medium was removed, and the cells were washed with $0.5 \times$ Na$_2$HPO$_4$-$0.5 \times$ NaH$_2$PO$_4$-$0.1 \times$ NaCl (pH 7.4). Membrane-bound radioactivity was removed by washing the cells with acidic solution [0.05 M glycine-HCl-0.1 M NaCl (pH 2.8)], which eliminated the charge-related binding of the complexes to the cell surface. The radioactivity in the medium and membrane-bound and acid-resistant internalized radioactivity was assessed by gamma counter (Aloka, Tokyo, Japan). The percentages of membrane-bound fraction and internalized fraction in the total bound radioactivity were calculated. The assay at $4^\circ$C could be performed only up to 3 h because cells died and detached thereafter.

**Biodistribution Study.** To develop an i.p. tumor model, $3\times10^6$ SHIN3 cancer cells were injected i.p. into 5-week-old female BALB/c nu/nu mice. Twenty days later, numerous small i.p.-disseminated tumors had formed, especially around the stomach, subphrenic region, at the hepatic and splenic hila, and on the mesentery. Seventy-four kBq (2 $\mu$Ci) of $^{111}$In-oligo/G4, $^{111}$In-oligo/G4-Av, $^{111}$In-oligo-Bt-Av, or carrier-free $^{111}$In-oligo were injected i.p. into groups of mice bearing i.p. tumors ($n = 6$–10; 125–167 ng each for $^{111}$In-oligo/G4, $^{111}$In-oligo/G4-Av, and $^{111}$In-oligo; 240–300 ng for $^{111}$In-oligo-Av, by oligo weight), and then biodistribution was examined. As a control, biodistribution in normal mice was compared for $^{111}$In-oligo/G4, $^{111}$In-oligo-Av, and $^{111}$In-oligo ($n = 4$–7). The mice were sacrificed by ether inhalation at 2, 10, or 2 h after i.p. injection of radioactive oligo. Blood, i.p.-disseminated tumors, and various organs were removed and weighed, and their radioactivity counts were measured by gamma counter. The % ID/g for these data were determined, and the results were normalized to a 20-g mouse, using following formula:

% ID/g (normalized) = [Radioactivity in the organ (blood)/total injected dose/organ (blood) weight (g)] × [body weight (g)/20] × 100.

All animal experiments were carried out in accordance with the regulations of the Kyoto University animal facility regarding animal care and handling.

**Scintigraphy by $^{111}$In-Oligo-Carrier Complexes.** A total of 1.85 MBq (50 $\mu$Ci)/2.2 $\mu$g of $^{111}$In-oligo-carrier complexes or carrier-free $^{111}$In-oligo were injected i.p. into i.p. tumor (SHIN3)-bearing mice or normal mice. Scintigrams were obtained at 0, 2, and 10 h and 1, 2, and 3 days after injection. Four days after injection of the oligo-carrier complexes, the mice were sacrificed, and the locations of the i.p.-disseminated tumors were examined and correlated with the scintigrams. In addition, to further confirm that the foci of radioactivity shown on the images actually represented delivery of $^{111}$In-oligo to the organs and/or tumors, the organs and i.p. tumors in tumor-bearing mice were taken out, washed with $H_2O$ to remove unbound $^{111}$In-oligo (-carrier complexes), and placed side by side. Scintigrams were then obtained of these samples and compared with corresponding mouse scintigrams obtained from the live mice.

**Statistical Analyses.** A one-way ANOVA with Fisher’s protected least significant difference test was used for statistical analyses of the internalization assay and the biodistribution study. All tests were two-sided, and a probability ($P < 0.05$) was considered significant.

**RESULTS**

**Internalization Assay.** The tumor cells incubated at $37^\circ$C showed an internalization of radioactivity (Fig. 2), whereas those incubated at $4^\circ$C did not (data not shown). The percentages of $^{111}$In-oligo/G4, $^{111}$In-oligo/G4-Av, and $^{111}$In-oligo-Av internalized at 24 h were 34, 51, and 56% of the total bound fraction, respectively, all of which were significantly higher than that of $^{111}$In-oligo (23%; $P < 0.01$ for $^{111}$In-oligo/G4 and $P < 0.001$ for $^{111}$In-oligo/G4-Av and $^{111}$In-oligo-Av). Significantly more $^{111}$In-oligo/G4-Av internalized than $^{111}$In-oligo/G4 ($P < 0.01$).

$^{111}$In-oligo/G4 or $^{111}$In-oligo/G4-Av bound to the cells (both membrane-bound and internalized radioactivities) at $37^\circ$C was 12–13% of the total radioactivity at 1 h. The total bound radioactivity decreased thereafter to 9 and 5% for $^{111}$In-oligo/G4 and $^{111}$In-oligo/G4-Av, respectively.
oligo/G4 and 111In-oligo/G4-Av, respectively, at 24 h. In contrast, the amount of 111In-oligo-Av bound to the tumor cells did not decrease over time and was 11% at 24 h (data not shown).

**Biodistribution Study.** Biodistribution data in normal and tumor-bearing mice at 2 and 24 h postinjection are shown in Figs. 3 and 4, respectively. In normal mice, 111In-oligo without carrier accumulated highly in the kidneys at 2 h (7.5% ID/g), representing a rapid renal excretion of 111In-oligo. When G4 was used as a carrier, the complex accumulated in the spleen (7.3% ID/g) and stomach (6.6% ID/g) at 2 h in addition to the kidney, all of which quickly decreased with time. In contrast, the high accumulation of 111In-oligo-Av in the liver (6.1% ID/g), spleen (9.6% ID/g), and stomach (7.0% ID/g) at 2 h further increased after 24 h had elapsed. The liver uptake of 111In-oligo-Av was significantly higher than that of 111In-oligo/G4 or 111In-oligo ($P < 0.0001$).

In the tumor-bearing mice, G4, G4-Av, or Av delivered significantly larger amounts of 111In-oligo to the i.p.-disseminated tumors than did 111In-oligo without carrier (Fig. 4; $P < 0.05$, $< 0.01$, and $< 0.0001$ for 111In-oligo/G4, 111In-oligo/G4-Av, and 111In-oligo-Av, respectively). In-oligo/G4; □, 111In-oligo/G4-Av; ■, 111In-oligo-Av; □, 111In-oligo.

![Fig. 3 Biodistribution of 111In-oligo complexes or 111In-oligo in normal mice 2 h (A) and 24 h (B) post-i.p. injection. □, In-oligo/G4; □, 111In-oligo-G4-Av; ■, 111In-oligo.](image)

![Fig. 4 Biodistribution of 111In-oligo complexes or 111In-oligo in i.p. tumor-bearing mice 2 h (A) and 24 h (B) post-i.p. injection. Conjugation of 111In-oligo with G4, G4-Av, or Av significantly increased the delivery of 111In-oligo to the tumors compared with carrier-free 111In-oligo at 24 h ($P < 0.05$, $< 0.01$, and $< 0.0001$ for 111In-oligo/G4, 111In-oligo/G4-Av, and 111In-oligo-Av, respectively). □, In-oligo/G4; □, 111In-oligo/G4-Av; ■, 111In-oligo-Av; □, 111In-oligo.](image)
Intraperitoneal Tumor Targeting of Antisense DNA

Intraperitoneal Tumor Targeting of Antisense DNA

The three oligo-carrier complexes synthesized for this study by the G4 and/or the Bt-Av system showed a high tumor accumulation for both the in vitro and the in vivo i.p. tumor models. For synthesis of [111In-oligo-Av, 3'-biotinylated oligo was used because biotinylation of the 3' end of an oligonucleotide, followed by binding to Av, has been reported to provide protection against serum 3'-exonuclease degradation (30, 31).

The internalization study showed a gradual internalization of the complexes to the tumor cells in the presence of serum. However, the level of the total bound [111In-oligo/G4 and [111In-oligo/G4-Av decreased with time. This decrease was possibly caused by some release of [111In-oligo from the complexes at the cell surface prior to internalization. Contrary to this possibility, the total bound [111In-oligo-Av did not decrease, suggesting that the binding of oligo-Bt to Av was stronger than that of oligo to either G4 or G4-Av.

In both normal and tumor-bearing mice, carrier-free [111In-oligo demonstrated only renal and urinary bladder uptake as a result of rapid excretion through the kidney. By complexing or conjugating [111In-oligo with G4, G4-Av, or Av, we significantly increased the delivery of [111In-oligo to the tumors. The hepatic or splenic accumulation of the complexes may be attributable to the uptake by reticuloendothelial systems; however, the high gastric uptake demonstrated was unexpected, and the reason is unclear. [111In-oligo/G4 and [111In-oligo/G4-Av showed decreasing tumor uptake over time, whereas [111In-oligo-Av maintained high tumor accumulation up to 24 h. These findings seem to be comparable to the in vitro study results, which also found a decrease of the total tumor cell binding with the [111In-oligo/G4 and [111In-oligo/G4-Av complexes. The markedly high uptake of [111In-oligo/G4-Av to the tumor at 2 h postinjection is not surprising because [111In-oligo/G4-Av may accumulate in the tumor by virtue of both dendrimer and Av mechanisms. Although the tumor uptake of [111In-oligo/G4-Av rapidly decreased to a level close to that of [111In-oligo/G4 at 24 h postinjection, it was still as high as 14.5% ID/g at 24 h. An extremely high i.p. tumor uptake of i.p.-injected Av has been reported (25), in addition to the findings that the i.p. injection can provide higher tumor uptake in i.p. tumor models because of greater accessibility compared with the i.v. route (32). Another potential reason for the high tumor uptake shown in this study is that after i.p. inoculation, SHIN3 cells form small tumors with a broader surface, which may lead to higher tumor uptake compared with that of other larger tumors (33).

At the mixing ratio of 1:100 for [111In-oligo to G4 (or G4-Av) used in this study, the complexes formed were positively charged and readily bound to the negatively charged cell membrane. The complexes are considered to be in a clustered form, similar to that of DNA-polysine complexes (34), and oligo are in a condensed form, which facilitates cellular uptake of the oligo (19). In previous ultrafiltration experiments using complexes with 1:100 or 1:1 DNA:generation 3 dendrimer (M, 6909) molar ratios, 80–90% of the complexes were in the range of 10–1000 kDa range, and 30% of the complexes were <30 kDa. Some fractions of the complexes that are large (~1000 nm in diameter; Ref. 17) are suspected to internalize inefficiently.

Unlabeled oligo was as low as 0.8% ID/g at 24 h. The high tumor uptake shown in this study is that after i.p. injection of [111In-oligo/G4 (A and B), [111In-oligo-Av (C and D), and [111In-oligo (E and F). i.p.-disseminated tumors were visualized with [111In-oligo/G4 (A) and [111In-oligo-Av (C; arrows).

Scintigrams of i.p. tumor-bearing (A, C, and E) and normal (B, D, and F) mice 24 h after i.p. injection of [111In-oligo/G4 (A and B), [111In-oligo-Av (C and D), and [111In-oligo (E and F). i.p.-disseminated tumors were visualized with [111In-oligo/G4 (A) and [111In-oligo-Av (C; arrows).

ID/g: P < 0.01 and < 0.05 compared with [111In-oligo/G4 and [111In-oligo/G4-Av, respectively). The tumor uptake of carrier-free [111In-oligo was as low as 0.8% ID/g at 24 h. The high tumor radioactivity uptake resulted in very high tumor-to-nontumor radioactivity ratios for [111In-oligo/G4, [111In-oligo/G4-Av, and [111In-oligo-Av (data not shown).

Scintigraphy of [111In-oligo-Carrier Complexes. Tumor targeting of the three [111In-oligo-carrier complexes also correlated with scintigraphy. The tumors shown by scintigraphy were confirmed when mice were sacrificed and the locations of the tumors were compared with the scintigraphic findings. Small tumors that accumulated and agglutinated around the stomach and subphrenic region, at the hepatic and splenic hila, and on the mesentery were visualized as if they were large tumors. Because of splenomegaly of the tumor-bearing mice and accumulated tumors at the splenic hilum and around the stomach, the intestine was slightly shifted to the right in the peritoneal cavity. Consequently, the mesenteric tumors were shown on the right side in the scintigrams. Scintigrams of the organs and tumors taken out from the mice also showed a high visualization of tumors when [111In-oligo-carrier complexes were used (images not shown). Fig. 5 shows the scintigrams of normal and i.p. tumor-bearing mice at 24 h after i.p. injection of [111In-oligo/G4, [111In-oligo-Av, and free [111In-oligo. [111In-oligo/G4 faintly showed the stomach, and [111In-oligo-Av accumulated in the liver in addition to the bladder in normal mice, whereas in the tumor-bearing mice, the i.p.-disseminated tumors were clearly visualized with sufficient contrast to the normal organs. The scintigram of free [111In-oligo showed only renal and bladder uptake, and no other organ or tumor was identified. The scintigram at 0 h revealed immediate renal excretion of [111In-oligo after i.p. injection in both tumor-bearing and normal mice (images not shown).

DISCUSSION

The three oligo-carrier complexes synthesized for this study by the G4 and/or the Bt-Av system showed a high tumor accumulation for both the in vitro and the in vivo i.p. tumor models. For synthesis of [111In-oligo-Av, 3'-biotinylated oligo was used because biotinylation of the 3' end of an oligonucleotide, followed by binding to Av, has been reported to provide protection against serum 3'-exonuclease degradation (30, 31).

The internalization study showed a gradual internalization of the complexes to the tumor cells in the presence of serum. However, the level of the total bound [111In-oligo/G4 and [111In-oligo/G4-Av decreased with time. This decrease was possibly caused by some release of [111In-oligo from the complexes at the cell surface prior to internalization. Contrary to this possibility, the total bound [111In-oligo-Av did not decrease, suggesting that the binding of oligo-Bt to Av was stronger than that of oligo to either G4 or G4-Av.

In both normal and tumor-bearing mice, carrier-free [111In-oligo demonstrated only renal and urinary bladder uptake as a result of rapid excretion through the kidney. By complexing or conjugating [111In-oligo with G4, G4-Av, or Av, we significantly increased the delivery of [111In-oligo to the tumors. The hepatic or splenic accumulation of the complexes may be attributable to the uptake by reticuloendothelial systems; however, the high gastric uptake demonstrated was unexpected, and the reason is unclear. [111In-oligo/G4 and [111In-oligo/G4-Av showed decreasing tumor uptake over time, whereas [111In-oligo-Av maintained high tumor accumulation up to 24 h. These findings seem to be comparable to the in vitro study results, which also found a decrease of the total tumor cell binding with the [111In-oligo/G4 and [111In-oligo/G4-Av complexes. The markedly high uptake of [111In-oligo/G4-Av to the tumor at 2 h postinjection is not surprising because [111In-oligo/G4-Av may accumulate in the tumor by virtue of both dendrimer and Av mechanisms. Although the tumor uptake of [111In-oligo/G4-Av rapidly decreased to a level close to that of [111In-oligo/G4 at 24 h postinjection, it was still as high as 14.5% ID/g at 24 h. An extremely high i.p. tumor uptake of i.p.-injected Av has been reported (25), in addition to the findings that the i.p. injection can provide higher tumor uptake in i.p. tumor models because of greater accessibility compared with the i.v. route (32). Another potential reason for the high tumor uptake shown in this study is that after i.p. inoculation, SHIN3 cells form small tumors with a broader surface, which may lead to higher tumor uptake compared with that of other larger tumors (33).

At the mixing ratio of 1:100 for [111In-oligo to G4 (or G4-Av) used in this study, the complexes formed were positively charged and readily bound to the negatively charged cell membrane. The complexes are considered to be in a clustered form, similar to that of DNA-polysine complexes (34), and oligo are in a condensed form, which facilitates cellular uptake of the oligo (19). In previous ultrafiltration experiments using complexes with 1:100 or 1:1 DNA:generation 3 dendrimer (M, 6909) molar ratios, 80–90% of the complexes were in the range of 10–1000 kDa range, and 30% of the complexes were <30 kDa. Some fractions of the complexes that are large (~1000 nm in diameter; Ref. 17) are suspected to internalize inefficiently.
(12), which allows some $^{111}$In-DNA to be released from the complexes at the cell surface.

To determine the complexation ratio of G4 with oligo, a preliminary study using Sephadex G-50 gel filtration was performed. At a molar mixing ratio of 1:1 for $^{111}$In-oligo and G4, >70% of $^{111}$In-oligo was not complexed with G4. At higher G4 molar ratios, the ratio of $^{111}$In-oligo to G4 increased, and at 1:100, all of the $^{111}$In-oligo was complexed with G4; we consequently selected the mixing ratio of 1:100 for in the present study.

Recent studies have indicated that the concentration of the gene, the DNA-to-dendrimer charge ratio, and the size (generation) of the dendrimer, which are the determining factors for the size and charge of the entire complex (4), directly affect the transfection efficiency (16). The optimal charge ratio of DNA: dendrimer examined in a serum-free in vitro environment (12, 19) might not be directly comparable with experiments carried out in the presence of serum or in vivo. A higher dendrimer: oligonucleotide ratio increased transfection efficiency, especially at a higher serum concentration (16). Further detailed studies to determine adequate DNA:dendrimer charge ratio are required. Many investigators have compared transfection and transcription efficiencies between different dendrimer generations (12, 16, 19, 20, 35); however, the appropriate size to be used as a carrier has not been confirmed yet.

Imaging in conjunction with gene therapy is now in the developmental stage. A variety of imaging techniques, including positron emission tomography, gamma scintigraphy and magnetic resonance imaging, have been investigated (36). Imaging with radioisotopes has an advantage because of the high sensitivity compared with magnetic resonance imaging, which enables visualization of even a smaller amount of gene delivered to the target organ/tissue or a smaller amount of gene expressed in the target. The delivery of $^{111}$In-oligo to the i.p.-disseminated tumors by G4, G4-Av, or Av as a carrier could be successfully visualized. The scintigraphy of $^{111}$In-oligo-Av in normal mice illuminated the liver, which is in good agreement with the high liver accumulation of this complex shown in our biodistribution studies in normal mice. In the i.p. tumor-bearing mice, $^{111}$In-oligo-Av was delivered to the tumors, giving low liver accumulation. The scintigrams of the $^{111}$In-oligo/G4, $^{111}$In-oligo/G4-Av, and $^{111}$In-oligo-Av complexes successfully visualized tumors with sufficient contrast to the normal tissues, in accordance with the high tumor-to-nontumor radioactivity ratios obtained in the biodistribution studies. Thus, these $^{111}$In-oligo-carrier complexes have potential as tracers to monitor gene delivery. Neither the scintigrams nor the biodistribution data provide explicit evidence of internalization of $^{111}$In-oligo into the i.p. tumor cells; however, this condition is strongly suggested from the results of the in vitro internalization study.

One potential obstacle to using radiolabeled oligonucleotides as a tracer to monitor gene delivery is evidence that oligonucleotides undergo exocytosis in vitro in some cell lines (37, 38). If a radiolabeled oligonucleotide underwent cytosolic metabolism after internalization and the free oligonucleotide exocytosed, leaving radioactive metal chelate in the cytosolic component, the information obtained from the radioactivity would not accurately be related to the localization of oligonucleotide (39). The combination of a very high tumor uptake of the $^{111}$In-oligo-carrier complexes presented in this study combined with the results of previous reports of increased transfection/transcription efficiency of DNA complexed with dendrimer or Av compared with free DNA (12, 16, 17, 22) indicate that the amount of exocytosed radiolabeled DNA may be negligible. However, the fate of DNA complexed with dendrimer and/or Av after internalization is still unclear.

In this study, we used an antisense oligonucleotide as the primer for c-erbB-2 and SHIN3 cancer cells, which do not show high expression of c-erbB-2. $^{111}$In-oligo-carrier complexes were delivered to the tumor cells by non-sequence-specific targeting both in vitro and in vivo. The high tumor accumulation of $^{111}$In-oligo complexes indicates that the oligonucleotide carriers used in the present studies, i.e., G4, G4-Av, and Av, have a possibility of being vectors in a variety of i.p. tumor-targeting gene therapies, irrespective of the sequence. Double-stranded DNAs or phosphorothioate oligonucleotides, which would be more stable in vivo (38), can be conjugated to these vectors. Conjugation with a plasmid containing reporter genes would also enable detection of expression of the delivered gene. Additionally, dendrimers have their own potential for modification through their surface amino groups, so as to form conjugate with a tumor-specific antibody and to create a tumor-specific vector (40, 41).

In conclusion, G4, G4-Av, and Av complexes had been shown to effectively deliver $^{111}$In-oligo to i.p.-disseminated tumors. These complexes have a great potential for modification and can be a vector for a variety of oligonucleotides. These $^{111}$In-oligo-carrier complexes can also be tracers for imaging of correlation of gene delivery and have enabled the first tumor images with $^{111}$In-labeled oligo to be obtained.

ACKNOWLEDGMENTS

We thank Nihon Mediphysics, Japan, for kindly providing InCl$_3$.

REFERENCES


Intraperitoneal Tumor Targeting of Antisense DNA

Tumor Targeting and Imaging of Intraperitoneal Tumors by Use of Antisense Oligo-DNA Complexed with Dendrimers and/or Avidin in Mice

Noriko Sato, Hisataka Kobayashi, Tsuneo Saga, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/7/11/3606

Cited articles
This article cites 41 articles, 10 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/7/11/3606.full#ref-list-1

Citing articles
This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://clincancerres.aacrjournals.org/content/7/11/3606.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, contact the AACR Publications Department at permissions@aacr.org.