Adenoviral-mediated Delivery of Gastrin-releasing Peptide Receptor Results in Specific Tumor Localization of a Bombesin Analogue in Vivo

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ABSTRACT

Radioimmunotherapy is hindered by a variety of factors linked to the utilization of monoclonal antibodies. These limitations include restricted tumor penetration as well as low levels of intratumoral antigen expression. To address the latter problem, we used a gene therapy approach to induce tumor cells to express enhanced levels of receptor with high binding affinity for a radiolabeled peptide. In this regard, a radiolabeled bombesin analogue was used in conjunction with a recombinant adenoviral vector encoding the murine gastrin-releasing peptide receptor (mGRPr). A panel of human carcinoma cell lines was infected in vitro with the recombinant adeno vector encoding the mGRPr vector to examine the induced binding of a 125I-labeled bombesin peptide. All cell lines examined displayed high levels of induced peptide binding, with approximately 60-80% of the radioactivity bound to the cells, in a live-cell binding assay. The human ovarian carcinoma cell line SKOV3.ip1 was chosen for in vivo analysis of radiolabeled bombesin analogue tumor localization in biodistribution and pharmacokinetic studies in athymic nude mice. Genetic induction of mGRPr in vivo resulted in selective tumor uptake of the radiolabeled peptide and high tumor: blood ratios. The biodistribution results compared favorably to those obtained with 111In-labeled c21 anti-erbB-2 monoclonal antibody in animals bearing i.p. SKOV3.ip1 tumors that endogenously express erbB-2. Thus, a novel method to combine gene transfer and radioimmunotherapy may result in augmented tumor cell targeting of radiopharmaceuticals.

INTRODUCTION

Conventional radioimmunotherapy is limited by a number of factors including poor distribution of monoclonal antibodies through solid tumors, generation of host antibodies against the administered antibody, and bone marrow toxicity related to high blood concentrations of radioactivity (1). In addition, many tumors have heterogeneous and/or low levels of expression of tumor-associated antigens that are the targets for the radiolabeled monoclonal antibody (2, 3). Therefore, novel approaches aimed at overcoming these limitations are of great importance. To this end, this study investigates the utility of combining an efficient in situ gene therapy delivery method with a radioimmunotherapy strategy that uses a cell surface receptor with a high affinity for a radiolabeled peptide.

Radiolabeled peptides have been used in cancer diagnostics for imaging and are currently being developed to target growth factor receptors such as somatostatin receptors (4), vasoactive intestinal peptide receptor (5), and epidermal growth factor receptor (6). Peptides have many advantages over commonly used monoclonal antibodies including their small size, high binding affinities, rapid tumor localization, better tumor penetration, rapid blood clearance, and higher tumor: normal tissue ratios (7, 8). To this end, we have previously demonstrated that recombinant adeno vector-mediated delivery of the thyrotropin-releasing hormone receptor induced expression in various carcinoma cell types in vitro. This subsequently allowed cells to bind a 3H-labeled thyrotropin-releasing hormone peptide (9). However, to date, successful 111In-labeling of the 3-amino acid peptide for this receptor has not been possible. Therefore, we sought to transduce tumor cells to express a receptor that would have a high binding affinity for a peptide that could be labeled with a therapeutic radionuclide. In this regard, the mGRPr was chosen due to its high binding affinity for the 14-amino acid peptide bombesin (10). Additionally, endogenous expression of this receptor is limited to a small subset of neuroendocrine cells in the pulmonary and gastrointestinal tracts. Therefore, ultimate clinical utility would not be hindered by localization of the radioisotope to nontumor targets.

We have previously demonstrated that a modified 7-amino

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2 To whom requests for reprints should be addressed, at Department of Radiation Oncology, University of Alabama, 1824 6th Avenue South, Birmingham, AL 35294.

The abbreviations used are: mGRPr, murine gastrin-releasing peptide receptor; GRPr, gastrin-releasing peptide receptor; AdCMVGRPr, recombinant adenoviral vector expressing mGRPr; AdCMV LacZ, recombinant adenoviral vector expressing the Escherichia coli β-galactosidase gene; AUC, area under the curve; CEA, carcinoembryonic antigen; Cmax, pharmacokinetic model-predicted maximum concentration; CMV, cytomegalovirus; mI/P, meta-iodobenzyl; 123I-mI/P-bombesin, 123I-mI/P-Des-Met1'-bombesin7-13NH2; mot, multiplicity of infection; % ID/g, percent injected dose/grain; pfu, plaque-forming unit; t1/2, halflife of absorption; tmax, time of maximal concentration; t1/2, initial elimination half-life; t1/2, terminal elimination half-life.
acid analogue of bombesin was capable of high affinity binding to mGRPr (11). Importantly, utilization of this modified analogue in vitro resulted in a longer retention time within cells stably transfected to express mGRPr, due to less dehalogenation compared with 125I-Tyr4-bombesin. Thus, we used adenoviral vector-mediated transfer of mGRPr in a panel of human carcinoma cell lines in vitro to demonstrate the feasibility of this approach in a wide variety of tumor types. Additionally, a well-characterized murine model of human ovarian carcinoma (12-14) was genetically induced to express mGRPr in vivo to target the tumor with the radiolabeled bombesin analogue. The biodistribution and pharmacokinetics of the radiolabeled bombesin analogue were analyzed in tumor nodules and in a panel of normal organs at various times after injection. This strategy represents a novel manner with which a gene therapy delivery approach may be used to sensitize tumor cells to the effects of radioimmunotherapy.

MATERIALS AND METHODS

Cell Lines. The OVCAR-3, U251MG, LS174T, D54MG, A427, and WiDr human carcinoma cell lines were obtained from the American Type Culture Collection (Rockville, MD). The human ovarian carcinoma cell line OV-4 was obtained from Timothy Eberline (Brigham and Women’s Hospital, Boston, MA). The human ovarian carcinoma cell line SKOV3.ipi was obtained from Janet Price (Baylor University, Houston, TX). The murine fibroblast cell line, BNR-11, that stably expresses mGRPr was obtained from J. Battey (National Cancer Institute, Bethesda, MD). All cell lines were maintained in the appropriate culture media supplemented with 10% fetal bovine serum (MediaTech, Herndon, VA), 200 µg/ml l-glutamine, 100 µg/ml penicillin, and 25 µg/ml streptomycin. All cell lines were cultured at 37°C in a humidified atmosphere with 5% CO2.

Construction of AdCMVGRPr. The adenovirus expressing mGRPr was prepared using the standard two-plasmid homologous recombination technique of Graham and Prevec (15). Briefly, a DNA fragment containing the mGRPr gene (provided by J. Battey) was subcloned into the polylinker of the adenoviral shuttle vector pACCMVpLpARS [ + ; provided by R. Gerard (Katholieke Universiteit Leuven, Leuven, Belgium)]. This plasmid provides promoter/initiation signals derived from the CMV early promoter/enhancer and polyadenylation signals from SV40. The resulting recombinant adenovirus shuttle plasmid pAC-mGRPr was used to derive an E1-deleted, replication-incompetent, recombinant adenovirus using standard methodologies (16). Briefly, the shuttle plasmid and the adenoviral packaging plasmid pJM17 [provided by F. Graham (McMaster University, Hamilton, Canada)] were cotransfected into the E1A transcomplementing cell line 293 using the commercial cationic liposome vector DOTAP (Life Technologies, Inc. Gaithersburg, MD). Transfected cells were maintained until the onset of cellular cytopathic effects. The newly generated recombinant adenovirus was plaque-purified three times. Validation of single plaque was accomplished by direct PCR using primers specific for mGRPr. The recombinant adenovirus encoding the mGRPr gene, AdCMVGRPr, was expanded within 293 cells and purified by CsCl gradient centrifugation (16). Genomic DNA derived from the recombinant adenovirus was subjected to digestion with various restriction endonucleases and analyzed by agarose gel electrophoresis. Wild-type adenovirus WT300 [provided by T. Shenk (Princeton University, Princeton, NJ)] was used as a control for analysis of genomic DNA derived from AdCMVGRPr. Adenoviral vectors were titered within cell line 293, using plaque assay techniques for direct determination of viral pfu.

In Vitro Radiolabeled Binding Assay. Infections with recombinant adenoviruses were performed as described elsewhere (17). Cells were harvested for radiolabeled binding assay 48 h after adenoviral-mediated infection by removal at 37°C with 4 mM EDTA and 0.05% KCl. Detached cells were washed once with 0.1% BSA and PBS (pH 7.2), enumerated, and resuspended at 1 x 10^7 cells/ml. Each set of cells was aliquoted into 100-µl samples in duplicate polystyrene test tubes after the addition of 100,000 cpm (100 µl) of 125I-Tyr4-bombesin (DuPont NEN Research Products, Boston, MA). Nonspecific binding was determined by the addition of 1,000-fold molar excess of unlabeled bombesin. The solutions were mixed at room temperature for 1 h, rinsed with 0.1% BSA and 0.2 mM EDTA in 0.1 M PBS (pH 7.2), and centrifuged at 1,700 x g for 10 min. The supernatant was removed, and the cells were counted in a gamma counter to determine the amount of bound radioactivity. Bound radioactivity was then plotted as the percentage of cpm bound to the cells versus the total amount of cpm added. The human ovarian carcinoma cell line SKOV3.ipi expresses the cell surface receptor for erbB-2. Therefore, a noninternalizing monoclonal antibody (e21) against a cell surface epitope of erbB-2 was used [provided by C. Richter King (Onocologix, Inc., Gaithersburg, MD); Ref. 18] because of the nonexistence of a monoclonal antibody reactive with mGRPr. The e21 antibody was 125I-labeled using the Iodo-Gen method (19) and used in an in vitro live-cell binding assay with SKOV3.ipi cells. Cells were harvested as described above and incubated for 1 h with 100 µl of a 2 µg/ml solution of 125I-labeled e21 antibody. Cells were washed, and bound radioactivity was determined as described above. Scatchard analyses were performed using BNR-11 cells, uninfected SKOV3.ipi cells, and SKOV3.ipi cells infected with 100 moi AdCMVGRPr. Various concentrations of 125I-Tyr4-bombesin (± excess cold mIP-bombesin as a blocking agent) were added to an equal number of cells in triplicate in one to four assays and allowed to incubate at 4°C for 2 h. The cells were rinsed, centrifuged, and counted in a gamma counter as described above. The Kd and Bmax values were determined using the computer program LIGAND (20).

In Vivo Biodistribution of Radiolabeled Peptide. All biodistribution studies were performed in female BALB/c athymic nude mice obtained at 4–5 weeks of age from Frederick Research Laboratory (Frederick, MD). The peptide Des-Met’4-bombesin(7–13)NH2 was synthesized using solid-phase peptide synthesis at the University of Alabama at Birmingham Comprehensive Cancer Center Peptide Synthesis and Analysis Shared Facility and labeled with 125I-mIP as described elsewhere (17). Mice were injected i.p. with 2 x 10^7 SKOV3.ipi human ovarian carcinoma cells. Five days after cell inoculation, mice were injected i.p. with 1 x 10^6 pfu of AdCMVGRPr. Animals were then injected i.p. 48 h later with 2 µCi of 125I-mIP-bombesin. It was shown elsewhere that 125I-mIP-bombesin and 125I-Tyr4-bombesin had similar binding to AdCMVGRPr-infected cells in

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vitro, whereas $^{125}$I-mIP-bombesin had superior tumor uptake in this ovarian cancer model in vivo (17). Mice were sacrificed at 0.5, 2, 5, 15, and 30 min and at 1, 4, 8, 12, and 24 h after peptide injection ($n = 4$–10/group), and the following organs were dissected: blood, heart, lungs, liver, stomach, small intestine, spleen, kidney, skin, bone, muscle, uterus, peritoneal lining, and thyroid. In addition, tumor nodules were removed from each mouse. All organs and tumors were weighed, and the activity was counted in a gamma counter. The % ID/g for each tissue was calculated, except for that of the thyroid, in which only the percent injected dose was determined.

**In Vivo** Biodistribution of Radiolabeled Antibody. The e21 antibody was used for in vivo biodistribution analysis. Mice were injected i.p. with $2 \times 10^7$ SKOV3.ip1 human ovarian carcinoma cells and injected i.p. 5 days later with $5 \mu$Ci $^{131}$I-labeled e21. Mice were sacrificed at 5 min, 1 and 12 h, and 1, 2, 4, and 6 days after antibody injection ($n = 6$/group). Tumor and normal organs were dissected and analyzed as described above.

**Statistical Analysis.** Descriptive statistics were calculated to examine the distribution and variation of time concentration of radiolabeled antibody and peptide and tissue:blood ratio data (means and SDs). Means at each specific time point were calculated from multiple mice data for tissue concentration and tissue:blood ratios. Mean concentration data (% ID/g) over time was used to develop the appropriate pharmacokinetic models. The variability in uptake of the peptide and antibody in tumor, blood, and other normal tissues required that individual pharmacokinetic models be developed for each. Some tissues had immediate uptake, whereas others had an absorption phase. Some tissues were modeled best by a one-compartment system, whereas others required a two-compartment system. All pharmacokinetic parameters were estimated using the nonlinear regression procedure of the Statistical Analysis System programs (21, 22). Selection of the appropriate model was based on Akaike’s information, the minimization of the model’s mean squared error and SE of the estimated model parameters, the proportion of variation explained by the model, and graphical diagnostics of the model fit.

Two different pharmacokinetic models, a two-compartment elimination model and a one-compartment model with an absorption phase, were used to fit the time-concentration curves for the antibody. A two-compartment elimination model was determined to be the best model for bone, kidney, peritoneal lining, liver, muscle, small intestine, spleen, stomach, tumor, and uterus data. For each of these, the elimination $t_{1/2}$ values for each compartment were calculated along with $C_{max}$ and AUC. A one-compartment model with an absorption phase was determined to be the best model for lung, skin, and stomach data. For each of these, elimination $t_{1/2}$ and $t_{1/2 \text{Abs}}$ values were calculated along with $C_{max}$, $t_{max}$, and AUC. A two-compartment elimination model was determined to be the best model for abdominal lining and muscle data. For each of these, the elimination $t_{1/2}$ values for each compartment were calculated along with $C_{max}$ and AUC. A two-compartment model with an absorption phase was the best model for blood, kidney, and small intestine data. For each of these, elimination $t_{1/2}$ values for each compartment and the $t_{1/2 \text{Abs}}$ value were calculated along with $C_{max}$, $t_{max}$, and AUC.

It should be expected that different tissues would have different time-concentration functions for a given radioligand. When this variability is compounded by looking at time concentrations across many tissues for two different radioligands, multiple models are required. For each tissue and radioligand, the most appropriate time-concentration curve must be found to
Table 1: Pharmacokinetic parameters for biodistribution of 125I-mIP-bombesin in athymic nude mice bearing SKOV3.ip1 tumors infected to express AdCMVGRPr

<table>
<thead>
<tr>
<th>Site</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (% ID/g)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2A&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2B&lt;/sub&gt; (h)</th>
<th>AUC (% ID × h/g)</th>
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<tr>
<td>Abdominal lining</td>
<td>47.7</td>
<td>–</td>
<td>–</td>
<td>0.1</td>
<td>6.5</td>
</tr>
<tr>
<td>Blood</td>
<td>1.5</td>
<td>0.6</td>
<td>0.07</td>
<td>0.8</td>
<td>6.7</td>
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<tr>
<td>Bone</td>
<td>1.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>7.9</td>
</tr>
<tr>
<td>Heart</td>
<td>0.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>6.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>15.3</td>
<td>0.5</td>
<td>0.1</td>
<td>0.4</td>
<td>4.7</td>
</tr>
<tr>
<td>Liver</td>
<td>9.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.5</td>
</tr>
<tr>
<td>Lung</td>
<td>1.3</td>
<td>0.05</td>
<td>0.01</td>
<td>–</td>
<td>4.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>3.6</td>
<td>–</td>
<td>–</td>
<td>0.6</td>
<td>7.7</td>
</tr>
<tr>
<td>Skin</td>
<td>1.0</td>
<td>0.4</td>
<td>0.07</td>
<td>–</td>
<td>3.7</td>
</tr>
<tr>
<td>Small intestine</td>
<td>41.7</td>
<td>0.3</td>
<td>0.06</td>
<td>0.6</td>
<td>8.3</td>
</tr>
<tr>
<td>Spleen</td>
<td>10.9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.6</td>
</tr>
<tr>
<td>Stomach</td>
<td>10.2</td>
<td>0.05</td>
<td>0.004</td>
<td>–</td>
<td>11.7</td>
</tr>
<tr>
<td>Tumor</td>
<td>25.6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5.2</td>
</tr>
<tr>
<td>Uterus</td>
<td>29.2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>4.3</td>
</tr>
</tbody>
</table>

RESULTS

Infection of Human Carcinoma Cell Lines with AdCMVGRPr. To evaluate the efficacy of genetically inducing a cell surface receptor on carcinoma cells, a recombinant adenoviral vector encoding the mGRPr under the control of the CMV promoter, AdCMVGRPr, was constructed. The obtained virus was structurally verified through PCR and restriction endonuclease digestion analysis (data not shown). Functional validation of the virus was performed through in vitro gene delivery methods. Human lung, colon, and ovarian carcinoma cell lines, along with human glioma cell lines, were infected with 10 or 100 pfu/cell AdCMVGRPr, 100 pfu/cell AdCMVLacZ, or left uninfected (Fig. 1B). Uninfected human carcinoma cell lines demonstrated binding of 31.8% of the added radioactivity, and infected cell lines with 10 or 100 pfu/cell AdCMVGRPr resulted in an increase of binding to 52.1 and 80.9%, respectively. Uninfected OV-4 and SKOV3.ip1 cells showed 7.9 and 15.1% binding, respectively. Increasing the moi to 100 resulted in an increased binding of radioactivity in both instances, with OV-4 cells binding 59.7%, and SKOV3.ip1 cells binding 70.6%. Scatchard analyses were performed using the SKOV3.ip1 cell line due to its relevance to our in vitro ovarian tumor model and compared to the BNR-11 cell line stably expressing mGRPr. 125I-Tyr<sup>4</sup>-bombesin had similar K<sub>d</sub> values when determined using BNR-11, uninfected SKOV3.ip1, and AdCMVGRPr-infected SKOV3.ip1 cells (0.50, 0.74, and 0.89 nM, respectively). The number of receptors/cell increased by greater than 20-fold when comparing uninfected SKOV3.ip1 cells (28,903) with AdCMVGRPr-infected SKOV3.ip1 cells (687,583). This number of receptors/cell on infected SKOV3.ip1 cells was more than 8-fold greater than the number of receptors/cell on the positive control BNR-11 cell line (78,532). These results demonstrate that the number of GRPrs/cell can be greatly increased by infecting cells with the AdCMVGRPr vector. Thus, ovarian carcinoma cell lines were also induced to express mGRPr after AdCMVGRPr infection and bound radiolabeled bombesin.

To further investigate the applicability of this approach, a panel of human ovarian carcinoma cell lines was tested for the ability to bind 125I-Tyr<sup>4</sup>-bombesin after AdCMVGRPr infection. The cell lines OVCAR-3, OV-4, and SKOV3.ip1 were infected with 10 or 100 pfu/cell AdCMVGRPr, 100 pfu/cell AdCMVLacZ, or left uninfected (Fig. 1B). Uninfected OVCAR-3 cells demonstrated binding of 31.8% of the added radioactivity; however, infection with 10 or 100 pfu/cell AdCMVGRPr resulted in an increase of binding to 52.1 and 80.9%, respectively. Uninfected OV-4 and SKOV3.ip1 cells showed 7.9 and 15.1% binding, respectively. The OV-4 and SKOV3.ip1 cells showed similar binding at 10 pfu/cell of 20 and 18.4%, respectively. Increasing the moi to 100 resulted in an increased binding of radioactivity in both instances, with OV-4 cells binding 59.7%, and SKOV3.ip1 cells binding 70.6%. Scatchard analyses were performed using the SKOV3.ip1 cell line due to its relevance to our in vitro ovarian tumor model and compared to the BNR-11 cell line stably expressing mGRPr. 125I-Tyr<sup>4</sup>-bombesin had similar K<sub>d</sub> values when determined using BNR-11, uninfected SKOV3.ip1, and AdCMVGRPr-infected SKOV3.ip1 cells (0.50, 0.74, and 0.89 nM, respectively). The number of receptors/cell increased by greater than 20-fold when comparing uninfected SKOV3.ip1 cells (28,903) with AdCMVGRPr-infected SKOV3.ip1 cells (687,583). This number of receptors/cell on infected SKOV3.ip1 cells was more than 8-fold greater than the number of receptors/cell on the positive control BNR-11 cell line (78,532). These results demonstrate that the number of GRPrs/cell can be greatly increased by infecting cells with the AdCMVGRPr vector. Thus, ovarian carcinoma cell lines were also induced to express mGRPr after AdCMVGRPr infection and bound radiolabeled bombesin. In contrast, infection of the ovarian carcinoma cells with the control virus AdCMVLacZ did not increase binding over that obtained with uninfected cells, except, for an unknown reason, in the instance of the OV-4 cells (binding increased from 7.9 to 22.1%). Therefore, it is likely that a wide variety of tumor types will be amenable to genetic radioisotope-targeting strategies. To compare the efficacy of our genetic radioisotope-targeting strat-
Incubation of the SKOV3.ip1 cells with $^{125}$I-labeled e21 resulted in 96.3% of the total radioactivity bound to the cells (data not shown). This indicated that these carcinoma cells were highly reactive to the e21 antibody and that both this cell line and the e21 antibody were suitable for in vivo analysis.

**Biodistribution of $^{125}$I-mIP-bombesin and $^{131}$I-labeled e21 Antibody.** The modified bombesin analogue, $^{125}$I-mIP-bombesin, was used for this in vivo study due to our previous report demonstrating that the tumor localization of $^{125}$I-mIP-bombesin at 4 h postinjection was much greater than the localization of $^{125}$I-Tyr$^4$-bombesin (17). Here we have characterized the biodistribution and pharmacokinetic profile of this bombesin analogue. In addition, a comparison to a radiolabeled monoclonal antibody was undertaken. The pharmacokinetic parameters and representative curves for the biodistribution of $^{125}$I-mIP-bombesin in tumor-bearing athymic nude mice are shown in Table 1 and Fig. 2. In these studies, mice were injected i.p. with SKOV3.ip1 tumor cells and subsequently injected i.p. with AdCMVORPr 5 days later. $^{125}$I-mIP-bombesin was administered 48 h after AdCMVORPr. The results for tumor, liver, and spleen were best fit by a one-compartment bolus model, whereas the blood data were best fit by a two-compartment absorption model. The highest concentration of $^{125}$I-mIP-bombesin in tumor was 33.6 ± 18.2% ID/g at 30 s after injection, which declined to 2.5 ± 2.8% ID/g at 24 h after injection (Fig. 2A). In control studies with AdCMVLaCZ injection or no virus injection (17), the tumor uptake of $^{125}$I-mIP-bombesin was 0.5 ± 0.1% ID/g at 4 h after injection, which was significantly lower than that after AdCMVORPr injection in this study (13.9 ± 7.7% ID/g; $P < 0.001$). The clearance $t_{1/2B}$ for tumor of the radiolabeled bombesin analogue was 5.2 h, whereas the $t_{1/2B}$ for blood was 6.7 h (Table 1). The clearance $t_{1/2B}$ for other tissues ranged from 3.5 to 11.7 h. The tumor had a much larger AUC than that of blood and most other normal organs.

The pharmacokinetic parameters obtained from the one-compartment bolus model (bone, heart, liver, spleen, and tumor), two-compartment bolus model (abdominal lining, muscle, stomach, and uterus), one-compartment absorption model (lung and skin), and two-compartment absorption model (blood, kidney, and small intestine) and representative curves for the $^{131}$I-labeled e21 anti-erbB-2 monoclonal antibody administered i.p. to animals bearing 7-day-old SKOV3.ip1 tumors without AdCMVORPr infection are shown in Table 2 and Fig. 3. The clearance $t_{1/2B}$ values for tumor (199.5 h) and $t_{1/2B}$ for blood (49.6 h) were considerably longer than the values obtained with $^{125}$I-mIP-bombesin (Fig. 3, A and B). The clearance $t_{1/2B}$ values of the antibody in other tissues were considerably longer than the values obtained with the radiolabeled bombesin analogue (Table 2). The highest concentration of e21 antibody in tumor was 100.7 ± 82.1% ID/g at 1 h after injection. This value declined to 12.8 ± 5.7% ID/g by 24 h. The tumor AUC was larger than that of all normal tissues. The tumor: blood ratios at various times postinjection for the radiolabeled bombesin analogue and the e21 antibody are shown in Fig. 4. The peak
Localization of Bombesin in GRPr-transduced Tumors

that for the antibody at 12 and 24 h, respectively, principally due to the lower blood concentration and more rapid clearance of the radiolabeled bombesin analogue from blood compared to that of the antibody. Thus, these results support the concept that utilization of a radiolabeled peptide would limit the bone marrow toxicity problem encountered with radiolabeled monoclonal antibodies that circulate in the blood for long periods of time.

**DISCUSSION**

Human ovarian carcinoma results in the most deaths from gynecologic malignancies in the United States. Women frequently present with end-stage disease confined within the peritoneal cavity. In a murine model of peritoneal tumor, Rowlinson et al. (23) demonstrated that i.p. delivery of a radiolabeled monoclonal antibody resulted in about a 50-fold advantage in tumor uptake when compared to i.v. administration, and higher tumor:blood ratios were seen at early time points with regional administration. Additionally, i.p. delivery has been demonstrated to be more efficient in women with ovarian carcinoma (24). Therefore, regional treatment of ovarian carcinoma with i.p. delivery of radiolabeled ligands is the preferred route of administration. Additionally, a variety of gene therapy strategies have recently been undertaken for the treatment of ovarian carcinoma; in a previous study, we reported that recombinant adenoviral vectors are highly efficient at in situ transduction of peritoneal ovarian tumor, with greater than 80% of the tumor cells expressing the transgene (13). Moreover, a well-characterized murine model of human ovarian carcinoma has been developed (12) and used by us and other investigators for a variety of gene therapy approaches (12-14). To this end, peritoneal tumor cell death has been accomplished with i.p. delivery of recombinant adenoviral vectors expressing either an intracellular single-chain antibody directed against the cell surface recep-tor erbB-2 (13) or the herpes simplex virus thymidine kinase gene (14). In both instances, we were able to elicit a therapeutic effect with reduced tumor burden and prolonged survival, indicating that sufficient tumor cell transduction had been accomplished. However, no evidence of complete remission was demonstrated in either instance; therefore, methods to enhance current gene therapy strategies are warranted.

Strategies to amplify the biological effects of genetic transduction events would potentially allow enhanced therapeutic efficacy of gene therapy methods. By linking tumor transduction to induced binding of radiolabeled peptides, it is hypothesized that this effect may be achieved, because cells in proximity to bound ligand may be killed as a result of exposure to the local radiation field. When used with radiation therapy, uniform systemic incorporation of the genetic construct into tumor cells may not be necessary. Therefore, we sought to combine the use of genetic induction of a receptor and targeting of a radiolabeled peptide. This is the first comprehensive study of the pharmacokinetics of in vivo localization using this approach.

Conventional radioimmunotherapy has typically used monoclonal antibodies directed against tumor-associated antigens. The major limitations of this approach include low tumor uptake and high blood concentrations of radiolabeled antibody, contributing to bone marrow toxicity and lack of homogeneous tumor distribution. Additionally, patients develop an immune response against the administered antibody. In an effort to overcome the low-level antigen expression problem, the utility of systemically administered cytokines for up-regulating the expression of tumor-associated antigens, which has resulted in increased localization of radiolabeled antibodies in both animal models and patients, has been investigated (25). In an alternative approach, we have undertaken a strategy whereby gene transfer methods were used to genetically induce the expression of a receptor on tumor cells to produce increased localization using this approach. This significantly increases the expression of a tumor-associated receptor, which has resulted in increased localization of radiolabeled ligand. In this regard, we have previously demonstrated that tumor cells can be genetically induced to express a nonendogenous surface receptor (26). We demonstrated that it was possible to express CEA via intratumoral inoculation of D54MG s.c. glioma xenografts with an adenoviral vector expressing CEA. These infected tumor nod-

### Table 2  Pharmacokinetic parameters for biodistribution of 125I-labeled e21 monoclonal antibody in athymic nude mice bearing SKOV3.ip1 tumors

<table>
<thead>
<tr>
<th>Site</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (% ID/g)</th>
<th>t&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2AB&lt;/sub&gt; (h)</th>
<th>t&lt;sub&gt;1/2DA&lt;/sub&gt; (h)</th>
<th>AUC (% ID × h/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal lining</td>
<td>16.4</td>
<td>-</td>
<td>0.08</td>
<td>5.4</td>
<td>225.1</td>
</tr>
<tr>
<td>Blood</td>
<td>5.7</td>
<td>0.7</td>
<td>-</td>
<td>49.8</td>
<td>413.4</td>
</tr>
<tr>
<td>Bone</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>35.9</td>
<td>207.9</td>
</tr>
<tr>
<td>Heart</td>
<td>2.0</td>
<td>1.0</td>
<td>0.1</td>
<td>10.0</td>
<td>210.3</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.5</td>
<td>-</td>
<td>-</td>
<td>10.4</td>
<td>244.9</td>
</tr>
<tr>
<td>Liver</td>
<td>5.2</td>
<td>-</td>
<td>-</td>
<td>190.2</td>
<td>188.4</td>
</tr>
<tr>
<td>Lung</td>
<td>3.5</td>
<td>0.7</td>
<td>0.08</td>
<td>-</td>
<td>36.9</td>
</tr>
<tr>
<td>Muscle</td>
<td>1.7</td>
<td>-</td>
<td>-</td>
<td>13.5</td>
<td>217.8</td>
</tr>
<tr>
<td>Skin</td>
<td>3.7</td>
<td>1.8</td>
<td>0.3</td>
<td>-</td>
<td>28.1</td>
</tr>
<tr>
<td>Small intestine</td>
<td>5.3</td>
<td>-</td>
<td>-</td>
<td>6.6</td>
<td>117.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>12.5</td>
<td>-</td>
<td>-</td>
<td>7.4</td>
<td>784.1</td>
</tr>
<tr>
<td>Stomach</td>
<td>21.1</td>
<td>-</td>
<td>-</td>
<td>10.7</td>
<td>362.7</td>
</tr>
<tr>
<td>Tumor</td>
<td>64.6</td>
<td>-</td>
<td>-</td>
<td>9.7</td>
<td>1413.0</td>
</tr>
<tr>
<td>Uterus</td>
<td>13.2</td>
<td>-</td>
<td>-</td>
<td>11.5</td>
<td>366.3</td>
</tr>
</tbody>
</table>

**Note:** AUC = area under the curve; % ID = percent of injected dose.
Fig. 4  Tumor:blood ratios of $^{125}$I-mIP-bombesin and $^{31}$I-labeled e21 monoclonal antibody in athymic nude mice. See the legends to Figs. 2 and 3 for a description of the experiments.

ules were successfully able to localize $^{131}$I-labeled anti-CEA antibody at a level equivalent to that of human colon cancer xenografts naturally expressing CEA, in comparison to animals injected with the control AdCMVLacZ virus, which did not demonstrate antibody uptake. Thus, we were able to show that in vivo transduction of tumor was possible and that this transduction would result in the expression of a nonendogenous cell surface receptor targeted by a radiolabeled monoclonal antibody.

The clinical utilization of monoclonal antibodies, however, is still going to elicit problems related to poor tumor penetration and bone marrow toxicity as described above. Additionally, we show here that utilization of the $^{131}$I-labeled e21 monoclonal antibody resulted in a longer clearance $t_{1/2}$ from blood and normal tissues when compared to that of the radiolabeled bombesin analogue. The same results would be expected if an anti-GRPr antibody were available for study. In an effort to overcome these limitations, we investigated the efficacy of radiolabeled peptides. Peptides offer many advantages over antibodies including high affinity binding (7), high vascular permeability (8), rapid tumor localization, and better tumor penetration (8). Furthermore, peptides have rapid blood clearance and lower immunogenicity. Additionally, we have shown that $^{125}$I-mIP-bombesin resulted in higher internalization and longer intracellular retention in mGRPr-expressing cells when compared to that of $^{125}$I-Tyr$^4$-bombesin (17). Thus, the higher tumor uptake of the bombesin analogue in vivo may have been a result of its higher degree of internalization and longer intracellular residence time. Through efficient in situ gene transfer, the results illustrate preferential transduction of tumor cells i.p. and selective uptake and retention of $^{125}$I-mIP-bombesin. In addition, comparison with an $^{131}$I-labeled monoclonal antibody reactive with the erbB-2 antigen on the human ovarian carcinoma cell line SKOV3.ip1 demonstrated that higher tumor: blood ratios were obtained with the radiolabeled peptide. Thus, these studies demonstrate the potential for in vivo transduction of tumor with new receptors to serve as a target for radiolabeled peptide therapy. In the context of a solid tumor growing in a human host, it may be possible to obtain high enough and uniform enough levels of viral infection and receptor expression
through the use of the conditional replication-enablement system for adenovirus (27, 28).

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Adenoviral-mediated delivery of gastrin-releasing peptide receptor results in specific tumor localization of a bombesin analogue in vivo.

M E Rosenfeld, B E Rogers, M B Khazaeli, et al.


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