Preclinical Evaluation of the Breast Cancer Cell-Binding Peptide, p160

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Abstract

Purpose: Selective delivery of drugs into the target tissue is expected to result in high drug concentrations in the tissue of interest and therefore enhanced drug efficacy. To develop a peptide-based radiopharmaceutical, we investigated the properties of a peptide with affinity for human breast cancer, which has been selected through phage display.

Experimental Design: The bioactivity of the p160 peptide (VPWMEPAYQRFL) was evaluated in vitro and in vivo. The specific binding to human breast cancer MDA-MB-435 cells was confirmed in competition experiments. Internalization of the peptide was investigated with confocal microscopy. Furthermore, the biodistribution of [131I]labeled p160 was studied in tumor-bearing mice. In vivo stability was evaluated at different periods after tracer administration using high-performance liquid chromatography analysis.

Results: The binding of [125I]labeled p160 was inhibited up to 95% by the unlabeled peptide with an IC50 value of 0.6 µmol/L. In addition, 40% of the total bound activity was found to be internalized into the human breast cancer cells. Although a rapid degradation was seen, biodistribution studies in nude mice showed a higher uptake in tumor than in most of the organs. Perfusion of the animals caused a reduction of the radioligand accumulation in the healthy tissues, whereas the tumor uptake remained constant. A comparison of [131I]p160 with a 131I-labeled Arg-Gly-Asp peptide revealed a higher tumor-to-organ ratio for [131I]p160.

Conclusions: p160 has properties that make it an attractive carrier for tumor imaging and the intracellular delivery of isotopes or chemotherapeutic drugs.

Breast cancer is the most common cancer in women representing >30% of malignancies in women. Because breast cancer may exist for a long period as noninvasive or invasive but nonmetastatic disease, there is an urgent need for early diagnosis and therapy in these patients. This is addressed by different diagnostic techniques, such as mammography, magnetic resonance imaging, and spectroscopy, scintigraphy, or positron emission tomography. Treatment of breast cancer is stage dependent and includes surgical management, radiation therapy, chemotherapy, and hormonal therapy, whereas different experimental approaches, such as gene therapy and antiangiogenic therapies, are under investigation (1).

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used in tumor imaging (6, 7), antiangiogenesis approaches (8), and tumor targeting with chemotherapeutic drugs or radio-nuclides (9).

The identification of new efficient peptides with specific targeting abilities and reduced background binding is a major challenge in cancer-related peptide research. Peptides with tumor affinity can be identified via selections using complex random peptide libraries, containing a high number of peptides that are displayed on bacteriophages (10). Phage display libraries have been used to select phages expressing peptides on their surface with organ- or tumor-binding specificity (11–13).

A potential candidate peptide with promising targeting properties is the p160 peptide (14). The peptide p160 (VPVMEPAYQRL), identified through random peptide phage display, is a linear dodecapeptide with specificity for the breast cancer cell line MDA-MB-435 and the neuroblastoma cell line WAC 2. The bacteriophage t160, displaying the peptide p160, was isolated by selection on the neuroblastoma cell line WAC 2. Inhibition of phage binding through the chemically synthesized p160 peptide determined that the phage binding to WAC 2 cells is mediated through the displayed peptide. In addition, confocal light microscopy studies revealed an internalization of t160 in WAC 2 neuroblastoma cells.

For in vivo application, the peptide has to be chemically synthesized and used without the phage backbone. In this study, we investigated the properties of the peptide p160 on the human breast cancer cell line MDA-MB-435 as the phages were found to have high affinity for this cell line. For a systematic investigation of the pharmacologic properties of p160, binding, internalization, and organ biodistribution studies were carried out. Affinity and binding kinetics of p160 were determined to internalization, and organ biodistribution studies were carried out. Affinity and binding kinetics of p160 were determined to study its cellular handling and to optimize its properties for drug targeting purposes.

**Materials and Methods**

**Peptides.** The peptides p160 (VPVMEPAYQRL) and D-p160 (all amino acids in D-isofrom) were obtained by solid-phase peptide synthesis using Fmoc coupling protocols (15). All standard reagents and solvents for the peptide synthesis were purchased from Merck (Darmstadt, Germany) or Novabiochem (Laueßlingen, Switzerland). The FITC-RGD-4C (CDCRGDCFC) peptide was obtained from Synthem (Nimèxe, France).

Radiolabeling of p160 and FITC-RGD-4C with $^{125}$I or $^{131}$I was done using the chloramine-T method (16). The radioisotopes Na$^{22}$ and Na$^{111}$ were obtained from Amersham Pharmacia Biotech (Freiburg, Germany). For conventional and confocal laser scanning microscopy studies, FITC was coupled via an additional lysine at the COOH terminus of p160.

**Cell lines.** All cell lines were cultivated at 37°C in a 5% CO$_2$ incubator. The human breast cancer cell line MDA-MB-435 (National Cancer Institute, Frederick, MD) was cultivated in RPMI 1640 with Glutamax containing 10% FCS (Invitrogen, Karlsruhe, Germany) and 25 mmol/L HEPES. The human breast cancer cell line MCF-7 (American Type Culture Collection, Manassas, VA) was cultivated in DMEM with Glutamax containing 10% FCS (Invitrogen) and 25 mmol/L HEPES. Human umbilical vein endothelial cells (HUVEC) were isolated as described (17) and cultivated on 1% gelatin-coated cell culture flasks using medium 199 (Invitrogen) containing 20% FCS, 2 mmol/L glutamine, 100 IU/ml penicillin, 100 IU/ml streptomycin, and 2 ng/ml basic fibroblast growth factor (Roche Diagnostics, Mannheim, Germany).

**In vitro binding experiments and competition experiments.** Binding assays were done using breast cancer MDA-MB-435, MCF-7, or HUVEC cells as target. For the in vitro experiments, cells were plated in six-well plates in 3 mL volumes of medium, supplemented with 10% FCS and 25 mmol/L HEPES, at a density of 400,000 cells per well. After 24 hours of cultivation, the medium was replaced by 1 ml fresh medium (without FCS) containing 1 × 10$^7$ to 2 × 10$^7$ cpm $^{125}$I-labeled p160 (3.6 × 10$^{-10}$–7.1 × 10$^{-10}$ mol/L) and incubation was done at 37°C. After 1 hour, incubation was stopped by removing the medium and washing the cells thrice with PBS. Subsequently, cells were lysed with 0.5 ml of 0.3 mol/L NaOH and the radioactivity was measured with a gamma counter and calculated as percent applied dose per 10$^6$ cells. Competition experiments were carried out using the unlabeled p160 peptide as inhibitor for radioligand binding at different concentrations (10$^{-4}$–10$^{-10}$ mol/L). Same binding experiments were carried out using the peptides D-p160 and octreotide as competitors for binding of the radioligand ($^{125}$I)p160. For kinetic analysis, $^{125}$I-labeled p160 was incubated with MDA-MB-435 breast cancer cells for different incubation periods varying from 10 minutes to 3 hours.

**Internalization experiments.** Internalization experiments were done as described (18, 19). Subconfluent cell cultures of MDA-MB-435 breast cancer cells were incubated with $^{125}$I-p160 for 60 minutes at 37°C and 4°C. Cells were incubated without or with an excess of unlabeled peptide (10$^{-3}$ mol/L). Cellular uptake was stopped by removing medium from the cells and washing thrice with 1 mL PBS. Subsequently, cells were washed twice with 1 ml of 20 mmol/L sodium acetate (pH 5.0) in PBS for 10 minutes at room temperature to remove the surface-bound radioactivity. The cells were washed again thrice with 1 ml ice-cold PBS and lysed with 0.5 ml NaOH (0.3 mol/L). Surface-bound and internalized radioactivity was measured with a gamma counter and calculated as percent applied dose per 10$^6$ cells.

**Confocal laser scanning microscopy using FITC-labeled p160.** For the confocal microscopy experiments, 50,000 MDA-MB-435 cells were seeded onto coverslips. After 24 hours of cultivation, the medium was replaced by fresh medium (without FCS) and FITC-Lys-p160 (10$^{-6}$ mol/L) was added to the cells. The FITC-labeled peptide was incubated with the cells for 30 minutes at 37°C. After incubation, the medium was removed and the cells were washed thrice with 1 mL medium. Subsequently, the cells were fixed with 2% formaldehyde for 20 minutes on ice. The cells were washed again thrice with medium and incubated with TO-PRO-3 (Molecular Probes, Eugene, OR, 1:1000 dilution, 30 minutes) to stain the cell nuclei. Finally, the cells were washed thrice with 1 mL PBS and the coverslips were put on slides using fluorescent mounting medium (DAKO, Carpinteria, CA). The experiments were also carried out using the unlabeled p160 peptide (10$^{-4}$ mol/L) as competitor for the binding of FITC-Lys-p160. Samples without FITC-Lys-p160 were analyzed to determine auto-fluorescence of the MDA-MB-435 cells. After treatment of the cells, confocal imaging was done on an inverted microscope (Leica DM IRBE) with a confocal laser scanning unit (Leica SP2 MP).

**In vivo studies.** Biodistribution was done on 9-week-old female BALB/c nu/nu mice obtained from Charles River WIGA (Sulzdorf, Germany) and housed in VentiRacks. For the in vivo studies, breast cancer MDA-MB-435 tumors were transplanted in Matrigel-Matrix (Falcon) s.c. into the anterior region of the mouse trunk. The tumors were allowed to grow for ~2 weeks to a volume of 1.0 cm$^3$, and 0.1 mL $^{131}$I-labeled p160 (~1 MBq) was injected via the tail vein. At 1 hour postinjection, the animals were sacrificed and dissected. Samples of tumor, blood, and selected tissues were removed, drained of blood, and weighed and the radioactivity in each organ was determined with a gamma counter (LB 951G, Berthold, Germany) and calculated as percent injected dose per gram tissue (%ID/g). To determine the uptake in blood-free organs, perfusion experiments were carried out. For the perfusion studies, $^{125}$I-p160 was injected in tumor carrying mice as described. At 1 hour postinjection, the animals were anesthetized by an i.p. injection of 5 mg Ketanest.
(Parke-Davis, Berlin, Germany) and 400 µL of 0.2% Rompun (BayerVital, Leverkusen, Germany). A catheter was put in the ascending aorta through a small cut in the left ventricle of the heart, and perfusion was done with 25 mL of 0.9% NaCl through a cut in the liver. After perfusion, samples of tumor and organs of interest were removed, weighed, and counted for radioactivity. Radioactivity concentration was expressed as %ID/g. Organ distribution of $^{131}$I[FITC-RCG-4C] was done without perfusion in BALB/c nu/nu mice carrying MDA-MB-435 breast cancer tumors. All animal experiments were carried out in conformity with the German law for protection of animals and are in compliance with European laws.

**Metabolism.** The serum stability of p160 was investigated by high-performance liquid chromatography. $^{131}$I-labeled p160 was injected in the tail vein of a BALB/c nu/nu mouse. At different time points varying from 1 to 60 minutes, blood samples were taken and centrifuged to collect serum. Serum was deproteinized by protein precipitation with equal volume of acetonitrile. After centrifugation for 15 minutes at 12,000 $\times$ g, serum proteins were pelleted and the supernatant was injected into an analytic LiChrosorb RP-select B 5 µm, 250 × 4 mm (Merck) high-performance liquid chromatography column (20). Tris/phosphate and methanol were used as eluents to separate the peptide and its fragments according to their hydrophilicity.

**Data analysis and statistics.** Statistical comparisons between groups were done by the unpaired Student’s t test using the Sigmatstat program (Jandel Scientific, Erkrath, Germany). $P \leq 0.05$ was considered statistically significant.

**Results**

**In vitro binding experiments and competition experiments.** To determine the affinity of p160, competition binding assays with the human breast cancer cell line MDA-MB-435 were done. For the characterization of the binding of p160, the peptide was synthesized by Fmoc solid-phase peptide synthesis and labeled with $^{125}$I. The radioligand $[^{125}\text{I}]$p160 was incubated with MDA-MB-435 cells for 1 hour at 37°C with or without unlabeled peptide as competitor ($10^{-4}$ mol/L). No FCS was present in the medium during the incubation to avoid degradation by serum proteins and to allow analysis of the peptide characteristics without the influence of additional variables. After incubation, the cells were washed and lysed and the bound radioactivity was calculated as percent applied dose per 10⁶ cells. The $^{125}$I-labeled p160 peptide showed a binding capacity of 1.5% to 2% of the applied dose per 10⁶ cells. The unlabeled peptide p160 at a

![Image](https://example.com/image1.png)
concentration of $10^{-4}$ mol/L caused an up to 95% decrease of the binding of the radioligand $^{125}\text{I}p160$. Using the peptides D-p160 and octreotide as competitor at the same concentration, the binding of the radioligand could not be competitively abolished (Fig. 1A). The same binding experiments with the breast cancer cell line MCF-7 revealed a binding capacity of $\sim 7\%$ of the applied dose per $10^6$ cells, which could be inhibited by unlabeled p160 but not by the peptides D-p160 and octreotide (Fig. 1C). Performing the binding experiments on HUVECs, the binding capacity was found to be lower. Only $0.4\%$ of the applied dose per $10^6$ cells was measured on the HUVEC cells. Furthermore, the binding of $^{125}\text{I}p160$ on HUVECs was not competitively abolished by the unlabeled p160 peptide at a concentration of $10^{-4}$ mol/L (Fig. 1B).

Kinetic studies of $^{125}\text{I}p160$ in MDA-MB-435 cells, with incubation periods varying from 5 minutes to 3 hours, revealed a time-dependent increase of the radioligand uptake for incubation periods up to 10 to 20 minutes. Thereafter, a time-dependent decrease of $^{125}\text{I}p160$ uptake was noticed, with the bound activity reduced to the background level after 3 hours of incubation (Fig. 1D).

The affinity of p160 in MDA-MB-435 cells was evaluated through competition studies using the unlabeled p160 peptide as competitor for radioligand binding at different concentrations varying from $10^{-4}$ to $10^{-10}$ mol/L. At a competitor concentration of $10^{-4}$ mol/L, up to 95% of the binding of $^{125}\text{I}p160$ were inhibited. At concentrations $<10^{-9}$ to $10^{-10}$ mol/L, the bound activity reached the level of un competitively bound. Evaluation of the competition binding data gave an $K_d$ value of $0.6 \pm 0.9$ $\mu\text{mol/L}$ with a $K_d$ of 0.86 $\mu\text{mol/L}$ (Fig. 2). The Scatchard analysis of the data led to a maximum number of binding sites of $1.03 \times 10^{-11}$ $\mu\text{mol/cell}$.

**Internalization and confocal laser scanning microscopy.** In vitro internalization studies were done to determine the rate of internalization of $^{125}\text{I}p160$ in MDA-MB-435 human breast cancer cells. After 1-hour incubation at $37^\circ\text{C}$, the cells were subjected to an acid wash procedure with sodium acetate (pH 5.0) to remove membrane-bound ligand molecules. Internalization was investigated both under conditions where internalization is active ($37^\circ\text{C}$) and under conditions where it is suppressed ($4^\circ\text{C}$). To determine nonspecific uptake, incubation with an excess of the unlabeled p160 peptide ($10^{-4}$ mol/L) was done. The results of the internalization experiments at $37^\circ\text{C}$ showed that 40% of the total bound activity were found to be internalized into the MDA-MB-435 cells (Fig. 3). After incubation at $37^\circ\text{C}$ with an excess of the unlabeled peptide, the internalized activity was measured to be decreased to the level of 2.5% of the internalized activity without the presence of competitor. After 1-hour incubation at $4^\circ\text{C}$, the internalization of $^{125}\text{I}p160$ was strongly suppressed to the value of 5% to 10% of the internalized activity at $37^\circ\text{C}$, whereas the presence of the unlabeled peptide at a concentration of $10^{-4}$ mol/L resulted in a total suppression of the radioligand uptake (Fig. 3).

The results of the binding and internalization studies of the radioactive p160 were confirmed by the results of the confocal laser microscopy studies. After 30-minute incubation of the FITC-Lys-p160 with MDA-MB-435 cells, an intensive fluorescence signal in the cells was found. Further investigation revealed a concentration of the fluorescence in irregular clusters at the periphery of the cells (Fig. 4A). After incubation with excess of the unlabeled p160 peptide as competitor, no FITC fluorescence was detected (Fig. 4B). To exclude autofluorescence of the MDA-MB-435 cells, confocal laser microscopy studies were done without treatment of the cells with FITC-labeled p160, revealing no fluorescence signal at all (data not shown).

**Metabolism.** In vivo investigation of the stability of p160 in serum was done through injection of $^{131}\text{I}$-labeled p160 in mice and high-performance liquid chromatography analysis of serum samples taken from the animal at different time periods. The stability studies revealed a fast degradation of p160 by serum proteases. Immediately after injection, only the full-length peptide eluted at 17.7 minutes (Fig. 5). After 2-minute circulation of $^{131}\text{I}p160$ in blood, a first degradation product of p160 was detected yielding a fragment that eluted at 13.2 minutes. After 5-minute circulation in the blood, a second
A fragment appeared at 4.0 minutes, and the amount of this fragment increased with time. After 30 minutes, only the 4-minute fragment remained and was still present after 1 hour.

**In vivo studies.** Biodistribution experiments of p160 labeled with $^{131}$I were done in female BALB/c nu/nu mice carrying human breast cancer MDA-MB-435 tumors s.c. into the trunk. The organ biodistribution in MDA-MB-435 tumor carrying mice at 1 hour after i.v. injection of $^{[131]}$I-p160 showed a tumor accumulation of 6%ID/g (Fig. 6). The uptake in the tumor was higher than in heart, spleen, liver, and brain and almost the same compared with the kidneys. Only the blood value (8%) was higher than the accumulation in the tumor (data not shown). The lung tissue showed almost the same radioactivity concentration as the tumor tissue, but the perfusion experiments showed a >60% decrease in lung radioactivity. To reduce blood background in the tumor and the other organs, biodistribution studies were done followed by perfusion of the mice with 0.9% NaCl. The perfusion experiments showed a reduction of the uptake in all organs, whereas the uptake in the tumor remained almost constant (Fig. 6). Heart, lung, and liver showed a statistically significant decrease of unperfused to perfused organ with $P < 0.005$. The reduction of the uptake in the healthy tissues but not in the tumor after perfusion results in an increase of the tumor-to-organ ratios. The tumor-to-liver ratio was 1.5 before perfusion and 4 after perfusion (data not shown).

Organ distribution of $^{[131]}$IFITC-RGD-4C in BALB/c nu/nu mice, carrying MDA-MB-435 breast cancer tumors, revealed a similar RGD-4C uptake in the tumor and most of the organs (blood, heart, spleen, and brain) like $^{[131]}$I-p160. RGD-4C showed a higher accumulation in kidney and liver (data not shown), resulting in lower tumor-to-organ ratios. The tumor-to-kidney ratio of $^{[131]}$IFITC-RGD-4C was calculated to be 10 times lower than the tumor-to-kidney ratio of $^{[131]}$I-p160. The tumor-to-liver ratio of $^{[131]}$IFITC-RGD-4C was also only half the tumor-to-liver ratio of $^{[131]}$I-p160 (Table 1).

**Discussion**

Although chemotherapy and hormonal therapy play an important role in the treatment of breast cancer, the results of prospective studies show that a significant number of patients does not respond to these therapeutic modalities (21). Drug resistance, in addition to side effects of chemotherapy and hormonal therapy, necessitates the search for specific tumor targeting agents. A novel target for the treatment of breast cancer is the HER-2 protein, which has been reported to be overexpressed in breast malignancies and is associated with aggressive tumor growth (22). In this respect, a tumor-specific liposome system has been developed for the delivery of anti-HER-2 antisense oligonucleotides, which are known to sensitize breast cancer cells to chemotherapy (23). The specific targeting of drug-carrying liposomes into breast cancer cells required an
addition of folate receptor ligands on the surface of the liposomes, as it is known that the human α-isof orm folate receptor is overexpressed in tumors (24). This example of how tumor specificity can promote tumor therapy shows the importance of the identification of molecules specifically binding to breast cancer cells. These can offer new specific ligands that can be conjugated on liposomes and play the role of lead structure for efficient tumor targeting.

P160 is a peptide with specificity for the breast cancer cell line MDA-MB-435 identified through phage display by Zhang et al. (14). The peptide p160 was chosen for further investigation among the other peptides identified by Zhang et al. because of its high specificity. Binding studies with phages expressing the p160 motif revealed that from various tumor and normal cell lines only the breast cancer cell line MDA-MB-435 and a subset of neuroblastoma cell lines were targeted. Further evaluation of the properties of the chemically synthesized p160 showed that this specificity was mediated by the peptide moiety of the phages expressing p160. Our data show that p160 binds to the MDA-MB-435 breast cancer cells but does not bind to primary endothelial HUVEC cells.

The hypothesis that the cellular binding of p160 could be mediated through a specific receptor was strongly supported by two results. First, the uptake in MDA-MB-435 breast cancer cells was reduced with increasing concentration of the unlabeled p160 peptide as competitor, whereas studies with unspecific competitors like octreotide and D-p160 showed no effect. Second, we found evidence for an internalization of the radiolabeled and the FITC-labeled p160, which was inhibited in the presence of the unlabeled peptide. This result indicates that a receptor-mediated process might be involved in the internalization of p160. This hypothesis was sustained by internalization studies at 4°C, revealing a suppression of the internalization as expected for a receptor-mediated endocytic process. Confocal laser scanning microscopy showed a concentration of FITC-Lys-p160 in irregular clusters at the periphery of the cells, which might be caused by the accumulation of peptide-receptor complexes in endocytosis-specialized areas of the cell membrane.

A prerequisite for the use of an agent as targeting vehicle is a selective binding to the tissue of interest and limited uptake by healthy tissues. After i.v. administration in tumor-bearing mice, [131I]p160 showed a higher uptake in tumors than in most normal organs. In addition, perfusion experiments showed a specific binding to tumor tissue. The perfusion decreased the uptake selectively in the normal tissues, resulting in higher tumor-to-organ ratios. This indicates the contribution of the blood pool to the high radioactivity values especially in the highly perfused organs lung and kidney. Consequently, these tissues showed the greatest decrease after perfusion, although the radioactivity level in the tumor remained almost constant. The perfusion experiment also suggests specific uptake into tumor tissue and only unspecific accumulation in the other organs. Although the perfusion has no therapeutic relevance, it reveals peptide characteristics necessary to generate a stable and more active compound. The high uptake in the kidneys can be explained by renal excretion of the peptide. The elevated blood values might be explained by various mechanisms. One possibility is the interaction of p160 with serum proteins such as albumin. Another possibility could be the rapid degradation of 131I-labeled p160, which might lead to labeled peptide fragments that are unable to bind to the tumor but circulate in the bloodstream. The instability of p160 is not uncommon. Peptides, identified through phage display technology, are presented at the surface of filamentous bacteriophages and are therefore protected against proteolysis. The chemically synthesized linear peptides are not shielded by the macromolecular

![Fig.5. Metabolites analysis by high-performance liquid chromatography in serum collected at time points from 2 to 60 minutes after i.v. injection of 131I-labeled p160. Before chromatography, the serum proteins were precipitated with acetonitrile and centrifugation at 12,000 × g for 15 minutes. t0 = 0 minute.](cancer therapy: preclinical)

![Fig.6. Organ distribution of p160 in female BALB/c nu/nu mice carrying MDA-MB-435 tumors. Black columns, activity concentration (%ID/g) in tumor and control organs after 1-hour circulation of 131I-labeled p160 in the mice. Gray columns, radioactivity concentration (%ID/g) in tumor and control organs after perfusion of the animals (n = 3 animals per experiment).](cancer therapy: preclinical)
Table 1. Tumor-to-organ ratios calculated from the organ distribution of $^{[131]}	extit{I}$p160 in unperfused female BALB/c nu/nu mice carrying MDA-MB-435 tumors ($n = 7$ animals) and the organ distribution of $^{[131]}	extit{I}$FITC-RGD-4C in unperfused BALB/c nu/nu mice carrying MDA-MB-435 tumors ($n = 3$ animals)

<table>
<thead>
<tr>
<th>Tumor-to-organ ratio</th>
<th>$^{[131]}	extit{I}$p160</th>
<th>$^{[131]}	extit{I}$FITC-RGD-4C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>2.25</td>
<td>1.95</td>
</tr>
<tr>
<td>Lung</td>
<td>0.89</td>
<td>0.93</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.39</td>
<td>1.91</td>
</tr>
<tr>
<td>Liver</td>
<td>2.04</td>
<td>1.05</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.98</td>
<td>0.09</td>
</tr>
<tr>
<td>Muscle</td>
<td>3.68</td>
<td>3.23</td>
</tr>
<tr>
<td>Brain</td>
<td>177</td>
<td>21.8</td>
</tr>
</tbody>
</table>

phage, which might result in reduced serum stability (25). Therefore, one major issue of further investigation is the stabilization of p160. In this respect, different methods can be used. One of those is the cyclization of the peptide through a disulfide bridge between two conjugated cysteine molecules at the NH$_2$- and COOH-terminal of p160, whereas a second method is the exchange of amino acids with unnatural amino acids that cannot be recognized by serum proteases, like D-amino acids or N-methylated amino acids. PEGylation is another modification that could be exploited. PEGylation has been used successfully to prolong peptide half-life in vivo (26). The use of peptides coupled to liposomes or other carriers might prove to be favorable in two ways: the large liposome particle might protect the peptide against degradation, as in the case of the phage-bound peptide, and the peptide could facilitate specific accumulation of the particle in the tumor (27).

The biodistribution of p160 was compared with the biodistribution of the RGD-4C peptide. The three- amino acid motif RGD is known to bind integrins (28, 29). The RGD-4C peptide was compared with p160 in this study for two reasons. First, the MDA-MB-435 cells are known to express integrins on their surface and are potential targets for RGD peptides (30). Second, the RGD-4C peptide was found to accumulate to integrin-expressing tumors using in vivo phage display (31). The comparison revealed a similar organ distribution of p160 and the RGD-4C peptide, except for a higher liver and kidney accumulation of RGD-4C. It has been shown that the pharmacokinetics of RGD peptides can be improved in several ways. Particularly, glycosylation of a cyclic RGD peptide resulted in significantly reduced liver uptake and increased tumor accumulation (32). Coupling of the chelator diethylentriaminepentaacetic acid to RGD caused a shift from predominantly liver clearance to renal clearance (33). These and other modifications led to the development of attractive RGD peptides for tumor therapy and can be used as model for further development of p160. However, it should be noted that despite its rapid degradation the unmodified p160 reached similar uptake values as RGD-4C and may therefore have great potential in further development. To allow use of p160 as a tumor imaging agent, certainly, the radioactivity level in the blood needs to be reduced. A first step will be the addition of a chelator to the peptide to allow labeling with radiometals. Chelated radiometal complexes are usually very firmly attached; therefore, the noise caused by deiodination events will be eliminated. In addition, the interaction of p160 with serum proteins has to be analyzed to assess a possible depot effect. An improvement of peptide stability will allow to take advantage of the rapid blood clearance typical for small peptides.

In conclusion, the peptide p160 seems to be a promising candidate for tumor imaging and cancer therapy. The uptake experiments in vitro show that the binding of the radiolabeled peptide to the breast cancer cells might be mediated through a specific receptor, whereas the organ distribution in tumor-bearing mice showed a higher binding to the tumor than to the other organs, which is favorable for cancer treatment.

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