A New Prostate Carcinoma Binding Peptide (DUP-1) for Tumor Imaging and Therapy

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ABSTRACT

Purpose: Prostate carcinomas belong to the most widespread tumors, and their number is increasing. Imaging modalities used for diagnosis, such as ultrasound, computed tomography, and positron emission tomography, often produce poor results. Radiolabeled peptides with high sensitivity and specificity for prostate cancer would be a desirable tool for tumor diagnosis and treatment.

Experimental Design: We used phage display and the prostate-specific membrane antigen–negative cell line DU-145 to identify a peptide. The isolated DUP-1 was tested in vitro for its binding specificity, kinetics, and affinity. Internalization of the peptide was evaluated with confocal microscopy. The tumor accumulation in a nude mouse model was analyzed with 131I-labeled DUP-1 in PC-3 and DU-145 prostate tumors as well as in the rat prostate tumor model AT-1.

Results: The synthesized peptide showed rapid binding kinetics peaking at 10 minutes. It shows specific binding to prostate carcinoma cells but low binding affinity to non-tumor cells. Peptide binding is competed with unlabeled DUP-1, and a time-dependent internalization into DU-145 cells was shown. Biodistribution studies of DUP-1 in nude mice with s.c. transplanted DU-145 and PC-3 tumors showed a tumor accumulation of 5% and 7% injected dose per gram, and bound peptide could not be removed by perfusion. The rat prostate tumor model showed an increase of radioactivity in the prostate tumor up to 300% in comparison with normal prostate tissue.

Conclusions: DUP-1 holds promise as a lead peptide structure applicable in the development of new diagnostic tracers or anticancer agents that specifically target prostate carcinoma.

INTRODUCTION

More than 30% of newly diagnosed cancers in males are prostate cancer, making it a leading cause of death from cancer worldwide (1, 2). Despite the initial effectiveness of hormone therapy, many patients with metastatic disease eventually progress to an androgen-resistant state (3). Various new treatment modalities are currently being developed, but none has yet shown a survival benefit in patients with hormone-refractory prostate cancer (4).

New imaging procedures for diagnosis, treatment planning, and therapy of prostate cancer are necessary for accurate staging because current imaging methods are not satisfying i.e., positron emission tomography with 2-[18F]fluoro-2-deoxyglucose does not allow metabolic labeling in the majority of untreated primary prostate cancers (5). Preliminary studies using 11C-choline show potential for the primary staging of prostate cancer, but these findings have to be confirmed in larger clinical studies (6).

Molecular therapeutic approaches use PSMA as target molecule (8). PSMA is a transmembrane folate hydrolase with enhanced expression in prostate cancer tissue in comparison with benign and neoplastic epithelial prostate cells (9, 10). A weak extraprostatic expression of the protein has been noted in small intestine mucosa, brain, salivary glands, and a subset of renal proximal tubules (11, 12). Therefore, the monoclonal antibody Hu591, which recognizes the extracellular domain of PSMA, has been used for treatment (13, 14). However, heterogeneous expression of the target structure may lead to treatment failure (15).

Peptides are promising molecules to deliver radionuclides or therapeutic drugs into tumors. The application of a tumor-selective peptide requires enough binding sites, such as overexpressed receptors, high affinity of the ligand, and sufficient in vivo stability. Peptides that have by now been examined in detail are somatostatin (16), gastrin (17), luteinizing hormone-releasing hormone (18, 19), and bombesin (20, 21). The most prominent example for a tumor-specific peptide is octreotide (Sandostatin; ref. 22), which recognizes mainly the somatostatin receptor subtype 2, and is used for diagnosis (23) as well as for radioligand therapy (24, 25). Peptides also facilitate selective transport of cytotoxic compounds into tumor tissue. For example, the conjugation of a somatostatin analogue to the topoisomerase inhibitors doxorubicin or 2-pyrrolino doxorubicin resulted in an effective growth inhibition of somatostatin receptor–expressing tumors in vivo (26, 27). The coupling of doxorubicin to the luteinizing hormone-releasing hormone, a peptide with 10 amino acids, was evaluated in human epithelial
ovarian cancers (28). In a nude mouse model with luteinizing hormone-releasing hormone–expressing prostate tumors, the cytotoxic luteinizing hormone-releasing hormone analogue reduced tumor growth by 62% compared with castrated animals (29). Even large peptide nucleic acid sequences conjugated with octreotide are selectively taken up by somatostatin receptor–expressing tumors leading to the suppression of oncogene expression (30, 31).

In this study, a new peptide with specificity for PSMA-negative prostate tumor cell lines, such as DU-145 and PC-3, was identified by phage display techniques. Affinity and kinetics of this peptide were determined in cell binding assays. Confocal microscopy showed the internalization of the peptide in a time-dependent manner. Biodistribution experiments in DU-145 and PC-3 tumor carrying nude mice and rats were done showing high uptake of the peptide in tumor tissue, recommending this peptide as a promising lead structure for improved targeting of prostate carcinomas.

MATERIAL AND METHODS

Cell Lines. The human prostate carcinoma cell lines DU-145 and PC-3 (both American Type Culture Collection, Manassas, VA), the human prostate cell line (normal, immortalized with SV40) PNT-2 (European Collection of Animal Cell Cultures, Salisbury, United Kingdom), and the human embryonic kidney cell line 293 (American Type Culture Collection) were cultivated at 37°C in a 5% CO2 incubator in RPMI 1640 with Glutamax containing 10% FCS (both Invitrogen, Karlsruhe, Germany) and 25 mmol/L HEPES, Dunning R3327 subline AT-1 (American Type Culture Collection) tumors cells were cultivated in RPMI 1640 (Life Technologies, Eggenstein, Germany) supplemented with 292 mg/L glutamine, 100 IU/mL penicillin, 100 mg/L streptomycin, and 10% FCS. Human umbilical vein endothelial cells were isolated as described in the literature (32). Cultivation was done on gelatin (1%)–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).

Selection of Tumor Cell Binding Peptides. The phage display library was a linear 12–amino acid peptide library (Ph.D.12, New England Biolabs, Beverly, MA). Each selection round was conducted as follows: 1011 transducing units were added to 293 cells for a negative selection. After 1 hour, the medium was collected and centrifuged for 5 minutes at 1,500 rpm, and the supernatant was transferred to DU-145 cells grown up to a size of 90% confluency. After 1 hour, the cells were washed four times with 10 mL HBSS(+)(Invitrogen) + 1% bovine serum albumin (BSA) and four times with 10 mL HBSS(−) + 1% BSA. The cells were then detached with 4 mL PBS + 1 mmol/L EDTA for 5 minutes and centrifuged for 5 minutes at 1,500 rpm. The cell pellet was washed thrice in 1 mL HBSS(−) + 1% BSA and lysed with 1% Triton X-100. Lysate (10 μL) was used for titration of the phages. The remaining lysate was amplified in 50 mL ER2537 bacteria according to the manufacturer’s protocol. For the next selection round, 1011 transducing units from the previous selection round were used. Six selection rounds were done followed by ssDNA isolation from clones (QIAprep Spin M13 Kit, Qiagen, Hilden, Germany). The peptide was identified by sequencing.

Animals and Tumor Growth. Male 6-week-old BALB/c nu/nu mice and the male Copenhagen rats weighing 220 to 250 g were obtained from Charles River WIGA (Sulzfeld, Germany) and housed in VentiRacks. For inoculation of the tumors in nude mice, a Matrigel matrix/cell suspension (5 × 106 cells) was injected s.c. into the anterior region of the mouse trunk. Tumors were grown up to a size of 1.0 cm3. The rat prostate adenocarcinoma Dunning R3327 subline AT-1 (American Type Culture Collection) was transplanted s.c. into the leg of the Copenhagen rats by using a tumor piece (4 mm3) from a rat host. All animals were cared for according to the German animal guidelines.

Peptide. The DUP-1 peptide (FRPNRAQDYNTN) was obtained by solid-phase peptide synthesis using Fmoc chemistry. The radiolabeling was achieved by iodination using the chloramine-T method (33). The labeled peptide was purified by high-pressure liquid chromatography on a LiChrosorb RP-select B 5 μm, 25 × 4 mm column (Merck, Darmstadt, Germany) using Tris-phosphate and methanol as eluents. The specific activities obtained were 90 GBq/μmol for the 125I-labeled peptide and 110 GBq/μmol for the 131I-labeled peptide. For fluorescence microscopy, FITC was coupled via an additional lysine at the COOH terminus.

In vitro Binding Experiments. Cells (n = 200,000) were seeded into six-well plates and cultivated for 24 hours. The medium was replaced by 1 mL fresh medium (without FCS). When using the competitor, unlabeled peptide (10−4 – 10−11 mol/L) was preincubated for 30 minutes. 125I-labeled peptide was added to the cell culture (1–2 × 106 cpmp per well) and incubated for the appropriate incubation times varying from 1 minute to 4 hours. The cells were washed thrice with 1 mL PBS and subsequently lysed with 0.3 mol/L NaOH (0.5 mL). Radioactivity was determined with a γ-counter and calculated as percentage applied dose per 106 cells. If BSA or dry milk powder were used as blocking agents, it was added to a final concentration of 1% in medium without FCS.

Stability Experiments. Serum stability measurements were done with unlabeled and 131I-labeled DUP-1. Aliquots of the peptide were incubated in human serum for several time points at room temperature or 37°C. After incubation, 1 volume of acetonitrile was added to the sample to precipitate serum proteins, which were pelleted by centrifugation. The supernatant was then analyzed by reverse-phase high-pressure liquid chromatography. Samples of were taken and analyzed by matrix-assisted laser desorption ionization-time of flight mass spectrometry.

Conventional and Confocal Laser Scanning Microscopy Using FITC-Labeled DUP-1. DU-145 cells were seeded subconfluently onto coverslips and cultivated for 24 hours. The medium was replaced by fresh medium (without FCS). For microscopy, FITC-Lys-DUP-1 (10−5 mol/L) was added to the medium and incubated for 10 and 60 minutes at 37°C. Subsequently, the cells were washed with 1 mL medium and fixed with 2% formaldehyde for 20 minutes on ice. For the pulse-chase experiment with confocal laser scanning microscopy, 5 × 10−4 mol/L FITC-Lys-DUP-1 were added.
to the medium for 10 minutes. The cells were washed thrice with 1 mL PBS and incubated with 1 mL fresh medium containing $5 \times 10^{-5} $ mol/L dextran-Alexa568 (10,000 molecular weight, fixable, Molecular Probes, Eugene, OR) for time points from 10 to 60 minutes. Subsequently, the cells were fixed, incubated with TO-PRO-3 (Molecular Probes, 1:1,000 dilution, 20 minutes) for cell nucleus staining. Then, the cells were analyzed using a Leica SP1 CLSM (Leica Microsystems Heidelberg, Mannheim, Germany).

**Organ Distribution with Radioiodinated DUP-1.** $^{131}$I-DUP-1 was injected i.v. into male nu/nu mice (2.8 $\times$ 10$^7$ cpm per mouse), carrying the s.c. transplanted human prostate tumors DU-145 or PC-3. After 5, 15, 45, and 135 minutes postinjection, the mice were sacrificed. The organs were removed and weighed and the radioactivity was determined using an automated NaI(Tl) well counter (CobraII, Canberra Packard, Meriden, CT). The percentage of injected dose per gram (ID/g) of tissue was calculated. For the perfusion experiments, the mice were anesthetized with 5 mg Ketanest (Parke-Davis, Berlin, Germany) and 400 $\mu$L of 0.2% Rompun (BayerVital, Leverkusen, Germany) both injected i.p. After full anesthesia, the mice were perfused through the heart with 0.9% NaCl (25 mL) and tumor and control organs were removed and weighed. For the biodistribution in male COP rats bearing Dunning R3327 subtype AT-1 tumors, $^{131}$I-DUP-1 (5 $\times$ 10$^7$ cpm per rat) was injected i.v., the animals were sacrificed after 5 and 15 minutes, and the organs were removed and weighed.

**RESULTS**

Selection of a Peptide Binding to the Prostate Carcinoma Cell Line DU-145. For the selection of tumor specific peptides, in vitro selection rounds were done on DU-145 cells. For each round, $\sim 10^{11}$ transducing units of the phages were added to a cell culture dish with 293 cells for a negative selection for 60 minutes followed by positive selection with DU-145 cells for 60 minutes. The unbound phages were washed off and bound phages were recovered by lysing the DU-145 cells. After six rounds, single phage clones were selected and amplified and ssDNA was isolated for sequencing. Among 24 clones sequenced, all peptides showed the same sequence. For in vitro evaluation of the peptide, we used the identified peptide as its inverse form. Because the inverse peptide showed up to 40% better binding, we used the FRPNRAQDYNTN (DUP-1) peptide for further evaluation (Fig. 1A).

**Binding, Competition, Binding Kinetics, and Stability of DUP-1 to Prostate Tumor Cell Lines.** The peptide DUP-1 was prepared by Fmoc solid-phase synthesis and labeled with $^{125}$I. Cells were incubated for 10 minutes with the $^{125}$I-labeled peptide with or without unlabeled peptide as competitor (10$^{-4}$ mol/L). Radioactivity of the lysed cells was calculated as percentage applied dose per 10$^6$ cells. A high binding capacity with up to 12% of the applied dose per 10$^6$ cells was found for the prostate tumor cells DU-145 and PC-3 but not for the immortalized benign prostate cells PNT-2 or for the primary cultures of human umbilical vein endothelial cells (Fig. 1B).

The binding capacity of PC-3 cells was not significantly higher in comparison with DU-145 cells. To show the specificity of this binding, cells were preincubated with $10^{-4}$ mol/L DUP-1 followed by the radiolabeled DUP-1. The recovered lysates showed that the unlabeled DUP-1 could competitively inhibit (up to 95%) $^{125}$I-DUP-1 binding. Unlabeled octreotide at the same concentration did not prevent $^{125}$I-DUP-1 from binding (data not shown). The DUP-1 peptide, which contains of the same amino acids but in their D-conformation instead of the L-conformation, did not bind to DU-145 cells (data not shown). To evaluate the inhibition and to determine the IC$_{50}$ value, different competitor concentrations (unlabeled DUP-1) were added to the cells before the $^{125}$I-labeled peptide was added. Concentrations of $10^{-4}$ to $10^{-5}$ mol/L inhibit $>95\%$ of the binding of $^{125}$I-DUP-1 to DU-145 cells (Fig. 2A). At a competitor concentration of $10^{-8}$ to $10^{-9}$ mol/L, the binding of DUP-1 was marginally enhanced by the presence of the competitor, whereas at concentrations below $10^{-10}$ mol/L the binding value reached the level of uncompeted binding again. The IC$_{50}$ was calculated as $1.77 \times 10^{-7}$ mol/L. Similar results were obtained with PC-3 cells (Fig. 2B). To evaluate the time course of peptide binding, $^{125}$I-labeled peptide was added to the cells and incubated for time points ranging from 1 minute to 4 hours. After the incubation, the cells were lysed and the radioactivity as percentage applied dose per 10$^6$ cells was calculated (Fig. 2C and D). Binding of DUP-1 is rapid and the highest binding rate is reached after 5 minutes. Thereafter, the amount of bound peptide decreases and reaches a basal level of $\sim 1.5\%$ applied dose per 10$^6$ cells. The stability of the peptide was evaluated in heparinized human serum at 25°C and 37°C. High-pressure liquid chromatography analysis showed the peptide is rapidly degraded with a half-life of $\sim 2$ minutes (Fig. 2F).
Internalization of FITC-Labeled DUP-1. To study the internalization process, FITC-Lys-DUP-1 was added to the medium and the cells were analyzed by microscopy (Fig. 3A). Labeling of the cell membrane with FITC-Lys-DUP-1 was observed after 10-minute incubation, whereas after 60-minute incubation the peptide was intracellularly localized. To allow a more detailed analysis of the peptide localization, a pulse-chase experiment was done and analyzed by confocal microscopy. A 10-minute pulse with $5 \times 10^{-4}$ mol/L FITC-Lys-DUP-1 was applied to DU-145 cells followed by the removal of unbound peptide and the incubation in the presence of $5 \times 10^{-4}$ mol/L dextran-Alexa568 (chase; Fig. 3B). Immediately after addition of the dextran dye, FITC-Lys-DUP-1 was found to remain bound to the cell membrane. Ten minutes later, most of the peptide was still bound, but no internalized dextran was detected. Thirty minutes after coincubation, FITC-Lys-DUP-1 bound either intracellularly or to the cell membrane; and (c) yellow spots, characterizing internalized vesicles that contain FITC-Lys-DUP-1 as well as dextran-Alexa568. A series of images obtained from different layers of a DU-145 cell showed the vesicle-like structure of the fluorescent spots (data not shown).

Biodistribution of Radiolabeled DUP-1. To investigate the biodistribution of DUP-1 in vivo, the peptide was labeled with $^{131}$I and injected i.v. into male nu/nu mice, carrying human prostate tumors (DU-145 or PC-3). The biodistribution in DU-145 tumor carrying mice showed that DUP-1 accumulated after 5 minutes in the tumor to a level of $\approx 5\%$ ID/g, which is higher than in the other organs, with the exception of kidney and blood (Fig. 4A). This level was stable up to 45 minutes before a distinct decrease was noted. PC-3 tumors showed a higher tumor uptake, amounting up to 7% ID/g in the tumor, but with a faster washout resulting in values comparable values of the DU-145 tumor at 45 minutes postinjection (Fig. 4B). The higher uptake in PC-3 tumors at 5 minutes and the faster washout in PC-3 tumors at 135 minutes were statistically significant in comparison with DU-145 tumors ($P < 0.05$). To reduce blood background in various organs, animals carrying DU-145 tumors were perfused with NaCl (Fig. 4C). Radioactivity was reduced in most organs, whereas the tracer accumulation of 5% ID/g remained constant.
with or without perfusion in the tumor for 5 and 15 minutes. This leads to an increase of most tumor-to-organ ratios as shown in Table 1. At 5 minutes, lung, liver, and muscle showed a statistically significant difference of unperfused to perfused organ (P < 0.05), and at 15 minutes, heart, lung, and liver showed a statistically significant difference of unperfused to perfused organ (P < 0.001). To show prostate binding of 

\[ ^{131}I\text{-DUP-1} \]

biodistribution experiments were done in Copenhagen rats bearing rat prostate adenocarcinoma Dunning R3327 subline AT-1 (Fig. 4D). The prostates in young mice used in our experiments were too small for reliable measurements. For this reason, we used the AT-1 rat prostate tumor model. The biodistribution in the rats was comparable with the data obtained in mice, with all organs showing a decrease of radioactivity over time, except for the tumor. The radioactivity in the prostate tumor increased up to 300% after 15 minutes in comparison with normal prostate tissue. The tumor-to-muscle ratio for DU-145 tumors in mice was 3.02 at 15 minutes and the prostate-to-muscle ratio for the rats was 0.99 at 15 minutes postinjection.

DISCUSSION

In this study, we introduce a new lead structure with specific binding to prostate carcinoma cell lines in vitro and selective accumulation in prostate carcinomas in vivo. This 12–amino acid lead structure can be the launching platform for the development of an improved mode of delivery for radionuclides or pharmaceutical drugs to prostate tumors. Phage display is a successful tool for identifying novel peptides with high specificity (34, 35) and has been employed to isolate a NG2 proteoglycan-binding peptide to target tumor neovasculature (36) or to identify specifically binding peptides for human lung tumor cells (37).

The peptide DUP-1 contains a motif that facilitates binding to different prostate carcinoma cell lines but not to a benign prostate cell line or to human umbilical vein endothelial cell. This specificity for prostate carcinoma cells is reproduced in animal experiments. DUP-1 shows enhanced uptake even in undifferentiated rat prostate adenocarcinomas (AT-1) versus normal prostate tissue. The rat model showed comparable tumor-to-muscle ratio for the AT-1 tumors in rats as for the DU-145 tumors in mice with 2.54 and 3.02 at 15 minutes postinjection, respectively. With a tumor-to-prostate ratio of \(~3\) at 15 minutes, DUP-1 is a promising molecule for the diagnosis of suspected prostate carcinoma. However, data in humans are needed to assess its potential for the differential diagnosis between tumor and benign hyperplasia. The tumor cell affinity of DUP-1 was also supported by perfusion experiments with animals bearing s.c. transplanted DU-145 and PC-3 tumors. Because no binding to primary cultures of endothelial cells was observed in vitro, we assumed that the peptide is able to penetrate through the basal membrane followed by direct binding to the tumor cells (38). This hypothesis was sustained by the biodistribution data obtained with the perfused animals showing that most organs display reduced radioactivity levels compared with the unperfused animals, whereas the tracer accumulation in the tumor remains unaffected. This indicates that the high activity value observed in the tumor is due to specific binding. The total amount of radioactivity obtained with DUP-1 in the tumors at 15 minutes for DU-145 and PC-3 was 5% and 7% ID/g, respectively. This is significantly higher compared with 3.65% ID/g delivered into tumors of the MDA-MB-435 xenograft model with \(^{125}\text{I-RGD} \) (39).

In vitro, we observed a rapid internalization of FITC-Lys-DUP-1. The experimental settings used in the pulse-chase experiment revealed internalization into cells, because unspecifically bound peptide was removed to ensure that only bound peptide can be internalized during the following incubation period. Dextran-Alexa586 does not bind to the cells (data not shown) and high concentrations of this dye allow visualization of internalized molecules only. Confocal laser scanning microscopy showed intracellularly localized vesicle-like structures. In addition, confocal micrograph slices through the cells showed small areas of localized fluorescence (data not shown), which was attributed to endocytotic vesicles. After 60 minutes, the size of the vesicles increased, indicating a fusion of the endocytotic vesicles to endosomes. This internalization is useful for both imaging and potential therapeutic applications of DUP-1 derivatives.
Although the binding site is unknown, it is unlikely that DUP-1 targets PSMA, because DU-145 and PC-3 cells are PSMA negative (40). The competitive binding with unlabeled DUP-1 points to saturable cell surface site, which after binding leads to an internalization process. DUP-1 has no sequence similarity to bombesin or luteinizing hormone-releasing hormone or to any other peptide or protein sequence available as confirmed by a search in different protein databases such as European Molecular Biology Laboratory, SwissProt, etc. The target structure for DUP-1 will be investigated in further experiments using display cloning procedures (41).

The elevated blood values can be due to various reasons. One possible explanation is the interaction of DUP-1 with serum albumin. The use of 1% BSA as blocking solution strongly inhibited the binding of DUP-1, suggesting that BSA bound the peptide and prevented binding (data not shown). Similar results were obtained with human albumin. A second reason is the relative low stability of the peptide, which might lead to labeled peptide fragments circulating in the bloodstream before they are secreted via the kidneys. Analyses of serum stability of DUP-1 in vitro with high-pressure liquid chromatography has proven degradation of DUP-1 within 10 minutes (data not shown). Similar results were obtained with the blood of mice and rats (data not shown).

This in vivo instability of peptides resulting from phage display libraries was expected. Peptides displayed on the phage surface are protected from proteolysis and may be displayed in a defined conformation. This may result in reduced stability and different binding properties of the corresponding peptides (42). The focus of this work therefore was to find lead peptide structures, which are able to bind selectively PSMA-negative.

**Fig. 4** Biodistribution of DUP-1 in male BALB/c nu/nu mice carrying DU-145 tumors (A; n = 9 animals per time point) or PC-3 tumors (B; n = 3 animals per time point). Animals were injected i.v. with $^{131}$I-DUP-1 and the radioactivity was measured in tumor and control organs after 5, 15, 45, and 135 minutes. C, male BALB/c nu/nu mice carrying DU-145 tumors (n = 6 animals per time point) injected i.v. with $^{131}$I-DUP-1. After 5 and 15 minutes, the animals were perfused and the radioactivity of tumor and control organs was determined. D, male COP rats (n = 3 animals per time point) bearing a Dunning R3327 subline AT-1 tumor were injected i.v. with $^{131}$I-DUP-1. After 5 and 15 minutes, the animals were sacrificed and the radioactivity in various organs was measured. Bars, SE.

**Table 1** Tumor-to-tissue ratios from %ID/g for unperfused and perfused animals carrying DU-145 tumors

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<th>Tumor-to-organ ratio</th>
<th>5 min Unperfused</th>
<th>5 min Perfused</th>
<th>15 min Unperfused</th>
<th>15 min Perfused</th>
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<tr>
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prostate cancer cells. In a next step, the lead sequence DUP-1 is now used to derivatize and optimize the in vivo stability by simultaneously maintaining the binding characteristics. This is expected to result in better target/nontarget ratios. Among the structure evolution steps, we consider sequence fragmentation, cyclization, D-amino acid substitution, and NH2- or COOH-terminal end modifications (43–45). These modifications should result in enhanced stability as well as in reduced binding to plasma proteins.

In conclusion, due to its high and specific binding to prostate carcinoma cells in vitro and in vivo together with rapid internalization, DUP-1 represents a promising structure useful for diagnosis and treatment of prostate cancer. DUP-1 or parts of it may be used for coupling with radioactive isotopes and anticancer agents or even for the modification of the envelope of virus particles such as adeno-associated virus to obtain tumor specific infection.

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