Cancer Therapy: Preclinical

Targeted Drug and Gene Delivery Systems for Lung Cancer Therapy

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Abstract

Purpose: To evaluate the efficacy of a novel docetaxel derivative of deslorelin, a luteinizing hormone–releasing hormone (LHRH) agonist, and its combination in vitro with RGD peptide conjugated nanoparticles encapsulating an antiangiogenic, anti–vascular endothelial growth factor (VEGF) in H1299 lung cancer cells and/or xenografts in athymic nude BALB/c mice.

Experimental Design: The in vitro and in vivo efficacy of the deslorelin-docetaxel conjugate was evaluated in H1299 cells and xenografts in athymic nude mice. Coadministration of deslorelin-docetaxel conjugate and RGD-Flt23k-NP was tested in vivo. Tumor inhibition, apoptosis, and VEGF inhibition were estimated in each of the treatment groups.

Results: The conjugate enhanced in vitro docetaxel efficacy by 13-fold in H1299 cells compared with docetaxel at 24 hours, and this effect was inhibited following reduction of LHRH receptor expression by an antisense oligonucleotide. Combination of the conjugate with the RGD-Flt23k-NP in vivo resulted in an 82- and 15-fold tumor growth inhibition on day 39 following repeated weekly i.v. injections and a single intratumoral (i.t.) injection, respectively. These effects were significantly greater than individual targeted therapies or docetaxel alone. Similarly, apoptotic indices for the combination therapy were 14% and 10% in the i.v. and i.t. groups, respectively, and higher than the individual therapies. Combination therapy groups exhibited greater VEGF inhibition in both the i.v. and i.t. groups.

Conclusions: Docetaxel efficacy was enhanced by LHRH receptor–targeted deslorelin conjugate and further improved by combination with targeted antiangiogenic nanoparticle gene therapy. Combination of novel targeted therapeutic approaches described here provides an attractive alternative to the current treatment options for lung cancer therapy. [Cancer Res 2009;15(23):7299–308]

Lung cancer is the leading cause of cancer related deaths worldwide (1). In 2009, an estimated 219,440 new lung cancer cases and 159,390 deaths are expected in the United States alone (2). Non–small cell lung cancer (NSCLC) accounts for 87% of all cases (2, 3), with a dismal 5-year survival rate of 15% (1). Almost two thirds (70%) of patients present locally advanced disease or metastatic disease at the time of diagnosis (1, 4), the primary treatment of choice for which is chemotherapy (5). In 2002, based on phase III clinical trial results, the U.S. Food and Drug Administration approved the use of docetaxel in combination with cisplatin in chemotherapy-naïve NSCLC patients with unresectable locally advanced or metastatic disease (6). However, such as other chemotherapeutics, docetaxel dosing and efficacy are limited by its undesirable side effects, including febrile neutropenia and myelosuppression (7), necessitating the development of more efficacious derivatives of docetaxel. One potential solution to increase potency and minimize nontarget effects is targeting docetaxel delivery to tumors. Conjugating ligands targeting receptors specifically expressed or overexpressed on cancer cells is an attractive technique to achieve targeting of delivery systems. Deslorelin, a nonapeptide luteinizing hormone–releasing hormone (LHRH) superagonist has a molecular weight of 1,285 Da and exerts its effects through the LHRH-receptor (LHRH-R; refs. 8, 9). The LHRH-R, a member of the GPCR super family, is expressed in various tissues, including normal lung (10), and overexpressed on lung cancers cells (11, 12). We have established that deslorelin can be conjugated to docetaxel at the hydroxyl (-OH) group in the serine moiety (amino acid 4) through a glutamate linker (13). One objective of this study was to determine
whether the \textit{in vitro} and \textit{in vivo} efficacy of docetaxel can be enhanced by its conjugation to deslorelin in a lung cancer model. Cancer therapy benefits from a multipronged approach. In addition to targeted cytotoxic therapy, antiangiogenic therapies are expected to be beneficial in lung cancer therapy (14). The vascular endothelial growth factor (VEGF), a potent inducer of angiogenesis, is overexpressed in 61% to 92% of NSCLC patients (15). Studies have also shown that VEGF in the tumor microenvironment is capable of overcoming the effects of docetaxel, resulting in docetaxel resistance (16). Furthermore, VEGF causes impaired delivery of therapeutic agents to tumor cells (17), indicating that VEGF inhibition could improve the efficacy of anticancer agents. Indeed, the combination of bevacizumab (an anti-VEGF antibody) with chemotherapy has been shown to prolong progression-free survival, and overall survival in NSCLC patients (18). However, treatment with bevacizumab is associated with serious side effects, including hypertension, neutropenia, bleeding, thrombocytopenia, and rashes, compared with control groups (19). Hence, new targeted strategies are required for the inhibition of VEGF expression and angiogenesis.

In this study, we investigated a targeted anti-VEGF gene therapy with an anti-VEGF intracorper (Flt23k) based on nanoparticles (NP) with surface ligands targeting the integrin receptors. Flt23k is a recombinant construct of domains 2 and 3 of high-affinity VEGF-R1 fused with endoplasmic reticulum retention signal sequence [Lysine-Aspartic acid-Glutamic acid-Leucine (KDEL)] constructed in a pTracer plasmid (20). This construct sequesters VEGF by the VEGF receptor in the endoplasmic reticulum, inhibiting VEGF secretion and the VEGF autocrine loop (20), thereby inhibiting angiogenesis (21). Thus, gene therapy with targeted Flt23k NPs provides a potential alternative to existing and currently tested anti-VEGF therapies. The second objective of this study was to determine whether the efficacy of the deslorelin-docetaxel conjugate (D-D) can be enhanced by a combination therapy with novel integrin receptor–targeted antiangiogenic NPs encapsulating the anti-VEGF intracorper plasmid (Flt23k-NP). A seven amino acid RGD peptide sequence, Gly-Arg-Gly-Asp-Ser-Pro-Lys, which binds integrin receptors αvβ3 with high affinity and αvβ5 with low affinity (22, 23), was used as neovasculature targeting ligand on the surface of these Flt23k-NPs (RGD-Flt23k-NP).

This is the first report of the efficacy of D-D and a novel targeted anti-VEGF intracorper plasmid–encapsulated NPs in \textit{in vivo} in subcutaneous H1299 cell xenograft–bearing mouse model. Further, it shows the advantage of combining chemotherapies and gene therapies in treating cancer.

### Materials and Methods

#### Chemicals.
Deslorelin was a gift from Anaco Pharmaceut, Inc. Docetaxel was obtained from Chempacific. H1299 cells were kindly provided by Dr. Po-Wen Cheng (University of Nebraska Medical Center, Omaha, NE). Cell culture materials, reagents, and Lipofectin were obtained from Invitrogen. Chemicals for conjugation and buffers, poly (vinyl alcohol), 3-(N-Morpholino)propanesulfonic acid, and RGD peptide, were obtained from Sigma-Alrich. Flt23k plasmid was a gift from Dr. Balamurali Ambati (Morgan Eye Center, University of Utah, Salt Lake City, UT).

#### Synthesis of deslorelin-docetaxel conjugate.
D-D was synthesized, purified, and characterized as previously described (13).

#### Cell lines and cell culture.
NSCLC cell line, H1299, was used for \textit{in vitro} and \textit{in vivo} experiments. H1299 cells were maintained under incubation in a humidified incubator under 5% carbon dioxide in RPMI 1640 supplemented with 10% fetal bovine serum, 5% glutamine, and 10 mmol/L, penicillin-streptomycin.

#### Antiproliferative effects of D-D in H1299 lung cancer cells.
Antiproliferative effects in H1299 cells were determined following a previously described method (13). H1299 cells in 96-well tissue culture plates were treated with 1 to 250 μg/mL of docetaxel (1.25-300 nmol/L), 1 to 250 μg/mL of deslorelin (0.7-200 nmol/L), and 1 to 250 μg/mL of D-D (0.19-46 nmol/L docetaxel equivalent) in serum-free medium containing 10% fetal bovine serum, 5% glutamine, and 10 mmol/L, penicillin-streptomycin.

#### LHRH-R expression on H1299 cells and the influence of decreased LHRH-R expression on the efficacy of D-D.
LHRH-R expression on H1299 cells was determined following a Western blot analysis as previously described (24), following pretreatment with antisense oligonucleotides (AON) and sense oligonucleotides (SON) against LHRH-R. Pretreatment was carried out using a previously reported (25) and validated (11) SON and AON against LHRH-R as previously described (11). Untreated cells were used as controls. Efficacy of D-D under decreased receptor conditions following pretreatment with AON was carried out following a previously described method (13).

#### Preparation and characterization of RGD–peptide–conjugated, Flt23k plasmid–loaded PLGA NPs (RGD-Flt23k-NP).
NPs were formulated as per a previously described method using a water/oil/water double emulsion solvent evaporation technique (26). RGD peptide was conjugated to NPs using a process as described previously (26). NPs were characterized for particle size using dynamic light scattering. Particles were visualized using a scanning electron microscope following gold coating.

#### In vivo tumor xenograft studies using H1299 cells.
These studies were done with approval and in accordance with guidelines of the Institutional Animal Care and Use Committee at the University of Nebraska Medical Center. Tumor inoculation in male athymic nude BALB/c mice (Harlan Sprague-Dawley) and tumor volume measurement were carried out as previously described (13).
At 100 mm³ tumor volume, treatments with docetaxel (2.5 mg/kg), D-D (2.5 mg/kg of docetaxel equivalent), Flt23k-NP (100 μg of plasmid equivalent NPs), RGD-Flt23k-NP (100 μg of plasmid equivalent), and D-D (2.5 mg/kg of docetaxel equivalent) plus RGD-Flt23k-NP (100 μg of plasmid equivalent; as a single injection mixture) were administered. Treatments were administered as a single intratumoral (i.t.) dose (50 μL) using two 25-μL injections into two halves (halved along the length) of the tumor xenografts or as repeated weekly i.v. doses (100 μL) through the tail vein. PBS (vehicle) was administered i.v. as control. All animals were sacrificed and xenografts were isolated once tumor volumes in vehicle-treated mice measured 1,500 mm³. Immuno-histochemical analyses were done on these xenografts.

In vivo terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay. Apoptotic cells in tumor tissues were detected by a terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method using the DeadEnd Colorimetric kit (Promega) according to the manufacturer's instructions. Following incubation with the rTdT enzyme, the sections were developed with 3,3′-diaminobenzidine, and images were obtained using Hitachi HV-D25 digital camera and EPIX-XCAPLite software V2.2 for windows at ×10 magnification. Apoptotic indices were calculated by counting number of TUNEL-positive cells/total number of cells in a field ×100. A total of 10 fields were counted in a random and blinded manner.

Immunohistochemistry for VEGF expression and CD31 plus TUNEL dual staining in tumor xenografts. Immunohistochemistry for VEGF was done using a dianaminobenzidine method in formaldehyde-fixed/paraffin-embedded sections wherein antigen retrieval was carried out with 10 mmol/L citrate buffer (pH 6.0). VEGF staining was carried out by overnight incubation (4°C) of primary goat anti-VEGF antibody (1:200 dilution; R&D Systems) and secondary antibody rabbit anti–goat IgG (1:200 dilution). The sections were developed with 3,3′-diaminobenzidine and images were obtained using Hitachi HV-D25 digital camera and EPIX-XCAPLite software V2.2 for windows at ×10 magnification.

![Fig. 1.](image-url)
Dual staining for CD31 and TUNEL was carried out using an immunofluorescence method. CD31 staining was carried out by overnight incubation (4°C) of primary rabbit anti-CD31 antibody (1:200 dilution; Abcam), followed by Texas red–labeled goat anti-rabbit IgG secondary antibody for 2 h (1:200 dilution). TUNEL staining was carried out according to manufacturer's protocol (Promega). Following incubation with the rTdT enzyme, the sections were developed with Streptavidin-FITC and confocal images were obtained with a ×100 oil immersion lens on a Zeiss Axiovert 135 microscope equipped with a CARV optical module ("spinning disc confocal") and a Hamamatsu Orca (C4742-95) digital camera (6.7 × 6.7 μm of physical pixels).

Statistical analysis. The experiments were carried out with an n = 8 wells for MTT assays and n = 6 mice for in vivo studies. Data in all cases are expressed as mean ± SD. Comparison of mean values between different treatments was carried out using two-way ANOVA followed by Tukey’s post hoc analysis with the SPSS (version 8) software. The level of significance was set at P < 0.05.

Results

**D-D is more effective when compared with docetaxel.** Antiproliferative effects and IC50 values of D-D on H1299 cells are shown in Fig. 1A. Over periods of 24-, 48-, and 72-hour exposure, it is observed that D-D is significantly more cytotoxic compared with docetaxel alone. Further, drugs/conjugates exhibit cytotoxities in H1299 cells in the order: D-D > docetaxel > deslorelin at all three time points tested. The IC50 values of the drugs based on a fit to a sigmoidal inhibitory Emax pharmacodynamic model confirm this notion. Deslorelin did not have any cytotoxic effects on H1299 cells even at doses as high as 100 nmol/L. IC50 values could not be accurately determined for deslorelin because there was no measurable antiproliferative effect.

**LHRH receptor expression in H12299 cells is decreased by pretreatment with AON against LHRH-R.** The expression of LHRH-R in H1299 cells following pretreatment with AON against LHRH-R is shown in Fig. 1B. H1299 cells (untreated) exhibit LHRH-R expression. Pretreatment with an AON against LHRH-R in H1299 cells caused a decrease in LHRH-R expression. The level of expression was determined by a densitometric analysis of band intensities from three separate experiments. Based on the densitometric analysis, LHRH-R expression following pretreatment exhibits the order: untreated (control) ~ SON treated > AON treated.

**LHRH receptor contributes to the effects of D-D.** The effects of decreased expression of LHRH-R on antiproliferative efficacy and IC50 values of D-D are shown in Fig. 1C. Pretreatment with an AON against LHRH-R significantly decreased the cytotoxicity of D-D, which was not observed in SON-treated groups. These results indicate that deslorelin retains its LHRH-R interactive ability when conjugated with docetaxel. Deslorelin-treated groups did not exhibit any cytotoxicity in any pretreatment group even at doses as high as 100 nmol/L. The IC50 values for deslorelin could not be determined because there was no measurable antiproliferative effect.

**Characterization of NPs.** NPs were characterized by dynamic light scattering for three batches each of Flt23k-NP and RGD-Flt23k-NP. Flt23k-NP exhibited mean diameters of 284.92 ± 24.35 nm with a polydispersity index of 0.234 ± 0.028. RGD-Flt23k-NP exhibited a mean diameter of 291.48 ± 34.58 nm with a polydispersity index of 0.132 ± 0.023. The surface morphology of the NPs using scanning electron microscopy is shown in Fig. 2.

**Combination therapy enhances tumor growth inhibition.** A schematic of tumor inoculation, treatments, and mechanism of action is presented in Fig. 3. Treatment efficacy following i.t. and i.v. administration are shown in Fig. 4. Animals in vehicle-treated group attained a tumor volume of ∼1,509 ± 9 mm3 on day 39 (Fig. 4A), at which time all tumors were measured and animals in various groups were sacrificed for isolation and further characterization of xenografts. Mice treated with D-D in combination with an AON against LHRH-R exhibited a significant decrease in tumor volume compared with mice treated with D-D alone or AON alone. These results indicate that combining D-D with an AON against LHRH-R significantly enhances the antitumor efficacy of D-D.
both i.v. (Fig. 4B) and i.t. (Fig. 4C) exhibited tumor inhibition in the following order: D-D + RGD-Flt23k-NP > D-D ≥ RGD-Flt23k-NP > docetaxel ≥ Flt23k-NP >> PBS. Tumor growth reduction was observed in i.t. groups until day 16 from treatment initiation, followed by an increase toward baseline over the next 23 days before sacrifice. No significant changes were observed in body weight and morbidity was not observed, indicating that all treatments were well tolerated.

**Combination therapy enhances apoptosis in tumor and endothelial cells.** The treatment efficacy to induce apoptosis in the tumor cells (TUNEL assay) and endothelial cells (dual staining with CD31 and TUNEL) is shown in Fig. 5. TUNEL-positive cells were observed in all groups. As expected vehicle (PBS)-treated mice alone exhibited lesser TUNEL-positive cells and more necrotic regions. TUNEL-positive cells observed in various groups were in the following order: D-D + RGD-Flt23k-NP >
D-D > docetaxel > RGD-Flt23k-NP > Flt23k-NP >> PBS in both i.v. (Fig. 5A) and i.t. (Fig. 5B) groups. However, i.v. groups exhibited significantly higher TUNEL-positive cells compared with i.t. group at all times. The above trend was also observed in the apoptotic index calculated by counting the number of TUNEL-positive cells (Fig. 5C). CD31 and TUNEL dual staining was assessed qualitatively in the xenograft sections (Fig. 5D). As evident, the endothelial cells exhibited apoptosis in the treatment groups, with a rank order similar to that observed in the tumor cells.

**Combination therapy enhances anti-VEGF efficacy.** Anti-angiogenic effect by VEGF inhibition in various treatment groups are shown in Fig. 6. Immunohistochemical analysis exhibited VEGF inhibition in mice receiving treatments compared with vehicle-treated mice alone. However, groups treated with Flt23k-NP, RGD-Flt23k-NP, and D-D + RGD-Flt23k-NP exhibited greater VEGF inhibition compared with groups receiving docetaxel or D-D. VEGF inhibition was in the order: D-D + RGD-Flt23k-NP > RGD-Flt23k-NP > Flt23k-NP > D-D > docetaxel > PBS. The same trend was observed in mice treated both i.v. (Fig. 6A) as well as i.t. (Fig. 6B). However, VEGF inhibition was higher in i.v. groups compared with i.t. groups.

**Discussion**

By using a novel D-D and RGD-conjugated Flt3k anti-VEGF intraceptor plasmid–loaded biodegradable NPs (RGD-Flt23k-NP) for lung cancer therapy (Fig. 3), we report the following key findings: (a) D-D, a targeted chemotherapeutic agent, exerts better in vitro and in vivo cytotoxic effects compared with docetaxel alone, likely by targeting the receptors for LHRH. (b) RGD-Flt23k-NP, a targeted NP gene delivery system, improves in vivo therapeutic efficacy compared with nontargeted NPs of the anti-VEGF intraceptor plasmid. (c) Combination therapy of D-D, a targeted chemotherapeutic agent, with RGD-Flt23k-NP, a targeted NP antiangiogenic agent, provides enhanced antitumor efficacy compared with individual therapies or docetaxel alone.

Our antiproliferative studies showed that D-D is significantly more cytotoxic compared with docetaxel alone after 24, 48, or 72 hours of exposure, with D-D being 10- to 13-fold more potent compared with docetaxel. The IC50 of D-D was as low as 0.8 nmol/L after 72-h exposure to H1299 cells. Deslorelin by itself did not exhibit any cytotoxic effects in H1299 cells, which is consistent with our previous studies in prostate cancer cells (13).

Deslorelin, an LHRH agonist, exerts its pharmacologic effects through the LHRH-R (12). Our Western blot analysis confirms expression of LHRH-R in H1299 similar to LNCaP and PC-3, prostate cancer cell lines, suggesting that D-D can exert its effects through LHRH-R in H1299 cells. The AON against LHRH-R significantly reduced LHRH-R expression by ∼2-fold and the potency of D-D by 3.5-fold at the end of 72-h drug exposure in H1299 cells. Thus, D-D likely exerted its effects in H1299 cells, partly through a LHRH-R–mediated uptake of the conjugate. Our prior studies indicated that deslorelin undergoes cellular uptake and transport through LHRH receptors (11, 27, 28). Thus, our in vitro studies suggest that lower doses of conjugate can achieve the same effect as docetaxel alone. Alternatively, the conjugate at doses comparable with docetaxel clinical doses might achieve greater tumor reductions.

Although i.v. injections are more common clinically, i.t. injections are expected to localize and retain the dose better than i.v. injections. Therefore, we assessed a single i.t. injection and repeated weekly i.v. injections in this study. Weekly i.v. administrations of D-D improved tumor inhibition by ∼40% at the end of the study, compared with equivalent docetaxel therapy. Single i.t. administration (equivalent to a single, weekly i.v. dose), on the other hand, resulted in a significant ∼20% greater tumor inhibition at the end of the study, compared with equivalent docetaxel therapy. Thus, D-D is significantly more effective compared with docetaxel. This enhanced tumor reduction,
although interesting, warrants the use of additional approaches for further inhibiting lung tumor growth. Therefore, we assessed the usefulness of an antiangiogenic therapy by itself or in combination with D-D cytotoxic therapy.

Targeted anti-VEGF gene therapies are expected to be significantly safer compared with currently available VEGF antibody therapies. Polymeric NPs are effective carriers of macromolecules, protecting against macromolecule degradation in blood and/or tissues (29). Therefore, we designed integrin receptor-targeted polymeric NPs (Flt23k-NP) encapsulating the anti-VEGF intraceptor plasmid (Flt23k; ref. 20). The RGD-Flt23k-NP exhibited approximately 30% and 20% greater, statistically significant, tumor inhibition compared with Flt23k-NP alone in the i.v. and i.t. groups, respectively.

Because cytotoxic and antiangiogenic approaches treat different aspects of tumor progression, a combination of these two approaches might provide an additive effect. Therefore, we tested a combination of D-D and RGD-Flt23k-NP in vivo. It is noteworthy that following weekly i.v. injections, D-D + RGD-Flt23k-NP achieved 82-fold tumor inhibition compared with 38-, 36-, 50-, and 46-fold growth inhibition achieved by docetaxel, Flt23k-NP, D-D, and RGD-Flt23k-NP, respectively.

Fig. 5. In vivo assessment of apoptotic efficacy of various treatments in H1299 lung cancer xenografts in mice. Representative photomicrographs of apoptotic cells in (A) i.v. and (B) i.t. groups using TUNEL assay in one representative section of six xenografts. C, the apoptotic index following i.v. and i.t. treatments with docetaxel, D-D, Flt23k-NP, RGD-Flt23k-NP, and D-D + RGD-Flt23k-NP. Arrows, some TUNEL-positive cells in a field. Columns, mean (n = 6); bars, SD. D, representative photomicrographs of apoptosis in proliferative endothelial cells using a dual CD31 and TUNEL staining in one representative section of six xenografts.
relative to vehicle-treated group on day 39 after dose initiation. At this time, all i.v. treated groups maintained the tumor size below the baseline (~100 mm³) by 60% or better. On the other hand, following a single i.t. injection, the i.t. combination therapy with D-D and RGD-Flt23k-NP resulted in a -6% tumor volume compared with the baseline. These effects were superior to i.t. docetaxel and Flt23k-NP, the nontargeted counterparts.

The TUNEL assay suggested that D-D + RGD-Flt23k-NP achieved greater apoptosis compared with Fl23k-NP and RGD-Flt23k-NP in both the i.v. and i.t. groups. Further, the D-D + RGD-Flt23k-NP coadministration achieved greater apoptosis compared with docetaxel and D-D in both the groups. D-D enhanced apoptosis compared with docetaxel alone in both the groups. Fl23k-NP-treated groups also exhibited greater apoptosis compared with the vehicle treated groups, which increased further when treated with RGD-Flt23k-NP. In addition to the above measures for apoptosis in tumor tissue at the end of the study, we also assessed apoptosis in endothelial cells of the tumor vasculature. The qualitative rank order for apoptosis in endothelial cells was similar to what was observed above in tumor cells, with the combination therapy exhibiting the highest apoptotic activity. Although the above observed apoptotic activity of docetaxel is well known, it is interesting to note that Flt23k plasmid, capable of inhibiting VEGF secretion (20), also resulted in apoptotic activity. There is some evidence indicating that inhibition of VEGF leads to apoptosis (14). Bevacizumab administered i.p. inhibits tumor growth of lung adenocarcinoma xenografts by ~20% and leads to an increase in proapoptotic gene expression (30). Inhibition of VEGF is postulated to inhibit tumor growth and induce apoptosis through loss of an endothelial cell–derived paracrine factor (31, 32) or due to a direct interaction between VEGF and the tumor cell itself (14).

In this study, all tumor xenografts exposed to Flt23k plasmid treatments exhibited a significant VEGF inhibition at the end of the study. We also observed that VEGF inhibition was also
increased in the D-D groups. The highest VEGF inhibition was evident in the D-D + RGD-Flt23k-NP group, possibly due to VEGF inhibition by both docetaxel as well as the anti-VEGF intraperitoneal. Previous studies have shown that docetaxel itself can lead to VEGF inhibition (33). Docetaxel is known to inhibit VEGF in colorectal carcinoma cells (34). However, docetaxel by itself did not inhibit VEGF in the current study, possibly due to low drug levels at the end of the study. Docetaxel, a small molecule, might have been cleared to a large extent by the end of the study (day 39) after single i.t. injections. In i.v. injection groups, 5 days elapsed between last injection and the time of sacrifice. Docetaxel concentrations present in tumor tissue at this time might not have been adequate to inhibit VEGF. Furthermore, docetaxel at 10 mg/kg achieves only a slight VEGF inhibition (35). We have used only 2.5 mg/kg dose, which is equivalent to the human dose, which likely is not adequate to observe VEGF inhibition as observed with D-D. With D-D, greater drug delivery or drug retention is anticipated. Possibly for this reason, D-D induced apoptosis as well as VEGF inhibition.

The i.v. administration differentiated targeted systems much better compared with i.t. administration. Overall, repeated i.v. administrations of treatments were able to sustain tumor volumes well below baseline compared with single i.t. administration of treatments. The higher efficacy following i.v. injection compared with i.t. injections could be attributed to the frequent dosing and 6-fold greater overall dose administered in the i.v. groups. Our studies suggest that targeted chemotherapeutic and antiangiogenic gene delivery systems are both more effective approaches compared with nontargeted delivery systems for lung cancer treatment. Further, a combination of targeted chemotherapeutic and antiangiogenic therapy is superior to individual therapies. The assessed targeting features likely improve localization of the treatments to the tumor compared with non-targeted systems. The relative advantage of targeted systems even after i.t. administration suggests better uptake and/or retention of targeted systems at the tumor site compared with nontargeted systems.

In conclusion, conjugation with deslorelin enhances antican- ccer activity of the clinically used chemotherapeutic agent docetaxel in vitro and in vivo in lung cancer models. Further, combination of D-D with targeted anti-VEGF intraperitoneal gene therapy offers additive effects in inhibiting tumor growth and enhancing apoptotic index. Thus, this study advances novel targeted chemotherapeutic and antiangiogenic agents based on nanomaterials for cancer therapy.

Disclosure of Potential Conflicts of Interest

Patent application pending.

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