F3-Targeted Cisplatin-Hydrogel Nanoparticles as an Effective Therapeutic That Targets Both Murine and Human Ovarian Tumor Endothelial Cells In vivo

Ira Winer¹, Shouyan Wang², Youg-Eun Koo Lee², Wenzhe Fan², Yusong Gong³, Daniela Burgos-Ojeda³, Greg Spahlinger³, R. Kopelman², and Ronald J. Buckanovich¹,³

Abstract

Recent studies indicate that ovarian cancer may be highly responsive to antivascular therapeutics. We have developed an antivascular tumor therapeutic using the F3 peptide to target cisplatin-loaded nanoparticles (F3-Cis-Np) to tumor vessels. We show that although F3-Cis-Np bind with high specificity to both human ovarian tumor cells and tumor endothelial cells in vitro, they only show cytotoxic activity against the tumor endothelial cells. In vivo these nanoparticles bind primarily to tumor endothelial cells. Therapeutic studies in both flank and orthotopic i.p. murine ovarian tumor models, as well as human tumor xenograft models, show rapid tumor regression with treatment. Treatment was associated with significant vascular necrosis consistent with an antivascular effect. Furthermore, treatment was active in both platinum-sensitive and platinum-resistant cell lines. Importantly, we show that F3-Cis-Np bind to human tumor endothelial cells in vitro and to human tumor vessels in vivo. Therapy targeting human vasculature in vivo with F3-Cis-Np led to near complete loss of all human tumor vessels in a murine model of human tumor vasculature. Our studies indicate that F3-targeted vascular therapeutics may be an effective treatment modality in human ovarian cancer.

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Introduction

Ovarian cancer is a deadly disease for which there have been few new therapies. For a decade platinum and taxane chemotherapy regimens have remained the mainstay of therapy. Recent studies have suggested that angiogenic pathways are important therapeutic targets in ovarian cancer. Phase II trials suggest a significant response rate of ovarian cancer to anti–vascular endothelial growth factor (VEGF) therapy (1–4). This is unlike other solid tumors such as colon, lung, and breast cancer which showed no response to single agent anti-VEGF therapy. When anti-VEGF therapy is used in combination with chemotherapy in ovarian cancer, response rates are even higher (5, 6). Unfortunately, even when anti-VEGF therapy is used in combination with chemotherapy, response to the former is relatively short. This is due in part to host cell upregulation of alternate angiogenic pathways (7).

Targeting vascular cells directly represents a means to target numerous angiogenic pathways that act ultimately at the endothelial cell. In addition, unlike traditional angiogenic therapies that prevent new vasculature, and therefore typically lead to disease stabilization but not regression, targeting active tumor vascular cells could potentially lead to tumor necrosis and disease regression. Ovarian tumor vasculature has been shown to be unique from normal restsng vasculature (8, 9). Several peptides have been identified and developed which bind with relatively high affinity and specificity to tumor vessels. RGD (arginine-glycine-aspartic acid) motif–containing peptides have been developed which bind to integrin molecules that are upregulated on tumor vessels and sometimes tumor cells (10, 11). Similarly, asparagine-glycine-arginine peptides can be used to target CD13 isoforms expressed in tumor vasculature (12). The 31-amino-acid F3 peptide has also been shown to bind to nucleolin protein expressed on the surface of tumor endothelial cells as well as on the surface tumor cells (13–15).

These peptides have been used to target nanoparticles carrying various therapeutic payloads to the tumor, such as magnetic resonance imaging contrast agents and photodynamic drugs (16, 17). This approach allows the therapeutic agent to be concentrated at the tumor site, while reducing systemic exposure and potentially reducing drug-related side effects. RGD-targeted cisplatin nanoparticles were found to inhibit endothelial cell proliferation in vitro (18). RGD-targeted paclitaxel containing nanoparticles were shown to target ovarian cancer cells in vitro and in vivo and effectively restrict ovarian tumor growth (19). Similarly, in several tumor models including ovarian cancer, vascular-targeted liposomal doxorubicin was found to be a more effective therapeutic than traditional doxorubicin or liposomal doxorubicin.
in vivo

Our data strongly support the efficacy of vascular-targeted tic, these nanoparticles lead to significant tumor regression. Fisher Scientific. Cisplatin was purchased from SICOR pharmaceuticals. 3-(aminopropyl) methacrylamide (APMA) was obtained from Sigma Aldrich. polyethylene glycol dodecyl ether (Brij 30), 3-(acryloyloxy)-2-propen-1-yl methacrylamide (AOT) were purchased from Sigma Aldrich.

The model of glioblastoma multiforme has also been used to deliver nanoparticles to the tumor microenvironment. A rat model of glioblastoma multiforme has recently been developed (22, 23). This model uses human embryonic stem cells as a source of vascular cells; thus, the vascular cells are human. Vessels generated in this model are shown to have both human endothelial and vascular smooth muscle cells.

We used the F3 peptide to deliver cisplatin-loaded polyacrylamide (PAA) nanoparticles to tumor vessels in both murine and human ovarian cancer models. We show that these nanoparticles bind to murine and human tumor endothelial cells both in vitro and in vivo. When used as a therapeutic, these nanoparticles lead to significant tumor regression and then stabilization of tumor burden. Nanoparticles were effective in both xenograft and orthotopic tumor models. Our data strongly support the efficacy of vascular-targeted nanoparticle therapy in ovarian cancer and represent the first time that the nanoparticles were applied to human tumor vessels in vivo has been shown.

Materials and Methods

Nanoparticle preparation

Chemicals. Acrylamide, TEMED, ammonium persulfate (APS), polyethylene glycol dodecyl ether (Brij 30), 3-(acryloyloxy)-2-hydroxypropylmethacrylamide (AHM), hexane, and diocetyl sulfosuccinate (AOT) were purchased from Sigma Aldrich.

We used the F3 peptide to deliver cisplatin-loaded polyacrylamide (PAA) nanoparticles to tumor vessels in both murine and human ovarian cancer models. We show that these nanoparticles bind to murine and human tumor endothelial cells both in vitro and in vivo. When used as a therapeutic, these nanoparticles lead to significant tumor regression and then stabilization of tumor burden. Nanoparticles were effective in both xenograft and orthotopic tumor models. Our data strongly support the efficacy of vascular-targeted nanoparticle therapy in ovarian cancer and represent the first time chemo-nanoparticle binding to human tumor ves-

3-(aminopropyl)methacrylamide (APMA) was obtained from Polysciences Inc., and ethanol (190 proof) was obtained from Fisher Scientific. Cisplatin was purchased from SICOR Pharmaceuticals Inc., whereas sulfosuccinimidyl 4-[N-maleimidomethyl]cyclohexane-1-carboxylate (sulfo-SMCC), Traut’s reagent (2-IT), amine reactive FITC, and AlexaFluor 594 were from Thermo Scientific Inc. All solutions were prepared from 18 mol/L water purified by a Barnstead/Thermolyne Nanopure II system except where otherwise stated.

Blank nanoparticle polymerization. Hexane (45 mL) was added into a dried flask and stirred under a constant purge of argon. AOT (1.6 g) and Brij 30 (3.1 g) were added with continuous mixing under argon for 20 minutes. Acrylamide (0.711 g) and APMA (0.055 g) were dissolved in PBS (pH 7.4) by sonication. AHM (0.428 g) was added to the acrylamide solution and the mixture was sonicated for 5 minutes to obtain uniformity. The acrylamide solution was then added to the hexane mixture and vigorously stirred for 20 minutes under argon protection. Polymerization reaction was initiated with fresh ammonium persulfate (10% aqueous solution, 40 μL) and TEMED (40 μL), and vigorous mixing was continued for 12 hours. Hexane was then removed by rotary evaporation and nanoparticles were precipitated with ethanol. Surfactant and unreacted monomers were removed by washing with ethanol (5 × 160 mL) followed by washing with water (5 × 100 mL) in an Amicon ultrafiltration cell (200 mL, equipped with a BioMax 100 kDa cutoff membrane). The concentrated nanoparticles were lyophilized for two days before use.

Cisplatin-encapsulated and FITC- or AlexaFluor-linked nanoparticles. Cisplatin-encapsulated nanoparticles were polymerized as above except that 2 mL cisplatin (SICOR Pharmaceuticals Inc., obtained via the Department of Pharmacy at the University of Michigan) drug solution (1 mg/mL) was used to dissolve all the monomers and nanoparticles were protected from light during the production process. An inductively coupled plasma (Perkin-Elmer Optima 2000 DV with Winlab software) was used to determine the concentra-

For fluorophore-linked nanoparticles, 1 mg of FITC or 5 mg of AlexaFluor 594 were added into monomer solution and the mixture was kept stirring at 37°C for an hour before injection into hexane. Blank, fluorophore-conjugated, or cisplatin-encapsulated PAA nanoparticles were prepared with a final average size of 24.4 nm (polydispersity index = 0.120).

F3-targeted nanoparticles. Cisplatin-encapsulated, fluorophore-linked, or blank nanoparticles were suspended in PBS by sonication, sulfo-SMCC was added, solution was mixed for 60 minutes, and then SMCC-conjugated nanoparticles were washed three times using Amicon centrifugal filter unit. F3 peptide and 2-IT dissolved in distilled water was then added into the SMCC-conjugated nanoparticle suspension. The mixture was mixed overnight and then washed again to obtain the concentrated nanoparticles.

Cell lines and in vitro studies. ID8 and A2008 cells were a generous gift from Dr. George Coukos (Abramson Cancer Center, University of Pennsylvania, Philadelphia, PA), SKOV3, HEY1, and A2780 cell lines were a generous gift of Dr. Rebecca Liu (University of Michigan, Ann Arbor, MI). Tumor endothelial cell (TEC) cultures were freshly generated from VE-Cadherin+/CD146+ cells fluorescence associated cell sorting isolated from mechanically dissected tumors as previously described (24, 25). TEC were then grown in EBM2 media (Clonetech). Monocytes were isolated from 2 mL of ACK lysed donor blood based upon their ability to adhere to plastic. All cells except TEC were grown in RPMI/10% fetal bovine serum/5% penicillin-streptomycin medium and split two days prior to experiments to 40% confluency. For binding experiments, cells were incubated with a range of F3-targeted AlexaFluor 594–linked nanoparticles (F3-Alex-Np), or blank nanoparticles (10–100 μg/mL) for 4 hours with intermittent rocking. Cells were then washed three times with PBS, incubated for 30 minutes, and then imaged using a Nikon fluorescence microscope attached to CoolSNAP CCD camera (Roper Scientific). To determine in vitro killing efficiency, cells were incubated with F3-targeted blank nanoparticles (F3-Np), F3-targeted...
cisplatin-encapsulated nanoparticles (F3-Cis-Np; 0.15 μg/mL final cisplatin concentration), parental cisplatin compound [at 5 μg/mL final concentration for cell line experiments and at 1 μg/mL for TEC and peripheral blood mononuclear cell (PBMC) experiment], or mock treated with PBS. The cells were washed after 4 hours and then allowed to grow for a total of 72 hours prior to harvesting for cell counting via trypan blue exclusion.

**Mouse studies.** All mice were housed at the University of Michigan Medical School in the Unit for Laboratory Medicine and protocols were approved under the University Committee on the Use and Care of Animals. Tumor cell lines were grown in DMEM/10% fetal bovine serum/5% penicillin-streptomycin medium.

**Axillary and flank tumor models.** Cells (10 × 10⁶) were injected with 0.2 mL of PBS and 300 μL of matrigel (BD Biosciences). In the initial targeting experiments, axillary tumors were allowed to grow for 10 days and then 100 mg/Kg F3-targeted Alexa 488–linked nanoparticles (F3-FITC-Np) or Alexa 488–linked nanoparticles (FITC-Np) were administered i.v. Twenty-four hours after injection the mice were sacrificed, and tumors, liver, lung, kidney, heart, and spleen were harvested and examined for fluorescent nanoparticle uptake. For therapeutic studies Hey1 tumor cells were stably transduced with a DsRed-expressing lentivirus (plentiloxEV-DsRed virus, provided by the vector core at the University of Michigan). Tumor cells were injected s.c. into either the flank (ID8 studies) or axilla (SKOV3, A2780-GFP, and DsRED HEY1) of either C57Bl6 or nu/nu mice, respectively. Axillary injection was used in the case of the human tumor xenografts as we find axillary tumors have a greater microvascular density than flank tumors. Xenografts were allowed to establish as indicated and were treated with either (a) i.p. cisplatin at 250 μg/kg alone or (b) i.p. cisplatin combined with F3-Np via tail vein injection or (c) with F3-Cis-Np via tail vein injection (i.v.) at 100 mg/kg of nanoparticles (final cisplatin concentration 75 μg/kg). Mice were treated initially at day 10 and day 14 (all tumor xenografts) and day 21 (for ID8 and SKOV3 xenografts only). Tumor volume was monitored via caliper (grafts) and day 21 (for ID8 and SKOV3 xenografts only), or mock treated with PBS. The cells were treated initially at day 10 and day 14 (all tumor xenografts) and day 21 (for ID8 and SKOV3 xenografts only), or mock treated with PBS. The cells were washed after 4 hours and then allowed to grow for a total of 72 hours prior to harvesting for cell counting via trypan blue exclusion.

**Results**

**Targeting of F3-targeted nanoparticles to TECs and tumor cells in vitro**

Blank, fluorophore-conjugated, or cisplatin-encapsulated PAA nanoparticles were prepared with a final average size ranging from 20 to 30 nm (Fig. 1). The PAA nanoparticle is a hydrogel that has a high aqueous solubility and long plasmacirculation time, being suitable for in vivo applications (14, 16, 26). It also has excellent engineerability within both nanoparticle core and surface, which allowed conjugation of fluorophores and/or multiple targeting/visualization peptides for the current studies. F3 peptide was conjugated to the nanoparticles for targeting. This peptide, a subcomponent of the HMGN2 protein, has shown specificity for both human tumor cells and tumor vasculature (13–15). Alexa-fluor-594 was bound to the nanoparticles (Alex-Np) for visualization in initial targeting experiments. To test the ability of the F3 peptide to target ovarian tumor cells, both mouse and human ovarian cancer lines (mouse ID8 and human SKOV3, A2008, and A2780) were incubated with either F3-Alex-Np (100 μg/mL) or Alex-Np (1 mg/mL). We observed significant binding of the F3-targeted nanoparticles to all ovarian tumor cell lines tested (Fig. 2A). Little or no binding was observed with nanoparticles that lacked the targeting F3 peptide. We next tested the ability of F3-Np to bind to TECs (Fig. 2A). F3-Alex-Np showed strong binding to both mouse and human TECs. Once again, nontargeted nanoparticles showed little or no binding.

**In vitro cytotoxicity of F3-targeted cisplatin-encapsulated nanoparticles**

To determine the cytotoxic potential of F3-targeted nanoparticles, tumor cell lines were treated with either PBS, F3-Cis-Np, or blank F3-Np combined with cisplatin for four hours and then washed. The final concentration of cisplatin in the F3-Cis-Np was 0.15 μg/mL and that of cisplatin combined with the blank F3-Np was 1 μg/mL for TEC/PBMC experiments and 5 μg/mL for A2780 and SKOV3 experiments. The cells were then allowed to grow for an additional
72 hours and harvested. We then assayed the number of viable cells in each treatment group relative to the PBS control. Consistent with previous studies on nanoparticles targeting tumor cells, our experiments showed little cell death in the nanoparticle experimental groups (Fig. 2B; refs. 27–29).

We then repeated these experiments using TECs isolated from both murine ID8-VEGF ovarian tumors and human ovarian cancers. Unlike what was observed for the tumor cell lines, we found significant induction of cell death with F3-Cis-Np on both mouse and human TECs. No cell death was noted when treating control PBMCs (Fig. 2B). Thus F3-targeted nanoparticles seem to be more toxic to TECs than to tumor cell lines.

**F3-Np target tumor vessels in vivo**

To test the efficacy of F3 targeting in vivo we used the highly vascular ID8-VEGF ovarian tumors model (30). Mice bearing ID8-VEGF tumors were treated i.v. with increasing concentrations of either nontargeted Alexa488-Np or F3-targeted Alexa488 nanoparticles. Mice were sacrificed 24 hours after infusion, and multiple tissue and tumor specimens were harvested and examined via fluorescence for nanoparticle localization. Highest specific binding to tumor vessels without significant uptake in liver and kidney was determined at a dose of 100 mg/kg (range tested, 25–200 mg/kg; Fig. 2C and data not shown). At this dose, nontargeted FITC-Np showed little tumor-specific uptake, but were found at significant levels in the liver and kidney. In contrast, at this concentration we observed significant uptake of F3-FITC-Np in tumor vessels and some uptake within tumor parenchyma. We noted minimal uptake of F3-FITC-Np in kupffer cells of the liver and within the renal collecting tubules (Fig. 2C and data not shown). No significant binding was observed in other tissues, including the lung, heart, spleen, intestine, and brain (data not shown).

**Therapeutic efficacy of F3-Cis-Np**

Next, to test the therapeutic efficacy of the nanoparticles, ID8-VEGF tumors were grown in the axillas of mice for 14 days. Mice were then treated i.v. with two weekly doses of F3-Cis-Np, with a final cisplatin concentration of 70 μg/kg, i.p. cisplatin at 250 μg/kg or i.p. cisplatin at 250 μg/kg, combined with i.v. blank F3-Np (total n = 15 for cisplatin or cisplatin and F3-Np and n = 20 for F3-Cis-Np in two independent experiments) and then were sacrificed one week later. Following the initial administration of F3-Cis-Np we observed a rapid and significant decline in tumor volume. This was maintained throughout the experiment. Intraperitoneal cisplatin alone and i.p. cisplatin/i.v. F3-Np yielded identical results (data not shown). At the conclusion of the experiment, a 2.5-fold reduction in overall weight of the tumors and ~3.5-fold reduction in volume were noted when compared with i.p. cisplatin alone or i.p. cisplatin/i.v. F3-Np (Fig. 3A and data not shown). Histologic analysis of these tumors showed (a) large regions of hemorrhage and necrosis consistent with a potent antivascular effect and (b) a significant reduction in the size of tumor islets with an increase in stromal tissues (Fig. 3B).

To determine the potential toxicity of this therapy, we collected serum from three animals in each treatment group 24 hours after the last i.v. treatment. Serum creatinine (a marker of renal function), aspartate aminotransferase and alanine aminotransferase (markers of hepatic function), and complete blood counts were not significantly different

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**Figure 1.** Development of nanoparticles. A, schematic illustration of the production of both blank and cisplatin containing F3-targeted nanoparticles. B, scanning electron micrographs showing the size of the nanoparticle after modification.
among untreated and F3-Cis-Np–treated mice (Fig. 3C). This suggests that these nanoparticles are not excessively toxic to normal tissues. Further supporting the safety of the nanoparticles, unlike mice treated with systemic cisplatin, we observed no significant weight loss for F3-Cis-Np–treated mice or any other adverse effects (data not shown).

We next tested the impact of nanoparticles on tumor growth using an orthotopic i.p. tumor model. Tumors were

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**Figure 2.** Binding and cytotoxicity of F3-targeted nanoparticles. **A,** in vitro binding of F3-targeted Alexa-594 nanoparticles (F3-Alexa-Np) but not for nontargeted Alexa-594 control nanoparticles (Alexa-Np) to murine and human tumor endothelial cells (mTEC and hTEC) and murine (ID8) and human (SKOV3) tumor cell lines. **B,** percent viable cells (relative to PBS controls) following treatment with F3-Cis-Np, blank F3-Np, or cisplatin (1 μg/mL for TECs and 5 μg/mL for tumor cells) targeting human TECs, human peripheral blood monocytes, and ovarian tumor cells (SKOV3 and A2780). **C,** in vivo binding of F3-Alexa488-Np and nontargeted control Alexa-488 nanoparticles in murine ID8 flank tumors and kidneys of treated mice.
Figure 3. Therapeutic efficacy and toxicity of F3-cisplatin nanoparticles in a murine ovarian tumor model. A, tumor growth curves and weights of ID8 xenografts treated with F3-Cis-Np or blank F3-Np and systemic cisplatin (n = 15 animals per group). Arrows, time of treatment. B, tissue histology of tumors from tumors treated with blank F3-Np and systemic cisplatin (F3-Np + Cis) or F3-Cis-Np. Low-power image shows large area of vascular necrosis (top right) in treated tumors. High-power images show significant reductions in tumor islets in treated tumors (bottom right). C, lack of toxicity in F3-Cis-Np–treated animals as shown in alanine aminotransferase (ALT) and aspartate aminotransferase (AST), blood urea nitrogen (BUN), and serum creatinine from mice treated with F3-Cis-Np or F3-Np controls. Values in parentheses, normal ranges. D, Kaplan Meier curves indicating survival using an orthotopic i.p. tumor model: 1, comparing i.v. F3-Cis-Np versus i.v. control nanoparticles + systemic cisplatin delivered i.p. (F3-Np + Cis); 2, comparison of indicated treatment groups comparing i.v. and i.p. treatment of F3-Cis-Np and various controls. Greatest survival advantage was seen in the i.v. F3-Cis-Np group. Addition of i.p. F3-Cis-Np added no survival advantage (n = 10 animals per group). Arrows, time of treatment.
allowed to engraft for 10 days and then mice were treated with either i.p. cisplatin at 250 μg/kg along with F3-Np i.v., i.p. cisplatin alone, or F3-Cis-Np i.v. with a final concentration of 75 μg/kg cisplatin. We observed a significant increase in the overall survival of animals treated with the F3-Cis-Np as compared with i.p. cisplatin alone or along with F3-Np (Fig. 3D1 and data not shown). To determine if combining i.v. F3-Cis-Np with i.p. F3-Cis-Np therapy could improve survival further by targeting both tumor vasculature (via i.v. dosing) and tumor cells (via i.p. dosing), we repeated the orthotopic tumor studies and compared various combinations of blank F3-Np and cisplatin delivered i.v., i.p., or combined i.p. and i.v. versus F3-Cis-Np via i.v., i.p., or i.v. and i.p. administration at 150 μg/kg (for i.v. or i.p. alone) or 75 μg/kg for the both i.v. and i.p. treatments for a total cisplatin concentration of 150 μg/kg. Mice were treated on days 10, 14, 21, and 28. We observed a significant survival advantage for all groups that received F3-Cis-Np i.v. as compared with mice receiving blank F3-Np combined with cisplatin. Interestingly, combined i.v. and i.p. treatment with F3-Cis-Np did not show a significant difference in survival (Fig. 3D). This suggest that vascular exposure is the primary means of therapy as the addition of i.p. therapy that could directly target tumor cells had no added benefit.

We also tested the impact of F3-Cis-Np using s.c. human tumor cell line xenograft tumor models. We used cisplatin-sensitive Hey1 and A2780-GFP, and cisplatin-resistant SKOV3 (IC50 4 mmol/L) ovarian tumor cell lines (n = 11 in treatment and control groups). As the human tumor xenografts with Hey1 and A2780 cells grow more rapidly than ID8 cells we used a more frequent treatment schedule, treating mice on days 7, 10, and 14 after tumor engraftment with either F3-Cis-Np or blank Np and systemic cisplatin as described above. In the platinum-sensitive Hey1 and A2780-GFP tumors there was clear arrest of tumor growth during the course of therapy as indicated both by tumor volumes and in vivo biofluorescent imaging (Fig. 4A and B, and data not shown). Consistent with an antivascular effect rather than an antitumor effect, we observed a similar growth arrest with therapy of the platinum-resistant SKOV3 tumors. Tumors resected from these mice were extremely pale compared with controls (Fig. 4B). Histochemical analysis of F3-Cis-Np–treated tumors showed a significant reduction in microvasculature and significant tumor necrosis similar to that observed with the ID8 tumors (data not shown).

F3-Cis-Np target and eliminate human tumor vessels in vivo

Finally, we wished to determine if the nanoparticles could bind to human tumor vessels in vivo. We used a recently developed model of human tumor vasculature that utilizes Hey1 tumors cells injected into H9 ESC-derived teratomas (22, 23). In this model tumor vascular cells are derived from human ESC and thus are human in origin. We performed i.v. injections of F3-Alexa-Np in H9-ESC-Hey1 tumor–bearing mice. Importantly, we observed clear binding of the F3-Alexa-Np to the human CD31+ tumor vessels, confirming the ability of these F3-targeted nanoparticles to bind to human vessels in vivo. We therefore used this model to test the therapeutic efficacy of F3-Cis-Np. To track tumor cell

Figure 4. Therapeutic efficacy of F3-Cis-Np against human tumor xenografts. A, A2780 cisplatin-sensitive tumor xenografts treated with F3-Np + cisplatin or F3-Cis-Np; 1, tumor growth curves; 2, in vivo fluorescent imaging. B, cisplatin-resistant SKOV3 tumor xenografts treated with F3-Np + cisplatin or F3-Cis-Np; 1, tumor growth curves; 2, gross tumor pathology. F3-Cis-Np–treated tumors were significantly smaller and pale/avascular (n = 5/group in independent experiments).
growth we used immunofluorescent DsRed Hey1 tumor cells. Once again we treated animals with either i.v. F3-Np and systemic cisplatin or F3-Cis-Np \((n = 3\) controls and \(n = 3\) treatment groups in two independent experiments). Animals were treated on days 10, 14, 17, and 21. As in the previous tumor models, treatment with F3-Cis-Np led to an initial loss of fluorescence and then stabilization of disease, whereas tumors treated with systemic cisplatin showed progressive growth (Fig. 5).

**Discussion**

We used an F3-targeted polymeric nanoparticle formulation consisting of encapsulated cisplatin in a polyacrylamide nanoparticle to target tumor vessels as a cancer therapeutic. Although vascular-targeted nanoparticle studies have been reported previously, to our knowledge ours is the first to show the ability to bind to human tumor vessels *in vivo*.

*In vitro* studies showed specific binding to tumor and tumor endothelial cells. *In vivo* studies showed predominant binding to microvascular TECs with lesser uptake on tumor cells. Minimal nonspecific uptake in kupffer cells of the liver and renal excretion of nanoparticles were noted, but nonspecific binding was minimized with titration of dose. Minimal toxicity was noted with F3-Cis-Np treatment as evidenced by stable creatinine, liver function tests, and complete blood counts. This is not surprising given the total cisplatin dose used in the nanoparticle studies (75 or 150 \(\mu\)g/Kg) was \(\sim 5\%\) the traditional dose of cisplatin (1–5 mg/kg).

In addition to being safe, F3-targeted nanoparticles seem to be highly effective as a therapeutic. Although F3 peptides can bind both tumor cells and endothelial cells, our studies...
suggest that the primary effect of therapy was antivascular: Similar to prior studies, minimal in vitro cytotoxicity was noted with F3-Cis-Np treatment of human tumor cell lines (27–29). This may be due to the higher concentration of drug needed to kill tumor cells as evidenced by their higher IC50.

In contrast, we observed significant in vitro cytotoxicity of F3-Cis-Np on human TECs (Fig. 2C). We also observed a rapid impact of therapy, associated with large regions of necrosis and hemorrhage, and a loss of clearly defined microvasculature consistent with an antivascular effect. Moreover, F3-Cis-Np were effective even in cisplatin-resistant tumors. Finally, like with other antiangiogenics, continuous treatment was associated with stable disease and no further reduction of tumor was noted.

F3-Cis-Np therapy was effective in both solid tumor and i.p. tumor models. Although the impact of therapy on flank tumors was most dramatic, a clear survival advantage was noted in the i.p. tumor model. The survival advantage was primarily associated with i.v. treatment. Interestingly, the combination of both i.p. and i.v. nanoparticles did not show a clear advantage over i.v.-only nanoparticles. This may be because the cisplatin dose in the F3-Cis-Np is subtherapeutic to kill tumor cells as observed in vivo. Alternatively, although we did not see significant systemic nanoparticle exposure with i.p. delivery (data not shown), it is possible the nanoparticles delivered i.p. are still primarily taken up in the tumor vasculature.

Based on previous observations that F3 peptide can target tumor cells, we chose to load our nanoparticles with cisplatin, the most active anti–ovarian tumor cell agent. Given our observations that the F3-targeted nanoparticles seem to be primarily targeting tumor vascular cells, cisplatin may not be the most active chemotherapeutic. Cisplatin, a DNA-targeting agent active against actively proliferating cells, may spare established nondividing vascular cells. We hypothesize that F3-Np therapy with a microtubule targeting agent may show even greater activity. Furthermore, an iRGD peptide was recently described (21). This peptide targets the vasculature and is then cleaved to allow release of the peptide with a now-exposed neuropilin-1 binding motif that mediates penetration of the peptide through the vasculature to target tumor cells. Thus, a combination of F3-taxane nanoparticle and iRGD peptide–targeted cisplatin nanoparticles could be particularly effective, targeting neovascularization, established tumor vessels, and tumor cells. Given the extremely low doses of chemotherapeutic agents necessary for effectiveness of the nanoparticles as shown in the current study, such a combination could be possible with an acceptable side effect profile. Further experiments will be necessary to determine the appropriate sequencing for combining these agents; vascular disruption prior to the administration of antitumor agents could in theory reduce intratumoral drug delivery and thereby reduce efficacy. In this context, dynamic imaging studies may be useful to address the optimal scheduling of combinatorial therapy.

Finally, we show for the first time the ability of vascular-targeted nanoparticles to target human TECs, both in vitro and in vivo. We used a human ESC-based tumor model in which a subset of the tumor vascular cells is derived from the human ESC and consists of human tumor vessels (22). We have shown that the vessels in this model are indeed tumor vessels, expressing unique tumor vascular markers (31). Thus, we believe that this model represents an excellent tool for the study of therapeutics targeting human tumor vasculature.

In conclusion, we have shown that F3-targeted nanoparticles are a safe and potently effective therapeutic targeting human tumor endothelial cells. This proof-of-principle study shows the ability to overcome ovarian cancer chemoresistance using vastly reduced drug concentrations by targeting nanoplates to the tumor neovascularization/microenvironment.

Disclosure of Potential Conflicts of Interest

The authors have no conflicts of interest to report.

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9. Lu C, Bonome T, Li Y, et al. Gene alterations identified by expression...
Correction: F3-Targeted Cisplatin-Hydrogel Nanoparticles as an Effective Therapeutic that Targets Both Murine and Human Ovarian Tumor Endothelial Cells \textit{In vivo}

In this article (Cancer Res 2010;70:8674–8683), which was published in the November 1, 2010 issue of \textit{Cancer Research} (1), the name of the third author is misspelled. The correct name should be Yong-Eun Koo Lee. In addition, an indication that the first 2 authors contributed equally to the work was missing.

Reference

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