Vascular Endothelial Growth Factor Receptor 3 Is Involved in Tumor Angiogenesis and Growth

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Abstract

Vascular endothelial growth factor receptor 3 (VEGFR-3) binds VEGF-C and VEGF-D and is essential for the development of the lymphatic vasculature. Experimental tumors that overexpress VEGFR-3 ligands induce lymphatic vessel sprouting and enlargement and show enhanced metastasis to regional lymph nodes and beyond, whereas a soluble form of VEGFR-3 that blocks receptor signaling inhibits these changes and metastasis. Because VEGFR-3 is also essential for the early blood vessel development in embryos and is up-regulated in tumor angiogenesis, we wanted to determine if an antibody targeting the receptor that interferes with VEGFR-3 ligand binding can inhibit primary tumor growth. Our results show that antibody interference with VEGFR-3 function can inhibit the growth of several human tumor xenografts in immunocompromised mice. Immunohistochemical analysis showed that the blood vessel density of anti-VEGFR-3–treated tumors was significantly decreased and hypoxic and necrotic tumor tissue was increased when compared with tumors treated with control antibody, indicating that blocking of the VEGFR-3 pathway inhibits angiogenesis in these tumors. As expected, the anti-VEGFR-3–treated tumors also lacked lymphatic vessels. These results suggest that the VEGFR-3 pathway contributes to tumor angiogenesis and that effective inhibition of tumor progression may require the inhibition of multiple angiogenic targets. [Cancer Res 2007;67(2):593–9]

Introduction

The vascular endothelial growth factor receptor 3 (*Vegfr3*; *Flt4*) gene is essential for the remodeling and maturation of the embryonic blood capillaries, and, in its absence (*Vegfr3*−/−), embryos die around E10 due to cardiovascular failure (1). After this time point, *Vegfr3* expression in normal embryos becomes restricted to the lymphatic vessels, which develop from the largest embryonic veins (2). In addition to the lymphatic endothelium, fenestrated blood capillaries of some adult organs continue to express low amounts of VEGFR-3 (3). Activation of downstream signaling via the binding of VEGF-C or VEGF-D to VEGFR-3 is required for growth of the lymphatic vasculature (4). The VEGFR-3 receptor homologue in zebrafish embryos is also required for normal blood vessel development (5, 6).

In experimental tumor models, overexpression of VEGFR-3 ligands by tumor cells induced intratumoral and peritumoral lymphangiogenesis and increased metastasis to the regional lymph nodes (7–11), whereas a soluble form of VEGFR-3, which inhibits VEGF-C/VEGF-D signaling, inhibited both lymphangiogenesis and metastasis (9, 12–14). Furthermore, VEGF-C/VEGF-D produced by tumor cells were shown to induce sprouting of lymphatic capillaries and dilation of the draining peritumoral lymphatic vessels (9, 15), thus facilitating lymphatic metastasis, which could be blocked dose-dependently by inhibition of VEGFR-3 signaling using the soluble receptor (16).

Considering that VEGFR-3 is required for hypoxia-driven vascular development and that it interacts with VEGFR-2 (5, 17), and because its expression is up-regulated in angiogenic tumor vessels (18–20), we wished to determine if a monoclonal antibody targeting VEGFR-3 (mF4-31C1), which is capable of preventing ligand binding to the receptor (21), can inhibit primary tumor growth.

Materials and Methods

Mouse, cell lines, and tumors. NCI-H460-LNM35 cells, which are a subline of NCI-H460-N15, a human large-cell carcinoma of the lung (22), and LLC cells were maintained in RPMI 1640 and DMEM, respectively, supplemented with 2 mmol/L L-glutamine, penicillin (100 units/mL), streptomycin (100 μg/mL), and 10% fetal bovine serum (Autogen Bioclear, Calne, United Kingdom). Luciferase (Luc)-tagged sublines of NCI-H460-LNM35 were then established by transfection with AAV-Luc virus, and Luc+ clones were isolated by means of limiting dilution (16, 22). HT-29, SK-RC-29, BxPC-3, HPAC, and PC-3 cells were obtained from the American Type Culture Collection (Manassas, VA). Nude BALB/c (5–6 weeks of age) or severe combined immunodeficient (SCID; 7–9 weeks of age) mice were purchased from Taconic (Cologne, Denmark) or from Charles River Laboratories (Wilmington, MA). Mice were housed under pathogen-free conditions in microisolator cages with laboratory chow and water available *ad libitum*. All experiments and procedures were done in accordance with the Finnish legislation and the U.S. Department of Agriculture, Department of Health and Human Services, and NIH policies on humane care and use of laboratory animals. NCI-H460-LNM35 cells (1×10^7 per SCID mouse and 1.5×10^6 per nude mouse) and LLC cells (1.5×10^6 per mouse) were injected s.c. into the abdomen. All other s.c. xenografts were established by injecting cells (2×10^6 SK-RC-29 or HPAC; 5×10^6 HT-29, PC-3, or BxPC-3) in 50% Matrigel (Collaborative Research Biochemicals, Bedford, MA) s.c. into the flank.

Subcutaneous tumors were allowed to reach a threshold volume (100–500 mm^3) and then the mice were randomized by tumor volume into

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Table 1. VEGF-C, VEGF-A, and VEGFR-3 expression by tumor cell lines

Tumor type	Cell line	Ligand concentration			Receptor (VEGFR-3)*
		VEGF-C †	*F* ‡	VEGF-A †	
Epidermoid	A431	500		5,787	1
Breast	DU4475	0		150	1
	MCF-7	0		850	1
	MDA-MB-231	2,200	1/6	3,263	1
	MDA-MB-435	0		3,150	1
	MDA-MB-468	0		15,000	1
	MDA-MB-435 LM2	0		1,180	1
Gastrointestinal	CaCo2	0		250	1
	Colo205	0		4,500	1
	DIFI	0		700	1
	DLD-1	0		700	1
	GEO	0	0/12	4,066	1
	HCT-8	0		2,900	1
	HCT-116	0		2,025	1
	HT-29	0		1,348	1
	LoVo	0		7,000	1
	T84	0		7,137	1
	SW620	0		203	1
	NCI-N87	0		1,443	1
Head and Neck	Cal-27	6,729		5,000	1
	FADU	925	3/3	1,016	1
	Detroit 562	5,881		7,269	1
Leiomyosarcoma	SK-LMS-1	6,286	1/1	6,657	1
Ovarian	OVCAR5	0		3,090	1
	OVCAR8	0	1/4	359	3
	MDAH-2774	2,000		ND	1
	SKOV3	0		481	1
Pancreatic	BxPC-3	0		950	1
	HPAC	0	1/3	2,700	1
	L3.7pL	324		3,800	1
Prostate	Du145	0		550	1
	LNCaP	0	1/3	4,773	1
	PC-3	5,400		2,400	1
Renal	A498	380		1,000	1
	Caki-1	800	3/3	450	1
	SK-RC-29	1,333		30,400	1
NSCLC	A549	1,870		700	1
	NCI-H1650	0		4,500	1
	NCI-H292	0		4,750	1
	NCI-H460	2,278		3,200	1
	NCI-H441	0	2/10	30,666	1
	NCI-H226	0		2,250	1
	Calu-6	0		264	1
	NCI-H358	0		8,409	1
	NCI-H358	0		19,345	1
	NCI-H1975	0		6,493	1
SCLC	NCI-H82	0	0/1		1
Osteosarcoma	KHOS/NP	977	2/2	3,521	1
	MNNG/HOS	2,088		1,570	1
Glioblastoma	U-118 MG	46,875	2/2	26,938	1
	U-87 MG	7,094		16,250	1

Abbreviations: ND, not determined; NSCLC, non–small-cell carcinoma of the lung; SCLC, small-cell carcinoma of the lung.
*Mean fluorescence intensity.
†Concentration in media at 24 h (pg/mL/10^6 cells).
‡Frequency of cell lines expressing VEGF-C in culture.

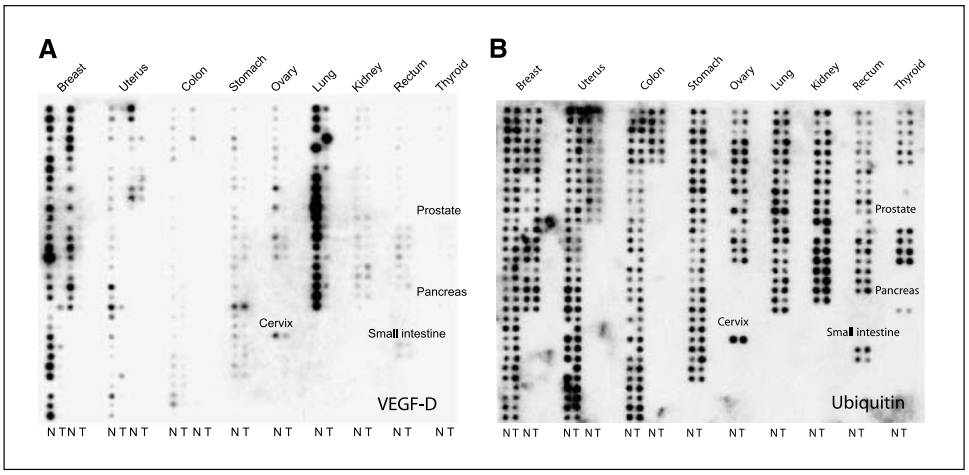

Figure 1. Expression of VEGF-D RNA by human tumors. *A,* to estimate the expression levels of VEGF-D in human tumor samples, we performed a dot blot analysis of VEGF-D mRNA in 241 human tumors (*T*) and corresponding normal tissues (*N*) from individual patients on a cDNA tissue array. Whereas VEGF-D was expressed in many normal tissues, its expression was in general down-regulated in tumors. No difference in the expression of the housekeeping gene *ubiquitin* was detected between the normal and tumor tissues in the same array (*B*).

treatment groups. Tumor-bearing mice were treated with antimouse VEGFR-3 antibody [mF4-31C1 (21), control immunoglobulin G (IgG), bevacizumab (ref. 35; obtained from the Helsinki University Hospital, Helsinki, Finland), or in combination every second day by i.p. or i.v. injection of the antibodies]. Tumor volumes were measured with a caliper and calculated according to the formula V = width × height × depth / 2, derived from the formula for the volume of an ellipsoid (24). Tumor volumes were analyzed using repeated-measures ANOVA with the JMP Statistical discovery package (v. 5.1, SAS Institute, Inc., Cary, NC).

***In vivo* imaging of primary tumors and quantification of bioluminescence signals *in vivo*.** Imaging was done at 1-week time intervals to detect primary tumor growth. For *in vivo* imaging, mice were injected i.p. with D-luciferin (Synchem, Kassel, Germany) at 150 mg/kg of mouse body weight. The light emitted from the bioluminescent tumors was detected with the IVIS Imaging System (Xenogen, Alameda, CA). Images of bioluminescent signals were acquired and the photonic signal intensities were subsequently quantified using the Living Image software (Xenogen).

Antibodies and immunohistology. Blood vessels were visualized by staining tissue sections with monoclonal antibodies against CD31 (rat anti-mouse or Syrian hamster anti-mouse antibodies from PharMingen) or MECA-32 (PharMingen). Goat anti-mouse VEGFR-3 (R&D Systems) was used to stain VEGFR-3 in the tumor sections. A polyclonal rabbit anti-mouse LYVE-1 antibody (25) was used to visualize lymphatic vessels. The primary antibodies were detected with the appropriate Alexa 488 or Alexa

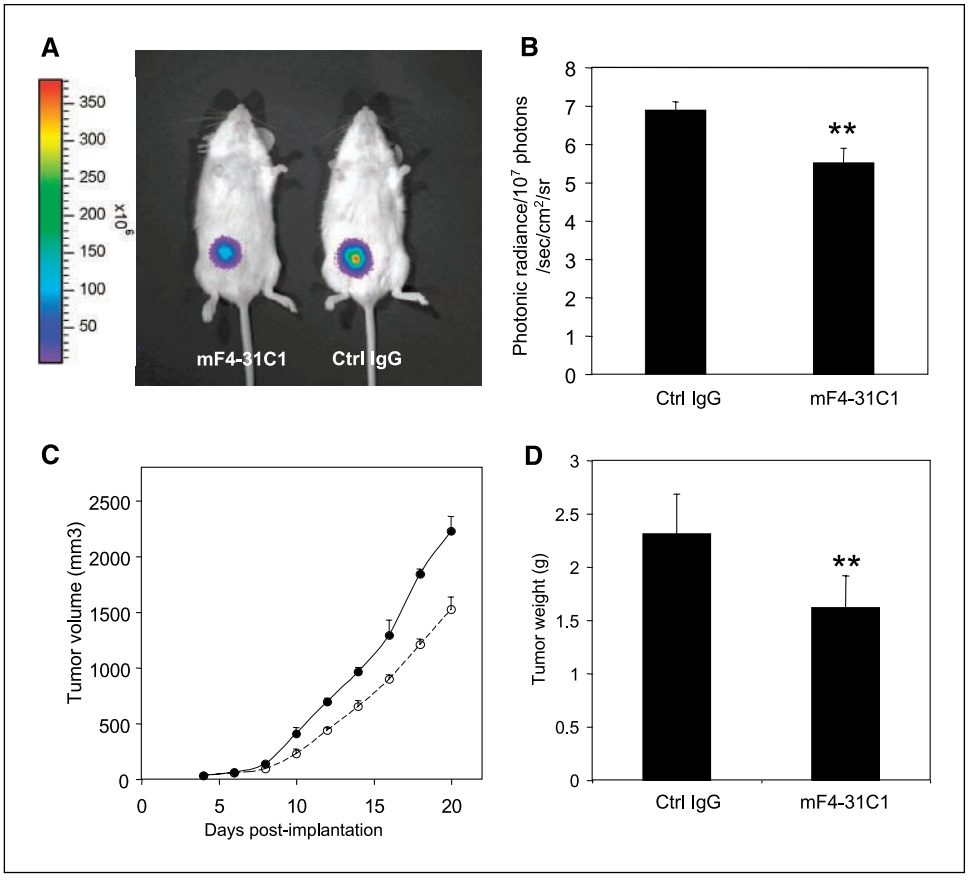

Figure 2. Antibody targeting of VEGFR-3 inhibits the growth of NCI-H460-LNM35 human lung carcinoma. *A,* tumor response to VEGFR-3 inhibition by mF4-31C1 antibodies was assessed by bioluminescent imaging 1 wk after s.c. inoculation of the luciferase tagged tumor cells into SCID mice. In this experiment, the treatment was started simultaneously with tumor cell implantation. Representative bioluminescent images of mF4-31C1- and rat IgG–treated (*Ctrl IgG*) primary tumors with an interpretative color bar. *B,* quantification of the average bioluminescent radiance after mF4-31C1 treatment ($P = 0.0037$; $n = 7$ in mF4-31C1, $n = 11$ in control IgG group). *C,* growth curves of mF4-31C1-treated (*dotted line with open circles*) and control IgG–treated (*line with solid circles*) tumors. *Bars,* SD. *D,* mean primary tumor weights at the end of the treatment; *bars,* SE.
**, $P < 0.01$.

594 (Molecular Probes)–conjugated secondary antibodies or by peroxidase immunohistochemistry using the trichostatin A detection method (Perkin-Elmer, Life Sciences). Whole-mount staining was done on 100-μm-thick frozen sections. The capture ELISA assays were done per manufacturer's instructions (R&D Systems). To assess the VEGF-D expression in different human tumors and corresponding normal tissues from individual patients, we used a tissue array (Cancer Profiling Array I, Clontech) following the manufacturer's instructions.

Necrosis and hypoxia. Hypoxic tumor tissue was detected by i.v. injection of the hypoxia marker pimonidazole hydrochloride (60 mg/kg; Hypoxyprobe kit, Chemicon International; ref. 40), followed by staining of tissue sections with FITC-conjugated monoclonal antibodies (Hypoxyprobe-1 Mab1, Chemicon International) that recognize pimonidazole adducts in hypoxic tissue proteins.

Image analysis. Sections from eight tumors from both treatment groups were viewed under a Leica DM LB microscope, and images were captured with an Olympus DP50 color camera. The samples were viewed at ×25 to ×100 magnifications. For quantitative analysis of the hypoxic and necrotic areas, the Image-Pro Plus software of MediaCybernetics was used. Necrotic areas were quantified from two to four sections per tumor (four tumors per treatment group) and the whole area of a section was used for quantification.

Results

Recent publications have suggested that VEGFR-3 is expressed in a large fraction of human cancers (27–30). Although we originally cloned VEGFR-3 from the HEL erythroleukemia cell line, our studies had indicated that this receptor is rarely expressed by nonvascular tumor cells in monolayer culture (23, 31, 32). We therefore carried out flow cytometric analysis on 52 tumor cell lines, of which only two (OVCAR8 and HEL) were positive for VEGFR-3 (Table 1). The VEGFR-3-specific staining with the hF4-3C5 antibody, which recognizes human VEGFR-3 (33), in the OVCAR8 or HEL cell lines was inhibited by incubating the cells with the corresponding immunogen (data not shown). Many of the tumor cell lines secreted VEGFR-3 ligand VEGF-C (18 of 52, 35%; Table 1), whereas none of the cell lines tested secreted VEGF-D as judged by a capture ELISA. All tumor cell lines secreted VEGF165 (detection limits in the capture ELISA analysis were 62 pg/mL for VEGF-D, 94 pg/mL for VEGF-C, and 15 pg/mL for VEGF165; Table 1). Analysis of RNA from 241 human tumors and corresponding normal tissues from individual patients indicated that many normal tissues expressed VEGF-D mRNA, with breast and lung tissues displaying notably high expression. Total VEGF-D mRNA was in general down-regulated in tumors (Fig. 1). It should be noted, however, that VEGF-D expression has been reported in several human tumor types and in tumor stromal cells, such as inflammatory cells (34). Indeed, the analysis of total RNA would not be sensitive enough to high regional expression of VEGF-D.

To determine if the inhibition of VEGFR-3 signaling by a blocking antibody against the receptor would have an effect on primary tumor growth, we treated tumor-bearing nude or SCID mice with the mF4-31C1 antibody. This antibody inhibits ligand binding and phosphorylation of murine VEGFR-3 and blocks VEGF-C-stimulated downstream signaling (21, 33).[4] We first studied the effect of m4F-31C1 in early phases of tumor growth by using luciferase-expressing NCI-H406-LNM35 cells in SCID mice (16, 22). Inhibition of tumor growth was evident already after four injections of mF4-31C1 antibodies (Fig. 2*A* and *B*). We then investigated later stages of primary tumor growth and expanded our study to other types of tumors. Our results showed that mF4-31C1 antibodies inhibited the s.c. growth of several different tumor cell lines in nude mice (Fig. 3). Variable inhibition of primary tumor growth by 30% to 44% occurred in xenograft models of human pancreatic carcinomas (BxPC-3 and HPAC), a renal carcinoma (SK-RC-29), a colon carcinoma (HT-29), a prostate carcinoma (PC-3), a large-cell lung carcinoma (NCI-H460-LNM35), and in a mouse lung carcinoma (LLC). The only tumor model tested, which seemed to be very resistant to the anti-VEGFR-3 treatment, was an aggressive, subclone of the human colon carcinoma SW480 (ref. 22; Supplementary Table S1).[5]

Figures 2*C* and 3 show examples of the growth curves of NCI-H460-LNM35, HT-29, BxPC-3, and PC-3 tumors treated with i.v. (Fig. 2*C*) or i.p. (Fig. 3) injections of the mF4-31C1 antibodies (*open circles, dotted line*) or with various controls (*solid circles*). Significant inhibition of tumor growth was obtained in 7 of 11 experiments with a dose of 40 mg/kg of mF4-31C1 (*P* values are shown in Supplementary Table S1). Half of this dose (20 mg/kg) was enough to significantly inhibit tumor growth in the NCI-H460-LNM35 xenograft model when administered into the tail vein (Fig. 2*C* and *D*). Although a lower dose (10 mg/kg; Fig. 3*B* and *C, solid boxes*) seemed to decrease tumor growth, this effect did not reach statistical significance.

Histologic analysis using antibodies against blood vascular endothelial markers MECA-32 or CD31 revealed that the mF4-31C1-treated tumors had significantly decreased blood vessel density in comparison with the control antibody–treated tumors (Fig. 4*A, B, E*, and *F*; $P \leq 0.001$), suggesting that the antitumor effect was through inhibition of tumor angiogenesis. Reduction in blood vessel density was observed both in the tumor periphery and in the core of the treated tumors (Fig. 4*F*). These results suggested that VEGFR-3 was expressed in the tumor blood vessels. To confirm this, we double stained tumor sections with antibodies against VEGFR-3 and vascular marker proteins CD31 and LYVE-1. Many of the blood vessel endothelial cells in the NCI-H460-LNM35 tumors were indeed positive for VEGFR-3 in frozen sections double stained for CD31 (Fig. 5*A*), whereas they lacked staining for the lymphatic marker protein LYVE-1 (data not shown). In addition, the mF4-31C1-treated tumors lacked intratumoral lymphatic vessels (Fig. 4*C* and *D*), whereas no effect was detected in the preexisting lymphatic vessels in the skin overlying the tumor (Fig. 4*C* and *D, white arrows*), in accordance with previous results (21).

The effect of mF4-31C1 antibody was more pronounced than the mere tumor volume suggested because the mF4-31C1-treated tumors showed significantly more necrosis than the control antibody–treated tumors (Fig. 5*B–D*). This suggests that anti-VEGFR-3 therapy led to tumor tissue necrosis. In several cases, only a thin peripheral zone of viable tumor remained adjacent to the border of the tumor mass and surrounding host tissues in the mF4-31C1-treated tumors. The hypoxia marker pimonidazole hydrochloride stained the rim of hypoxic cells between the necrotic area and the viable tumor mass in both mF4-31C1- and control antibody–treated tumors (Fig. 5*C* and *D, arrows*). In addition to that, the hypoxia marker stained tumor cells inside the tumor mass in the mF4-31C1-treated tumors (Fig. 5*D, arrowheads*), indicating the presence of hypoxia in additional areas. Furthermore, a reduced

[4] Unpublished observations.

[5] Dr. Tatiana Petrova, personal communication.

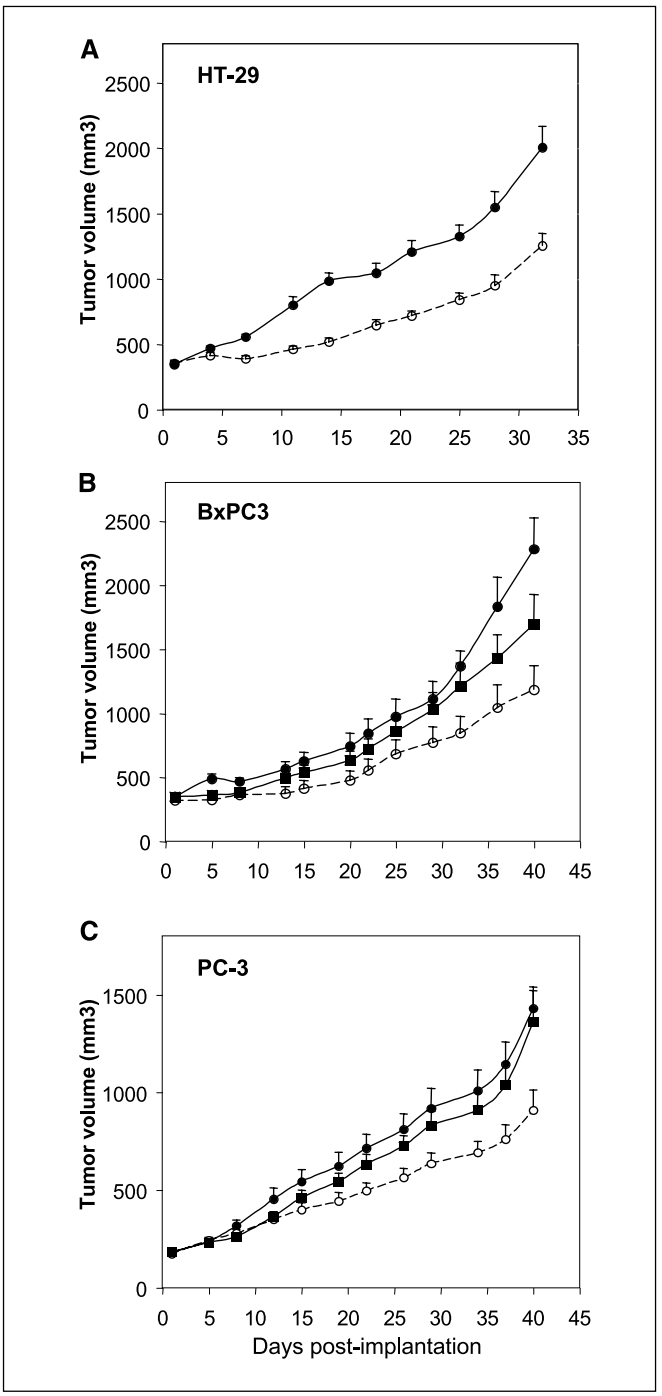

Figure 3. Anti-VEGFR-3 treatment inhibits primary tumor growth in several tumor models. Tumor cells were injected s.c. into nude mice. Tumor volumes were measured by using a caliper. Antibody treatment was started when the tumors were palpable. *Points,* mean tumor volumes during the treatment; *bars,* SE. *Line with solid circles,* control treatment (PBS); *dotted line with open circles,* mF4-31C1, 40 mg/kg; *line with solid boxes,* mF4-31C1, 10 mg/kg.

blood vessel density correlated with increased hypoxia around the vessels in the tumor mass (Fig. 5*E* and *F*).

Bevacizumab is a humanized monoclonal antibody against human VEGF, which has been approved for clinical use against metastatic colorectal cancer (35). Bevacizumab was very effective in inhibiting the growth of the NCI-H460-LNM35 xenografts at 5 mg/kg (~70% inhibition; $P = 0.002$). When a suboptimal dose (1.5 mg/kg) was used instead, bevacizumab inhibited primary tumor growth to a similar extent as the mF4-31C1 antibodies (35% and 45% inhibition, respectively), whereas a combination of both of these antibodies gave 62% inhibition of primary tumor growth (Supplementary Fig. S1). However, this difference was not statistically significant.

Discussion

Previous studies have shown that blocking the binding of VEGF-C and VEGF-D to VEGFR-3 using the soluble VEGFR-3-IgG Fc fusion protein inhibits tumor lymphangiogenesis and tumor metastasis to lymph nodes (9, 12–14, 16). We show here that treatment of tumor-bearing mice with the mF4-31C1 monoclonal antibody, which antagonizes ligand binding to VEGFR-3, inhibits primary tumor growth in several different experimental tumor models. Surprisingly, the efficacy of anti-VEGFR-3 treatment did not correlate with VEGF-C/VEGF-D expression by the corresponding

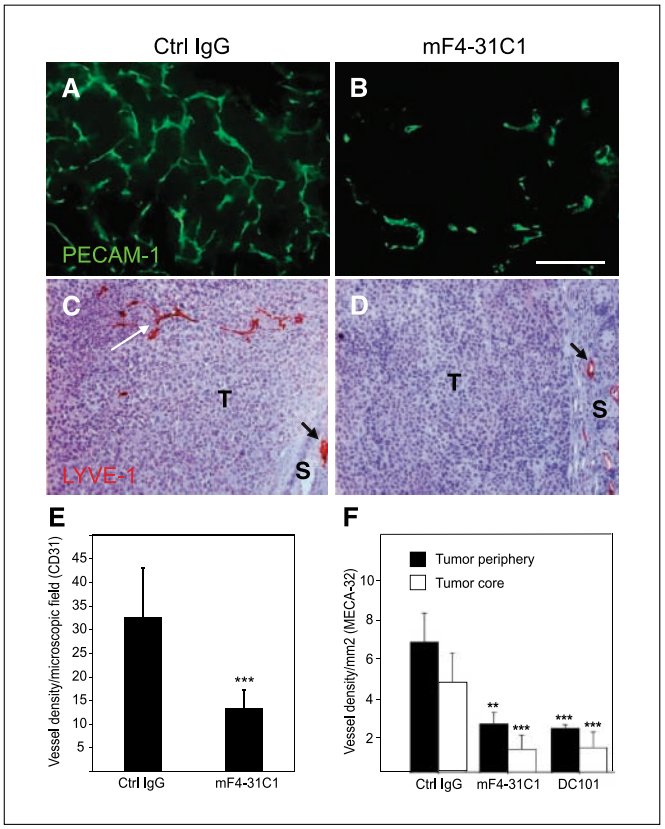

Figure 4. Decreased blood vessel density in anti-VEGFR-3–treated tumors. At the end of the treatment, the tumors were excised and prepared for histologic analysis. Blood vessels were stained with an anti-PECAM-1 antibody (*A* and *B, green*). Anti-VEGFR-3–treated tumors (*B*) showed a decreased density of PECAM-1–positive vessels when compared with control antibody–treated tumors (*A*). Staining with anti-LYVE-1 antibodies revealed that the mF4-31C1-treated tumors lacked intratumoral and peritumoral lymphatic vessels (*D*) whereas control antibody–treated tumors contained LYVE-1-positive vessels (*C, white arrow*). *C* and *D, black arrows,* normal lymphatic vessels in the skin overlying the tumor. *E,* quantification of PECAM-1–positive vessels in the treated NCI-H460-LNM35 tumor xenografts. *F,* quantification of MECA-32-positive vessels in the periphery and core of the treated SK-RC-29 tumor xenografts. Vessels were counted from three representative sections of each tumor (five per group). DC101, antimouse VEGFR-2 antibody. *Bars,* SD. *T,* tumor; *S,* skin. Magnification, ×200 (*A* and *B*); ×100 (*C* and *D*). Whole-mount staining of 100-μm sections (*A* and *B*) or staining of 7-μm sections on glass slides (*C* and *D*). **, $P < 0.01$; ***, $P < 0.001$. Bar, 50 μm.

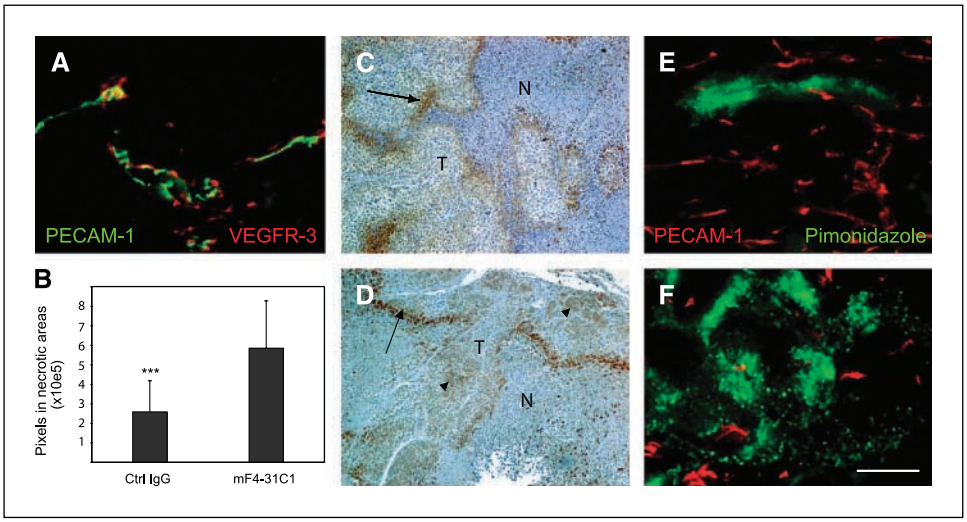

Figure 5. Anti-VEGFR-3–treated tumors show increased necrosis. *A,* sections from NCI-H460-LNM35 tumors were stained for VEGFR-3 (*red*). Blood vessels were visualized by anti-PECAM-1 staining (*green*). *B,* quantification of necrotic areas in mF4-31C1- and control antibody–treated tumors. *Bars,* SD. *C* and *D,* tumors were excised and hypoxic areas were visualized with a monoclonal antibody that recognizes pimonidazole adducts in hypoxic tissue (*C* and *D, brown*). *Arrows,* rim of hypoxic cells between the necrotic areas (*N*; *blue staining*) and viable tumor mass. Note that in the mF431C1-treated tumors, tumor cells inside the tumor mass stain brown for the hypoxia marker (*D, black arrows*). *E* and *F,* thick (100 μm) tumor sections were stained with antibodies against PECAM-1 (*red*) and pimonidazole adducts (*green*). The mF4-31C1-treated tumors (*F*) contained fewer blood vessels but larger hypoxic areas when compared with control antibody–treated tumors (*E*). *T,* viable tumor tissue. Magnification ×200 (*A*), ×100 (*C–F*). ***, $P < 0.001$. Bar, 50 μm.

tumor cell lines *in vitro* (Table 1). This may reflect the fact that VEGF-C/VEGF-D–negative tumor cell lines can induce ligand expression *in vivo* (13) and that inflammatory cells *in vivo* contribute significantly to VEGF-C/VEGF-D production (36). The inhibitory effect seemed to be through inhibition of angiogenesis because the anti-VEGFR-3–treated tumors contained significantly less blood vessels than the control antibody–treated tumors.

In a previous study, the rat anti-VEGFR-3 monoclonal antibody AFL4 was reported to inhibit tumor xenograft growth in mice by compromising the integrity of tumor blood vessels, leading to microhemorrhages (20). However, in our hands, the AFL4 antibody does not inhibit ligand binding or phosphorylation of VEGFR-3 nor does it block VEGF-C-stimulated cell proliferation (21). This means that the reported *in vivo* effects of the AFL4 are mediated by a mechanism other than the direct inhibition of ligand binding as with mF4-31C1 (21). For example, the AFL4 antibody could inhibit receptor dimerization or promote receptor internalization or antibody-dependent cytotoxicity. In our present study, we started the treatment when palpable, established tumors were detected in the mice, whereas in the study by Kubo et al. (20), the treatment was started simultaneously with tumor implantation and no significant inhibition was observed when treatment was started later. After the first submission of our manuscript, Roberts et al. (37) reported on decreased tumor growth in an orthotopic VEGF-C expression vector–transfected human mammary carcinoma xenograft in nude mice that were treated with the mF4-31C1 antibody for 6 weeks starting at the time of tumor cell inoculation. Their treated and untreated tumors were less than 100 and 150 mm^3 in size, respectively. This would correspond to a spheroid tumor diameter of 5.8 and 6.6 mm, respectively. Because such size range makes quantitative assessment very difficult in our experiments, we started the antibody treatment only after the tumors reached this size. Besides inhibiting tumor angiogenesis, the mF4-31C1 treatment blocked formation of tumor-associated lymphatic vessels that have been associated with lymphatic metastasis, whereas it did not affect preexisting lymphatic or blood vessels. Similarly, no effect on mature lymphatic vessels or blood vasculature was observed when the VEGFR-3 pathway was blocked by the soluble receptor produced transiently by adenovirus or adeno-associated virus (14, 26). Further work should define the mechanisms involved in the reduced tumor angiogenesis up on VEGFR-3 blockade.

Tumor angiogenesis is promoted by multiple pathways that include both oncogene-driven and tumor-associated, hypoxia-driven expression of proangiogenic factors such as VEGF and perhaps down-regulation of angiogenic suppressors (38). Tumor cells and stromal macrophages in several tumor types express VEGF-C or VEGF-D, and their receptor VEGFR-3 is commonly expressed in the angiogenic tumor vasculature and stromal macrophages (36), but not, in general, in tumor cells or in most normal vessels (39). Our results in this study suggest that VEGF-C/VEGF-D and their receptors contribute to angiogenesis and growth in at least some tumors. Monotherapy with a single antiangiogenic agent may not be sufficient to counteract the numerous angiogenic factors produced by cancer cells and/or stromal cells during tumor progression. The usefulness of VEGFR-3 blocking antibodies will next be further explored in combination studies with other antiangiogenic and antitumor agents. Indeed, in some tumor types, effective inhibition of tumor progression may require inhibition of multiple angiogenic targets.

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