

## Antitumor Effects of Synthetic VEGF-receptor Binding Antagonist, VGA1155

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**Abstract.** *Vascular endothelial growth factor (VEGF) plays key roles in tumor angiogenesis. Therefore, VEGF and its receptors are considered to be primary targets for anti-angiogenic strategy during cancer chemotherapy. Our previous study reported that VGA1155, a low-molecular-weight inhibitor of the binding of VEGF, inhibited VEGF binding to KDR/Flk-1 receptor-overexpressing cells. In the present study, the antitumor effects and antimetastatic effect of VGA1155 were examined in vivo. VGA1155 suppressed the growth of human lung, breast, colon and epidermoid cancers (LC-6, HT29, MX-1, Col-1 and A431) in the nude mouse xenograft model, and pulmonary metastasis of melanoma in the spontaneous metastasis model. These results suggest that VGA1155 has antitumor effects in vivo through the inhibition of VEGF binding to its receptors.*

Tumor angiogenesis, the generation of new blood vessels in response to angiogenic stimuli from tumor cells, promotes solid tumor progression by stimulating tumor cell survival, tumor invasion and metastasis (1). The growth of solid tumors is dependent on angiogenesis, as tumors generally cannot grow beyond the size of 1-2 mm in diameter without the formation of new blood vessels to supply nutrition and oxygen. Among a large number of pro-angiogenic factors, vascular endothelial growth factor (VEGF) and VEGFR2

(KDR/Flk-1) play key roles in tumor angiogenesis. Through KDR/Flk-1, VEGF stimulates endothelial growth, endothelial migration, capillary formation and *in vivo* angiogenesis (2-4). Therefore, VEGF and its receptors are considered to be primary targets for anti-angiogenic strategy during cancer chemotherapy. For example, SU5416, the first KDR/Flk-1 receptor tyrosine kinase inhibitor, suppressed the growth of colon cancer metastasis (5). Moreover, many other reports are available on KDR/Flk-1 receptor tyrosine kinase inhibitors such as SU6668 (6), ZD4190 (7) and PTK787 (8). However, these kinase inhibitors are required to enter the cytosol and have a higher chance of affecting cytosolic functional proteins or other biological co-factors existing constitutively. On the other hand, the anti-VEGF antibody, Bevacizumab, suppressed metastatic renal cancer (9). However, the cost involved in the industrial production of protein drug products containing monoclonal antibodies is likely to be very high.

Our previous study reported that a low-molecular-weight inhibitor of the binding of VEGF to its receptors, VGA1155 (10), inhibited VEGF binding to FLT-1 receptor-overexpressing cells and KDR/Flk-1 receptor-overexpressing cells with IC<sub>50</sub> values of 0.38±0.07 and 0.14±0.00 μM (mean±SE, n=3), respectively. This inhibitor is able to exert an anti-angiogenic effect without entering target cells (VEGF receptor-expressing endothelial cells) and offers certain advantages, including a low cost of synthesis. VGA1155 also inhibited VEGF-induced DNA synthesis and tube formation of vascular endothelial cells *in vitro*. Moreover, the VEGF inhibitor suppressed angiogenesis toward B16-BL6 melanoma on the back of C57BL/6 mice *in vivo* (11).

Therefore, it was of interest to verify the antitumor effect of VGA1155 through its anti-angiogenic features. In the present study, we examined the suppressive effects of VGA1155 on several human tumor growths in the nude mouse xenograft model and the antimetastatic effect in the spontaneous metastasis model.

**Abbreviations:** VEGF, vascular endothelial growth factor; PBS, phosphate-buffered saline; FLT, fms-like tyrosine kinase; KDR/Flk-1, kinase insert domain containing receptor/ fetal liverkinase; bFGF, basic fibroblast growth factor; IL-8, interleukin-8; PDGFR, platelet derived growth factor receptor.

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**Key Words:** VGA1155, vascular endothelial growth factor, anti-metastasis.

## Materials and Methods

**Materials.** VGA1155 (5-[N-methyl-N-(4-octadecyloxyphenyl)acetyl]amino-2-methylthiobenzoic acid) (Figure 1) was synthesized in our laboratory. VGA1155 was dissolved in isotonic phosphate buffer (pH 9.0).

**Cells and animals.** LC-6 human non-small cell lung cancer, A431 human epidermoid carcinoma, Col-1 human colon cancer and MX-1 human breast cancer cells were obtained from the Central Institute for Experimental Animals (Kanagawa, Japan). HT29 human colon cancer cells were purchased from the American Type Culture Collection (Manassas, VA, USA). B16-BL6 cells, a highly metastatic variant of B16 melanoma derived from the C57BL/6 mouse, were kindly provided by Dr. I.J. Fidler, MD, Anderson Cancer Center, Houston, TX, USA. BALB/c-nu/nu mice and C57BL/6 mice were purchased from CLEA Japan, Inc. (Tokyo, Japan) and Charles River Japan (Kanagawa, Japan), respectively. All experiments involving animals were carried out under protocols approved by the Taisho Pharmaceutical Co., Ltd., Animal Care Committee, Japan.

**Human tumor xenografts model.** LC-6 human non-small cell lung cancer, A431 human epidermoid carcinoma, Col-1 and HT29 human colon cancer and MX-1 human breast cancer were maintained by subcutaneous implantation into BALB/c-nu/nu nude mice (12). The tumors were excised from the mice, the necrotic portions were removed and minced, and tumor fragments of about 2 x 2 x 2 mm were prepared and implanted subcutaneously into the back of nude mice using a trocar (day 0). Intraperitoneal doses of VGA1155 were given for 21 successive days from day 1 to day 21, except for A431-implanted mice (day 11 to day 31). The tumor sizes were measured at least 3 times a week using a caliper, and tumor volumes were calculated by the following formula. The antitumor activity of the compounds was expressed by the T/C (%) of tumor volume.

Tumor volume (mm<sup>3</sup>) = 1/2 x (major diameter (mm)) x (minor diameter (mm))<sup>2</sup>

T/C (%) = [(Tumor volume of treated animals)/(Tumor volume of control animals)] x 100

**Melanoma metastasis model.** Pulmonary metastasis of B16-BL6 cells implanted into the right hind footpad was assayed, as previously described (13). Briefly, the primary tumors were surgically removed by amputation under anesthesia on day 21 post-implantation, and the volume of the primary tumor was measured. Two weeks after the amputation, the mice were sacrificed. The lungs were removed and fixed in Bouin's solution. The tumor nodes on the lung surface were counted under a dissecting microscope. VGA1155 was given intraperitoneally for 20 successive days from day 1 to day 20 post-implantation.

**Statistical analysis.** The statistical significance of the results of the nude mice xenograft model and the results of the metastasis model were analyzed by Student's *t*-test.

## Results

To evaluate the antitumor effect of VGA1155, several human solid tumors were selected for the nude mouse xenograft model. Intraperitoneal injection of VGA1155

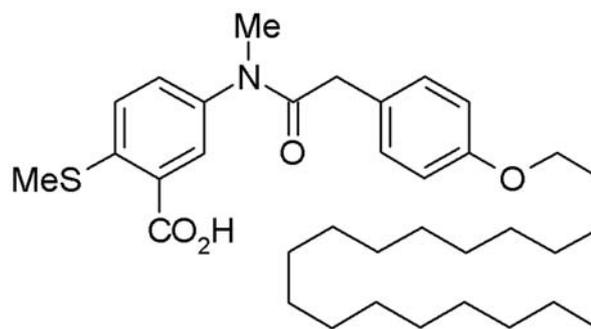


Figure 1. Chemical structure of VGA1155, (5-[N-methyl-N-(4-octadecyloxyphenyl)acetyl]amino-2-methylthiobenzoic acid). Molecular weight: 583.87.

(30 mg/kg) resulted in significant suppression of the growth of LC-6 human non-small cell lung cancer (T/C=45.3%) (Figure 2A). VGA1155 also showed significant inhibition of the growth of A431 human epidermoid carcinoma, HT29 human colon cancer and MX-1 human breast cancer (Figure 2B, C, D). Although the antitumor effect against Col-1 human colon cancer was not statistically significant, VGA1155 showed a tendency to suppress the growth of colon cancer (Figure 2E). However, VGA1155 did not affect the growth of SC-2 human gastric cancer and GL-5 human brain tumor in this model (data not shown).

Since VGA1155 inhibited angiogenesis *in vitro* and *in vivo* (11), which is considered to be associated with the formation of tumor metastasis (1, 14), the antimetastatic effect of VGA1155 was next investigated in the spontaneous metastasis model using mouse melanoma. In this model, B16-BL6 mouse melanoma was orthotopically implanted into the hind footpad of the C57BL/6 mouse and the size of the primary tumor on the hind paw and the number of metastases on the lung surface were examined. As shown in Figure 3, intraperitoneal administration of VGA1155 at a dose of 30 mg/kg daily for 20 days showed a tendency to suppress the growth of the primary tumor and pulmonary metastasis, but the effect was not statistically significant.

## Discussion

In the present study, it was demonstrated that VGA1155 significantly suppressed the growth of several human tumors in nude mice (Figure 2). It also showed a tendency to suppress pulmonary metastasis of orthotopically-implanted melanoma in mice. These results indicated that VGA1155, a synthetic VEGF binding antagonist with low molecular weight, was effective at suppressing the growth and metastasis of solid tumors *in vivo*. Since our previous study reported that VGA1155 suppressed angiogenesis *in*

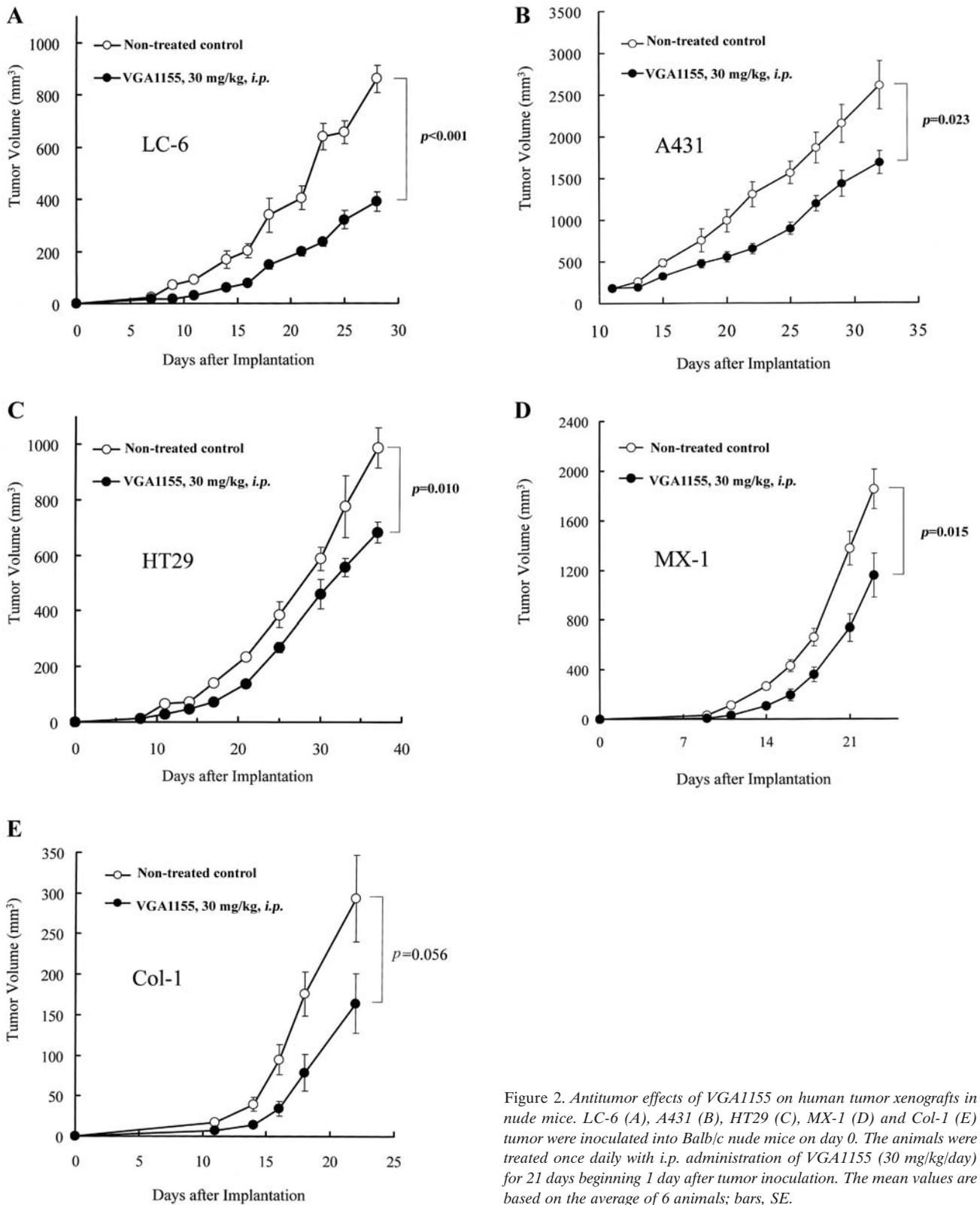


Figure 2. Antitumor effects of VGA1155 on human tumor xenografts in nude mice. LC-6 (A), A431 (B), HT29 (C), MX-1 (D) and Col-1 (E) tumor were inoculated into Balb/c nude mice on day 0. The animals were treated once daily with *i.p.* administration of VGA1155 (30 mg/kg/day) for 21 days beginning 1 day after tumor inoculation. The mean values are based on the average of 6 animals; bars, SE.

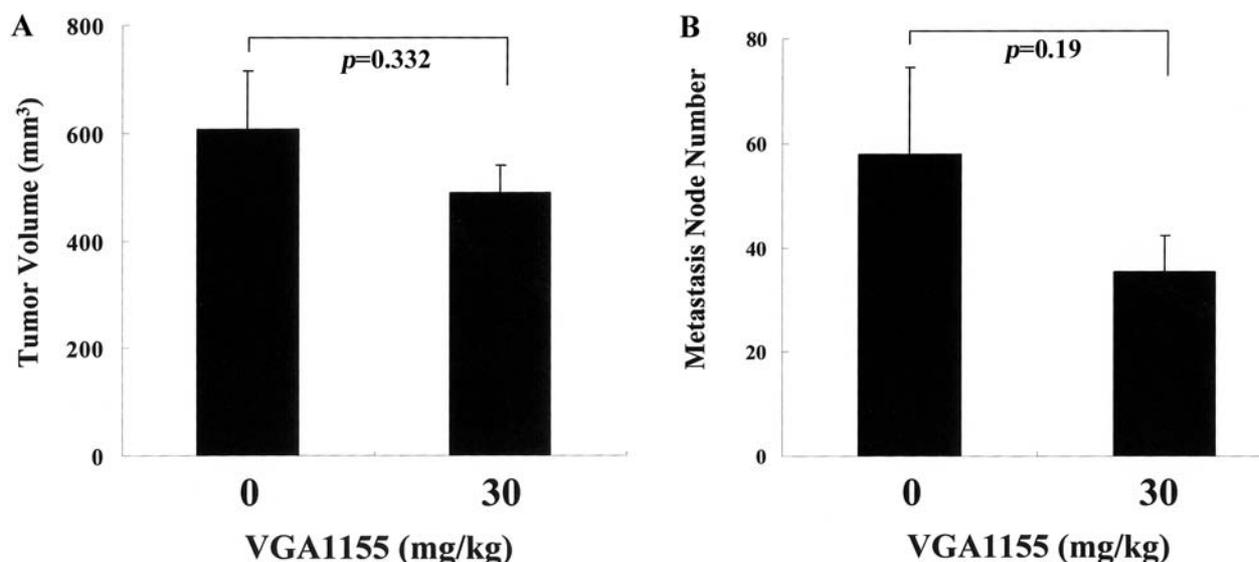


Figure 3. Inhibition effect of VGA1155 on lung metastasis induced by orthotopic subcutaneous implantation of mouse melanoma. The B16-BL6 cell suspension was orthotopically implanted into the right hind footpad of C57BL/6 mice. The primary tumors were surgically removed by amputation under anesthesia on day 21 post-implantation and the volume of primary tumor was measured (A). The animals were treated once daily with i.p. administration of VGA1155 (30 mg/kg/day) for 20 days before amputation. The mice were sacrificed 14 days after the amputation. The lungs were removed and tumor colonies on the surface were counted (B). The mean values are based on the average of 12 animals; bars, SE.

*in vitro* and *in vivo* (11), these antitumor or antimetastatic effects appear to be partly due to the anti-angiogenic property of VGA1155. It may be considered difficult for a low-molecular-weight compound, such as VGA1155, to inhibit the binding or interaction between large protein molecules; however, it is of prime interest that VGA1155 inhibited the binding between VEGF and its receptor, KDR/Flk-1, and consequently inhibited the growth and metastasis of tumors.

The present study indicated that the suppressive effect of VGA1155 on tumor growth may be weaker than that of conventional antitumor drugs with a cytotoxic feature, and that VGA1155 was not effective for some tumors, including gastric and brain tumors. Although further investigation will be needed to reveal the factors influencing the drug sensitivity of tumors, the efficacy of VGA1155 against tumors may depend on the contribution of VEGF to tumor growth *in vivo*. Davis *et al.* reported that CD105-positive vessels, which were distinguished as angiogenic tumor blood vessels, were refractory to the treatment of tumor xenograft with anti-VEGF antibodies. They suggested the existence of a VEGF-insensitive mechanism in tumor angiogenesis (15). A number of other angiogenic factors were reported, such as bFGF, PDGF, angiopoietin and IL-8 (4). The relationship of VEGF with these angiogenic factors may influence the success of anti-VEGF therapy.

Although the effects of VGA1155 in combination with other drugs was not examined here, other antitumor drugs may be needed to therapeutically augment the value of VGA1155. For example, SU11248, which is a new anti-angiogenic drug undergoing clinical study, has been found to simultaneously inhibit some receptor tyrosine kinases. It inhibited PDGFR- $\beta$ , FLT-3 and KIT as well as the VEGF receptor KDR/Flk-1, while the first anti-VEGF inhibitor, SU5416, specifically inhibited only the VEGF receptor tyrosine kinase (5, 16-18). Bevacizumab, the anti-VEGF antibody, increased the survival rate in combination therapy with irinotecan, fluorouracil and leucovorin, while administration of the antibody alone did not affect survival (9, 19).

Bevacizumab was approved by the Food and Drug Administration (USA) for use in metastatic colorectal cancer in combination with fluorouracil in 2004. The antibody prolonged survival by 4.7 months compared to the placebo control in the clinical combination study (19). Bevacizumab inhibits the binding of VEGF to its receptors on the cell surface, like VGA1155. However, VGA1155 is a low-molecular-weight inhibitor, which is economically advantageous because protein drugs, such as antibodies, are more expensive to mass-produce.

In conclusion, VGA1155, which inhibits the binding between VEGF and its receptor, suppressed tumor growth

and metastasis *in vivo*, thus indicating the potential of a low-molecular-weight compound to inhibit large protein ligand binding.

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