Antiangiogenic Effect by SU5416 Is Partly Attributable to Inhibition of Flt-1 Receptor Signaling

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Abstract

Interaction between vascular endothelial growth factor (VEGF) and its cognate receptors, KDR/Flk-1 and Flt-1, of vascular endothelial cells is expected to induce an angiogenesis “switch” in tumors and other angiogenesis-associated diseases. SU5416, a selective inhibitor of the KDR/Flk-1 tyrosine kinase, is known to be a potent inhibitor of tumor angiogenesis. In this study, we first observed that SU5416 inhibited Flt-1 tyrosine kinase activity at similar doses, in addition to inhibiting KDR/Flk-1 tyrosine kinase activity in response to VEGF. SU5416 inhibited cell migration of human vascular endothelial cells expressing both Flt-1 and KDR in response to VEGF and also inhibited the cell migration in response to placenta growth factor (PIGF), a specific ligand for Flt-1. Chemotaxis of monocytes expressing only Flt-1 was also inhibited by SU5416 in a dose-dependent manner. Moreover, SU5416 was found to inhibit tyrosine kinase of Flt-1 in response to PIGF in vitro. And angiogenesis induced by PIGF in a Matrigel plug assay was inhibited by administration of SU5416. The antiangiogenic effects by this VEGF receptor-targeting compound appeared to be mediated through interference not only with KDR/Flk-1 but also with Flt-1. Cell migration of vascular endothelial cells and mononuclear cells through Flt-1, thus, might play a key role in VEGF-induced tumor angiogenesis in concert with KDR/Flk-1.

Introduction

VEGF, one of the most potent angiogenic factors, regulates endothelial cell proliferation and vascular permeability (1, 2). Disruption of the mouse VEGF gene induces abnormal blood vessel development and lethality in embryonic maturation. Two VEGF receptor tyrosine kinases, Flt-1 (3) and KDR/Flk-1 (4), have been identified. Disruption of the murine Flk-1 gene results in a severe deficiency in vasculogenesis and production of hematopoietic cells (blood islands) during embryogenesis (5). In contrast, Flt-1 homozygous gene disruption results in an abundance of endothelial cells with abnormal vasculature. This suggests that Flt-1 plays an essential role in both vasculogenesis and angiogenesis (6). Hiratsuka et al. (7) reported that Flt-1 tyrosine kinase-deficient mice appeared to develop normal vessels, although VEGF-induced macrophage migration was attenuated. On the other hand, Hiratsuka et al. (8) subsequently reported that tyrosine kinase of Flt-1 was important for angiogenesis under certain pathological conditions, such as tumor angiogenesis. Carmeliet et al. (9) have further studied the role of PIGF, a specific ligand for Flt-1 (10, 11), and reported that vascular endothelial cells amplify their responsiveness to VEGF during the “angiogenesis switch” in many pathological disorders through up-regulation of PIGF-Flt-1 receptor signaling. An Flt-1-mediated signal elicits cell migration through actin reorganization via the activation of p38 mitogen-activated protein kinase in HUVE cells (12). Both KDR/Flk-1 and Flt-1, thus, appear to be required for angiogenesis under pathological conditions, including cancer, as well as vasculogenesis during embryonic development.

On the other hand, tumor enlargement often depends on angiogenesis, and VEGF and its receptors play an essential role in tumor angiogenesis (13). VEGF is secreted by tumor cells and by tumor-associated stromal cells, including endothelial cells, fibroblasts, and macrophages. Thus, both VEGF produced by the surrounding stroma (14) and that produced by tumor cells themselves (15) appear to play a key role in tumor angiogenesis. VEGF and its receptor molecules have been implicated in angiogenesis of various human tumor types (13, 16).

Since the initial report that angiogenesis is closely correlated with metastasis or prognosis in patients with breast cancer (17), numerous studies have consistently demonstrated that angiogenesis plays a major role in the malignant behavior of various tumor types such as breast cancer and glioblastoma and also in many other angiogenesis-associated diseases (13, 16, 18). Agents that specifically target tumor angiogenesis are being developed and are expected to modulate not only tumor enlargement but also metastasis or invasion in various malignant tumors (19). Furthermore,
because both KDR/Flk-1 and Flt-1 are expressed rather preferentially in vascular endothelial cells (20), development of tyrosine kinase inhibitors that target both KDR/Flk-1 and Flt-1 is expected to inhibit neoangiogenesis in malignant tumors. A synthetic antiangiogenesis compound, SU5416, was developed to target KDR/Flk-1 receptor kinase. Fong et al. (21) reported that SU5416 exerts its activity through inhibition of KDR/Flk-1 receptor kinase and that SU5416 showed antitumor activity in an experimental animal model system of tumor growth. Shaheen et al. (22) reported that SU5416 inhibited the growth of colon cancer metastasis in the liver. In this study, we first demonstrated that SU5416 also inhibited Flt-1 tyrosine kinase and that Flt-1 tyrosine kinase is required for cell migration by endothelial cells and also by monocyte/macrophages. The antiangiogenic activity of SU5416 thus appears to be because of inhibition of not only KDR/Flk-1 but also Flt-1 tyrosine kinase.

Materials and Methods

Materials. Human VEGF165, human PIGF-1, and mouse PIGF-2 were purchased from R&D Systems (Minneapolis, MN). SU5416 (chemical name: 3-[2,4-dimethylpyrrol-5-yl] methylidenyl]-iodolin-2-one) was provided from SUGEN (21, 23) and Taiho Pharmaceutical Co. A monoclonal antibody specific to phosphotyrosine (PY-20) was purchased from ICN Biochemicals (Cosa Mesa, CA). Anti-Flt-1 antibody and anti-KDR antibody were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Low growth factor Matrigel was purchased from Becton Dickinson (Bedford, MA).

Cell Lines and cDNA Transfection. A mouse NIH3T3 cell line overexpressing human KDR receptor was established previously (24) and was designated NIH3T3/KDR for the present study. Flt-1 expression vector BCMGS neo Flt-1 (25) was transfected into NIH3T3 cells followed by selection of G418-resistant clones to establish NIH3T3/Flt-1. Mouse renal cell carcinoma RENCA cells were kindly provided by Dr. I. J. Fidler and cultured in RPMI supplemented with 10% FBS, 60 μg/ml penicillin, and 60 μg/ml streptomycin. HUVE cells were purchased from Clonetics Co. (Walkersville, San Diego, CA) through Sanko Junyaku Co., Ltd. (Tokyo, Japan) and cultured in endothelial cell basal medium (EBM)-2 supplemented with 2% FBS.

Mouse Dorsal Air Sac Assay. A dorsal air sac assay was performed in 7–10-week-old male mice according to a method published previously (26, 27). RENCA cells (1 x 10^6 cells) were suspended in PBS and injected into a chamber consisting of a ring (Millipore Corp., Bedford, MA) covered with Millipore filters (0.45-μm pore size) on both sides. This chamber containing RENCA cells was implanted into a dorsal air sac produced in the mouse by injecting 10 ml of air. After implantation, SU5416 (25 mg/kg/day) was administered by i.p. injection. Five mice in each group were killed and carefully skinned on day 5. After the implanted chamber was removed from the s.c. air fascia, a ring without a filter was placed on the same site and photographed (27). We counted the number of meandered blood vessels within the chamber in the area of the air sac fascia. The angiogenic response was graded as 0, 1, 2, 3, 4, or 5 according to the number of newly curled thin blood vessels (zero, one, two, three, four, or more than five vessels, respectively).

Immunoblotting Assay. Confluent NIH3T3/Flt-1 or NIH3T3/KDR cells were cultured in serum-depleted DMEM for 48 h. The cells were then preincubated with SU5416 at concentrations ranging from 0.05 to 5 μM for 2 h followed by stimulation with 20 ng/ml VEGF for 5 min at 37°C. The cells were then rinsed with ice-cold PBS and lysed in lysis buffer (50 mM HEPES, 150 mM NaCl, 1% Triton X-100, and 10% glycerol containing 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 mM sodium vanadate). Cell lysates were subjected to SDS-PAGE and transferred onto Immobilon membranes (Millipore Corp.). After transfer, blots were incubated with the blocking solution and probed with antiphosphotyrosine (PY20) antibody, anti-Flt-1 receptor antibody, or anti-KDR antibody followed by washing. The protein contents were visualized using horseradish peroxidase-conjugated secondary antibodies followed by enhanced chemiluminescence (Amersham).

In Vitro Kinase Assay. Immunoprecipitated Flt-1 was washed four times with lysis buffer and twice with kinase assay buffer [20 mM HEPES (pH 7.4), 10 mM MgCl2, 2 mM MnCl2, 0.05% Triton X-100, and 1 mM DTT]. The agarose beads used for precipitations were suspended in kinase assay buffer containing 0.25 μCi/μl [γ-^32P] ATP and then incubated on ice for 10 min. The beads were then washed three times with kinase assay buffer, suspended in sample buffer, and heated for 5 min at 100°C. For SDS-PAGE, the gel was fixed in methanol:acetic acid, treated with 1 M KOH for 30 min at 55°C, fixed again, and examined by autoradiography.

Isolation of Human Monocytes and Chemotactic Activity. Human leukocytes from peripheral blood (200 ml) of healthy donors were collected, and mononucleated cells were separated using a lymphocyte separation medium (Litton Bionetics, Kensington, MD) and Beckman JE-5.0 elutriation system as described previously (28). Fractions enriched in monocytes (95%) were obtained at 3000 rpm and flow rates of 30–36 ml/min. More than 97% of the cells were viable. Monocyte chemotactic activity was measured using a 48-well chemotaxis chamber (Neuro Probe, Cabin John, MD) with a 5-μm pore-size polycarbonate filter as described previously (29).

VEGF or PIGF-induced Migration Assay of HUVE Cells. Cell migration assay was performed using a 24-well chamber with 1.33 μg/ml fibronectin-coated 8-μm polycarbonate filters (30). HUVE cells (3 x 10^5 cells) were suspended in EBM-2 containing 0.5% FBS and seeded in the inner chamber. In the outer chamber, serial dilutions of SU5416 with or without VEGF (20 ng/ml) in the same medium were added. After incubation for 3 h at 37°C, nonmigrated cells on the upper surface of the filter were removed, and the cells that had migrated under the filter were counted manually by examination under the microscope (31).

Mouse Matrigel Plug Assay. The mouse Matrigel plug assay was performed as described previously (32). In brief, 0.3 ml of Matrigel supplemented with 400 ng of PIGF was s.c. inoculated into BALB/c mice. After the inoculation, SU5416 (25 mg/kg/day) was administered by i.p. injection. Four days
after the inoculation, the mice were sacrificed, and the gels were removed by retaining the overlying skin. These gels were then fixed in 10% buffered-formalin, embedded in paraffin, and sectioned at a 4-μm thickness. The sections were then deparaffinized and stained with Masson-Trichrome. The numbers of neovasculatures within the gels on day 4 were counted (32).

Results

SU5416 Inhibits Tumor-induced Angiogenesis in Vivo. Fong et al. (21) have reported previously that SU5416 shows antitumor activity against mice bearing various tumor types. To confirm this previous finding, we examined whether SU5416 could also inhibit tumor angiogenesis in an experimental model system assessed by a dorsal air sac assay in vivo. Implantation of a chamber containing murine renal cell carcinoma RENCA cells, which produce VEGF in the dorsal air sac, resulted in development of microvessels (as indicated by arrows) with curled thin structures and many tiny bleeding spots in addition to the preexisting vessels (Fig. 1A). Administration of SU5416 at 25 mg/kg/day for 5 days markedly reduced the development of such tumor cell-induced microvasculatures. However, treatment with SU5416 did not affect any preexisting vessels. The quantitative analysis demonstrated a significant reduction of neovascularization by administration of SU5416; neovascularization grades were 5 ± 0.2 and 1.8 ± 0.4 in the absence and presence of SU5416, respectively. SU5416 at 25 mg/kg/day was found to be effective against tumor-induced angiogenesis in vivo (Fig. 1), in agreement with the previous study (21).

SU5416 Inhibits Autophosphorylation of Flt-1, KDR, and Tyrosine Kinase Activity in Response to VEGF. Consistent with the previous study by Fong et al. (21), immunoblot analysis with antiphosphotyrosine antibody (PY20) after receptor activation by VEGF showed that SU5416 at ≥0.5 μM inhibited autophosphorylation of KDR receptor in its cDNA transfectant, NIH3T3/KDR with IC50 of 0.1 μM (Fig. 2A). In NIH3T3/Flt-1 cells, immunoblotting with PY20 indicated that only the upper of the two bands

Fig. 1. SU5416 inhibits tumor-induced angiogenesis in vivo. Inhibition of tumor angiogenesis by RENCA cells in the mouse dorsal air sac assay after systemic treatment with SU5416. Representative photographs of the mouse dorsal air sac assay, RENCA cells in untreated mice (A), and in mice treated with SU5416 at 25 mg/kg/day (B) are shown. pv, preexisting vessels; tn, tumor neovasculature.

Fig. 2. Effect of SU5416 on VEGF-induced KDR and Flt-1 receptor autophosphorylation and kinase activity of Flt-1. NIH3T3/KDR and NIH3T3/Flt-1 cells were grown to near confluence and then serum depleted for 48 h. The cells were preincubated with indicated concentrations of SU5416 for 2 h, followed by the addition of 20 ng/ml VEGF for 5 min at 37°C. Protein extracts were resolved by 7.5% SDS-PAGE and probed with antiphosphotyrosine (A; PY20) antibody, anti-KDR receptor antibody and antiphosphotyrosine (B; PY20) antibody, or anti-Flt-1 receptor antibody (C). In vitro kinase assay of Flt-1 receptor activity. Flt-1 was immunoprecipitated from equal amounts of cell lysates and incubated with [γ-32P] ATP. 32P-labeled Flt-1 was detected by autoradiography.
Targeting VEGF Receptor Tyrosine Kinases

Kinase activity was markedly blocked by SU5416 at 0.5 μM (Fig. 2B). Flt-1 autophosphorylation was enhanced by exogenous addition of VEGF. SU5416 at ≥0.5 μM almost completely inhibited the Flt-1 autophosphorylation (Fig. 2B). However, this compound did not affect cellular levels of Flt-1 receptor protein. We also examined whether SU5416 could inhibit in vitro kinase activity by Flt-1. As seen in Fig. 2C, its in vitro kinase activity was markedly blocked by ≥0.5 μM doses of SU5416. SU4516 could thus inhibit tyrosine kinase activity of Flt-1, as well as KDR/Flik-1.

**SU5416 Inhibits PIGF-induced Flt-1 Autophosphorylation.** PIGF is a specific ligand for Flt-1 but not for KDR/Flik-1 (10, 11). We next examined whether PIGF-induced tyrosine autophosphorylation of Flt-1 would be blocked. Treatment of NIH3T3/Flt-1 cells with 50 ng/ml PIGF enhanced the autophosphorylation of Flt-1 (Fig. 3) but not that of KDR (data not shown). SU5416 at 0.5–5 μM almost completely blocked the Flt-1 tyrosine autophosphorylation in response to PIGF (Fig. 3). SU4516 thus inhibited both PIGF- and VEGF-stimulated Flt-1 tyrosine autophosphorylation. Fig. 3 also shows that the upper and lower bands of Flt-1 are phosphorylated by PIGF and that phosphorylation of both bands is inhibited by similar doses of SU5416. It remains unclear why the lower band of Flt-1 is phosphorylated by PIGF but not by VEGF (Figs. 2B and 3).

SU5416 inhibits both migration of vascular endothelial cells and chemotaxis of human monocytes in response to VEGF or PIGF.

We next examined how inhibition of Flt-1 kinase activity and/or KDR/Flik-1 kinase activity can modulate the migration of vascular endothelial cells and monocytes. VEGF is well known to stimulate migration of HUVE cells through its interaction with its cognate receptor KDR/Flik-1 and/or Flt-1 (12). In contrast, PIGF is known to be a ligand and induce autophosphorylation of Flt-1 (33). We assumed that inhibition of Flt-1 tyrosine kinase activity could abrogate the specific signal transduction by PIGF.

As shown in Table 1, VEGF or PIGF stimulated cell migration of HUVE cells 2–3-fold, respectively. And this detected as Flt-1 was phosphorylated specifically in response to VEGF with IC50 of 0.1 μM (Fig. 2B). Flt-1 autophosphorylation was enhanced by exogenous addition of VEGF. SU5416 at ≥0.5 μM almost completely inhibited the Flt-1 autophosphorylation (Fig. 2B). However, this compound did not affect cellular levels of Flt-1 receptor protein. We also examined whether SU5416 could inhibit in vitro kinase activity by Flt-1. As seen in Fig. 2C, its in vitro kinase activity was markedly blocked by ≥0.5 μM doses of SU5416. SU4516 could thus inhibit tyrosine kinase activity of Flt-1, as well as KDR/Flik-1.

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**Table 1** The effect of SU5416 on cell migration of vascular endothelial cells by VEGF or PIGF

<table>
<thead>
<tr>
<th>Growth factors</th>
<th>SU5416 (μM)</th>
<th>Migrated cell activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>212 ± 23.5</td>
</tr>
<tr>
<td>VEGF</td>
<td>0</td>
<td>444 ± 58.3</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.5</td>
<td>319 ± 33.8</td>
</tr>
<tr>
<td>VEGF</td>
<td>5</td>
<td>253 ± 19.8</td>
</tr>
<tr>
<td>PIGF</td>
<td>0</td>
<td>338 ± 18.0</td>
</tr>
<tr>
<td>PIGF</td>
<td>0.5</td>
<td>273 ± 15.3</td>
</tr>
<tr>
<td>PIGF</td>
<td>5</td>
<td>234 ± 20.6</td>
</tr>
</tbody>
</table>

* Mean number of triplicate dishes ± SD. In this assay, VEGF at 20 ng/ml or PIGF at 50 ng/ml was used.

**Table 2** Effect of SU5416 on VEGF- or MCP-1-induced chemotaxis of human monocytes

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>SU5416 (μM)</th>
<th>Migrated cell activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>67.8 ± 3.74</td>
</tr>
<tr>
<td>VEGF</td>
<td>0</td>
<td>127 ± 14.2</td>
</tr>
<tr>
<td>VEGF</td>
<td>0.5</td>
<td>106 ± 18.6</td>
</tr>
<tr>
<td>VEGF</td>
<td>5</td>
<td>78.2 ± 37.4</td>
</tr>
<tr>
<td>MCP-1</td>
<td>0</td>
<td>327 ± 16.6</td>
</tr>
<tr>
<td>MCP-1</td>
<td>5</td>
<td>283 ± 37.4</td>
</tr>
</tbody>
</table>

* Mean number of triplicate dishes ± SD. In this assay, either VEGF at 20 ng/ml or MCP-1 at 100 ng/ml was used.

**VEGF- or PIGF-specific migration was almost completely blocked by SU5416 at ≥0.5 μM.** Chemotaxis of human monocytes is known to be enhanced in response to C-C chemokines, MCP-1 and macrophage inflammatory protein-1α, as well as in response to VEGF (34). As shown in Table 2, VEGF induced a 2-fold and MCP-1 induced a 5-fold increase in the chemotaxis of human monocytes. As monocytes express only Flt-1, the VEGF-stimulated chemotaxis is Flt-1 tyrosine kinase dependent. The VEGF-stimulated chemotaxis of monocytes was almost blocked completely by SU5416 at ≥5 μM. However, even at 5 μM, SU5416 could not inhibit MCP-1-stimulated chemotaxis activity of monocytes. Three independent experiments using different monocyte preparations yielded data comparable with that in Table 2.

**SU5416 Inhibits PIGF-induced Angiogenesis in Vivo.** Finally, we examined whether SU5416 inhibits PIGF-induced angiogenesis using a Matrigel plug assay. Angiogenesis was enhanced in response to PIGF (Fig. 4B). Administration (i.p.) of SU5416 (25 mg/kg/day) for 5 days resulted in marked inhibition of PIGF-induced angiogenesis in Matrigel (Fig. 4C). Quantitative analysis demonstrated that SU5416 significantly reduced PIGF-induced neovascularization: Lumen numbers were estimated to be 6.6 ± 1.5, 20.8 ± 7.2, and 6.5 ± 3 in the control (Matrigel alone), 400 ng of PIGF and SU5416-
treated groups, respectively (Fig. 4D). The mouse Matrigel plug assay thus demonstrated that SU5416 inhibited PIGF-induced angiogenesis in vivo.

**Discussion**

SU5416 is a potent antiangiogenic agent that was developed by targeting one of the VEGF receptors, KDR/Flk-1, and that has demonstrated antitumor activity in mice bearing various tumor types (21, 22). In the present study, we also observed marked inhibition of VEGF-induced angiogenesis of mouse cornea (data not shown) and development of microvessels induced by implanted tumor cells in the dorsal air sac in mice when SU5416 was administered for 5 days. SU5416 inhibits KDR/Flk-1 kinase through its binding to the conserved ATP-binding site within the kinase domain of the receptor (21). We demonstrated that SU5416 at 0.5–5 μM inhibited not only KDR/Flk-1 receptor tyrosine kinase but also Flt-1 receptor tyrosine kinase in response to VEGF. Moreover, we also demonstrated that SU5416 at 0.5–5 μM could inhibit Flt-1 receptor tyrosine kinase in response to PIGF that bind only to Flt-1 receptors.

VEGF markedly stimulated cell migration of vascular endothelial cells, and this VEGF-induced migration was inhibited by SU5416. Because HUVE cells express both VEGF...
receptors, it remains unclear which receptor is responsible for SU5416-induced inhibition of vascular endothelial cell migration promoted by VEGF. Kanno et al. (12) have reported that DNA synthesis in VEGF-stimulated HUVE cells is mediated preferentially by KDR/Flk-1 and also that the migration of VEGF-stimulated HUVE cells is mediated by both Flt-1 and KDR/Flk-1 in a complex way. Inhibition of HUVE cell migration by SU5416 thus might be because of both VEGF receptors. On the other hand, Ziche et al. (35) demonstrated that PI GF was as effective as VEGF in promoting cell migration of endothelial cells, although slightly less potent. Consistent with this report, cell migration of HUVE cells was stimulated ~1.5-fold in response to PI GF, and this PI GF-induced migration by HUVE cells was also blocked in the presence of SU5416. Moreover, we demonstrated that SU5416 inhibited VEGF-induced chemotaxis of monocytes, which express only Flt-1. These results suggest that PI GF-induced migration of endothelial cells and VEGF-induced chemotaxis of monocytes are mediated through Flt-1 kinase.

In our present study, angiogenesis was induced in Matrigel in response to PI GF. Administration of SU5416 for 5 days resulted in successful inhibition of PI GF-induced angiogenesis as revealed by a mouse Matrigel plug assay. SU5416 thus could block angiogenesis through its inhibition of PI GF-Flt-1 receptor signaling. It has been reported that blood vessels of larger sizes (>-100 μm in diameter) were developed when PI GF-expressing cancer cells were transplanted in Flt-1 tyrosine kinase-deficient homozygous Flt-1 TK−/− mice, whereas vessels of smaller sizes (<50 μm in diameter) were developed when VEGF-expressing cancer cells were transplanted in Flt-1 TK−/− mice (7). This suggests that the angiogenesis pathway induced by VEGF could be different from that induced by PI GF. Carmeliet et al. (9) have reported recently that embryonic angiogenesis is not affected by deficiency of PI GF. However, the loss of PI GF impaired plasma extravasation and collateral growth during ischemia and angiogenesis under various pathological conditions, such as inflammation, wound healing, and cancer (9). In concert with VEGF, PI GF thus appears to play a key role in angiogenesis under pathological conditions, including cancer. SU5416 is thus expected to show its antiangiogenesis activity in vivo, possibly through a blocking of the angiogenesis pathway by not only KDR/Flk-1 but also Flt-1.

On the other hand, we also observed that SU5416 inhibited the human monocyte migration induced by VEGF. It remains uncertain whether this inhibitory effect is involved in the drug-induced antiangiogenesis in vivo. In a study by Hiratsuka et al. (7), VEGF-induced chemotaxis of macrophages was abolished when macrophages were derived from Flt-1 TK−/− mice. Activated monocytes/macrophages in the tumor stroma often produce angiogenic factors and proteolytic enzymes and modulate angiogenesis in the tumor environment (18, 36–38). Macrophage infiltration correlates with angiogenesis or malignancy in human breast cancers (39), human gliomas (40), renal cell carcinomas (41), and human melanomas (42). Infiltration of thymidine phosphorylase-positive (43, 44), hemoxygenase1-positive, or Cap43-positive monocytic (40, 41) cells is also closely associated with angiogenesis or prognosis in human gliomas and renal cell carcinomas. The inhibition of monocytic/macrophage infiltration in tumor stroma thus might play a key role in angiogenesis inhibition in some tumor types. Hiratsuka et al. (8) have reported recently the role of Flt-1 tyrosine kinase in pathological angiogenesis in Flt-1 TK−/− mice. They observed no apparent differences in the number of infiltrating macrophages in transplanted Lewis lung carcinoma (LLC)-PI GF-induced tumor at day 8. On day 16, however, the number of infiltrated macrophages were 3-fold larger than in Flt-1 TK−/− mice than in Flt-1 TK−/− mice. This suggests that PI GF might not play a major role in infiltration of macrophages in the model system. However, it still remains unclear how infiltration of monocytes/macrophages might be associated with angiogenesis. In the present study, no macrophage infiltration was observed in Matrigel plug assay. Additional in vivo study with any adequate assay model will be needed to determine whether SU5416 can affect the infiltration of macrophages in association with angiogenesis.

In conclusion, a potent antiangiogenesis and antitumor agent, SU5416, was developed by targeting KDR/Flk-1 tyrosine kinase. We demonstrated that SU5416 could inhibit Flt-1 tyrosine kinase, as well as KDR/Flk-1 tyrosine kinase. SU5416 also could inhibit not only migration of vascular endothelial cells in response to PI GF or VEGF but also migration of monocytic cells in response to VEGF in vitro. Moreover, SU5146 could inhibit angiogenesis in Matrigel induced by PI GF in vivo. Our results strongly suggest that the antitumor and antiangiogenesis effects of SU5416 are because of inhibition of not only KDR/Flk-1 but also Flt-1 tyrosine kinases.

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References


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