Gambogic Acid Inhibits Angiogenesis and Prostate Tumor Growth by Suppressing Vascular Endothelial Growth Factor Receptor 2 Signaling

Tingfang Yi, Zhengfang Yi, Sung-Gook Cho, Jian Luo, Manoj K. Pandey, Bharat B. Aggarwal, and Mingyao Liu

Abstract

Gambogic acid (GA), the main active compound of Gamboge hanburyi, has been previously reported to activate apoptosis in many types of cancer cell lines by targeting transferrin receptor and modulating nuclear factor-κB signaling pathway. Whether GA inhibits angiogenesis, which is crucial for cancer and other human diseases, remains unknown. Here, we found that GA significantly inhibited human umbilical vascular endothelial cell (HUVEC) proliferation, migration, invasion, tube formation, and microvessel growth at nanomolar concentration. In a xenograft prostate tumor model, we found that GA effectively inhibited tumor angiogenesis and suppressed tumor growth with low side effects using metronomic chemotherapy with GA. GA was more effective in activating apoptosis and inhibiting proliferation and migration in HUVECs than in human prostate cancer cells (PC3), suggesting GA might be a potential drug candidate in cancer therapy through angioprevention with low chemotoxicity. Furthermore, we showed that GA inhibited the activations of vascular endothelial growth factor receptor 2 and its downstream protein kinases, such as c-Src, focal adhesion kinase, and AKT. Together, these data suggest that GA inhibits angiogenesis and may be a viable drug candidate in antiangiogenesis and anticancer therapies. [Cancer Res 2008;68(6):1843–50]

Introduction

Gambogic acid (GA; C₃₈H₄₄O₈, MW 628.76), a polyprenylated xanthone, is the main active compound of Gamboge hanburyi (a traditional Chinese medicine) used as detoxification, homeostasis, antiinflammatory, and parasiticide medicines for thousands of years (1). Previous studies reported that GA-activated apoptosis in many cancer cell lines and inhibited human hepatoma SMMC-7721 tumor growth in vivo in a nude mouse model (2–7). Recent reports showed the molecular mechanism of apoptosis activation effect of GA by binding to the transferrin receptor (8) and suppressing nuclear factor-κB (NF-κB) signaling pathway (9). Whether GA inhibits angiogenesis, a crucial step in tumor growth and metastasis, is still unknown. Here, we examined the effects of GA on migration, invasion, and tube formation of endothelial cells and angiogenesis in vitro and in vivo.

Materials and Methods

Cell lines, cell culture, and reagents. GA was ordered from Gaia Chemical Corp. 98% by TLC/high performance liquid chromatography.

Note: T. Yi and Z. Yi contributed equally to this report.

Requests for reprints: Mingyao Liu, Institute of Biosciences and Technology, Texas A&M Health Science Center, 2121 W. Holcombe Boulevard, Houston, TX 77030. Phone: 713-677-5700; Fax: 713-677-5712; E-mail: mliu@ibt.tamhsc.edu.

©2008 American Association for Cancer Research.
A 25 mmol/L solution of GA was prepared in DMSO, stored at −20°C, and then diluted as needed. Human umbilical vascular endothelial cells (HUVEC) were kindly gifted from Dr. Xinli Wang (Cardiothoracic Surgery Division of the Michael E. DeBakey Department of Surgery at Baylor College of Medicine Hospital). The human prostate cancer cell line (PC3) was purchased from the American Type Culture Collection. VEGF was obtained from NIH experimental branch. HTScan VEGFR2 kinase assay kit was ordered from Cell Signaling Technology. Horseradish peroxidase (HRP)–labeled secondary antibody, TMB substrate, and stop solution were kindly gifted by Cell Signaling Technology. Streptavidin-coated yellow 96-well plates were kindly gifted by PerkinElmer Life Sciences. Matrigel was ordered from BD Biosciences. Mitomycin C was ordered from Roche.

**Proliferation assay.** HUVEC and PC3 cell proliferation assays with different concentrations of GA were followed the manual of CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega) with VERSAmax microplate reader (Molecular Devices).

**Flow cytometry fluorescence-activated cell sorting analysis.** About 2 × 10⁶ HUVEC and PC3 cells were treated with GA at 37°C, 5% CO₂ incubator for 24 h. The cells were collected and analyzed in a FACS Vantage SE DiVa flow cytometer (Becton Dickinson) with propidium iodide staining.

The cell population percentages at sub-G₁ were defined as apoptotic cell percentages.

**Migration assay.** HUVECs were allowed to grow into full confluence in 6-well plates precoated with 0.1% gelatin and then incubated with 10 μg/mL mitomycin C at 37°C, 5% CO₂ for 2 h to inactivate HUVECs. Monolayer inactivated HUVECs were wounded by scratching with 1 mL pipette tip. Fresh endothelial cell growth medium (ECGM) was added with or without 4 ng/mL VEGF and different concentrations of GA. Images were taken by Nikon digital camera after 7 to 10 h of incubation at 37°C, 5% CO₂. For PC3 cell migration, migration assays as in HUVECs were performed on the inactivated PC3 cells (with the same inactivation treatment as HUVECs). The migrated cells were quantified by manual counting, and percentage inhibition was expressed using untreated wells as 100%. Columns, mean; bars, SE (n = 3; t test, P < 0.005).

**Transwell migration assay.** The transwell (Corning Incorporated) were coated with 0.1% gelatin (Sigma) for 30 min at 37°C. After washing the transwells thrice with 1 × PBS, the bottom chambers (600 μL) were filled with ECGM with 4 ng/mL VEGF and the top chambers were seeded with 100 μL ECGM and inactivated HUVECs (4 × 10⁴ cells per well). The top and bottom chambers contained
the same series of concentrations of gambogic acid. HUVECs were allowed to migrate for 4 h at 37°C, 5% CO₂. After the incubation, cells on the top surface of the membrane (nonmigrated) were scraped with a cotton swab. Cells on the bottom side of the membrane (migrated cells) were fixed with 4% paraformaldehyde for 20 min and washed thrice with 1 × PBS. The cells were stained by H&E staining and then destained with 1 × PBS. Images were obtained using an OLYMPUS inverted microscope and with invading cells being quantified by manual counting. Percentage inhibition of invading cells was quantified and expressed on the basis of untreated cells (control) representing 100% (t test, P < 0.01).

**Tube formation assay.** Matrigel (BD Biosciences) was thawed at 4°C for overnight, and each well of prechilled 24-well plates was coated with 100 μL Matrigel and incubated at 37°C for 45 min. HUVECs (4 × 10⁴ cells) were added in 1 mL ECGM with various concentrations of GA. After 12 to 16 h of incubation at 37°C, 5% CO₂, endothelial cell tube formation was assessed with OLYMPUS inverted microscope. Tubular structures were quantified by manual counting of low power fields (25×), and inhibition percentage was expressed using untreated wells as 100% (t test, P < 0.001).

**Aortic ring assay.** Aortic ring assay was performed, as previously described with some modifications (19, 20). Forty-eight–well plates were covered with 100 μL of Matrigel at 4°C and incubated at 37°C, 5% CO₂ for 30 min. Aortas isolated from mice were cleaned of periadventitial fat and connective tissues, and cut into −1-mm-long to 1.5-mm-long rings. After being rinsed five times with endothelial cell–based medium, the aortas were placed on the Matrigel-covered wells and covered with another 100 μL of Matrigel. Artery rings were cultured in 1.5 mL of ECGM without serum for 24 h, and then the medium was replaced with 1.5 mL of ECGM with or without GA. The medium was changed every 2 d with the exact composition as described above. After 4 d of incubation, the microvessel growth was quantified by taking photographs with Olympus IX 70 invert microscope with a 4× objective lens. After images were acquired, the outgrowth area was delineated and measured with the Pro plus software (Media Cybernetics).

**Matrigel plug assay.** Matrigel (0.5 mL/plug) with neither VEGF nor GA, VEGF (4 ng/mL) but no GA, VEGF (4 ng/mL) and 0.1/0.2 μmol/L GA, and VEGF (4 ng/mL) and 0.2 μmol/L GA were injected s.c. in the midventral abdominal region of 5-week-old to 6-week-old C57BL/6 mice (five mice for each group). After 7 d, the mice were sacrificed and the plugs were removed. Each group had four to five Matrigel plugs. The Matrigel plugs were fixed with formalin and embedded with paraffin. The 5-μm sections were stained with H&E staining. The number of erythrocyte-filled blood vessels in high power field (HPF; 200×) was counted (plug number, 4–5; t test, P < 0.005).

**Xenograft mouse model.** The 5-week-old to 6-week-old severe combined immune deficiency (SCID) male mice (ordered from NIH) weighing ~20 g were divided into groups with five mice per group. PC3 cells were s.c. injected (2 × 10⁶ cells per mouse) into the mice (30). After the tumors had become established (~50 mm³), the mice were s.c. injected with or without 3 mg/kg GA everyday. The mice body weights and tumor sizes were recorded everyday, and the tumor sizes were determined by Vernier caliper measurements and calculated as length × width × height. After 15 d, mice with s.c. tumors not greater than 1.5 cm in diameter were sacrificed (21).

**Histology and immunohistochemistry.** Tumors were removed and fixed with Histochoice MB (Molecular Biology) tissue fixative (Amresco) and embedded with paraffin. Specific blood vessel staining was performed on the 5-μm sections with Chemicons blood vessel staining kit (vom Willebrand Factor, Chemicon International). Images were taken with ZEISS Axioskop 40 photo microscope. The number of blood vessels was counted (plug number, 4–5; t test, P < 0.005).

**VEGFR2 inhibition assay.** A 12.5-μL of the 4 × reaction cocktail containing 100 ng VEGFR2 (supplied from the HTScan VEGFR2 kinase assay kit, Cell Signaling Technology) was incubated with 12.5 μL/tube of GA for 5 min at room temperature. A 25-μL of 2× ATP/substrate peptide cocktail was added to the preincubated reaction cocktail/GA compound. After incubation at room temperature for 30 min, a 50-μL stop buffer (50 mmol/L EDTA, pH 8) was added per tube to stop the reaction. Then 25 μL of each reaction was transferred with 75 μL H₂O/well to a 96-well streptavidin-coated plate (PerkinElmer Life Sciences) and incubated at room temperature for 60 min. After washing the wells thrice with 200 μL/well PBS/T (0.05% Tween 20 in 1 × PBS), a 100-μL primary antibody [phosphorylated tyrosine monoclonal antibody (pTyr-100), 1:100 in PBS/T with 1% bovine serum albumin (BSA)] was added per well. After being incubated at room temperature for 60 min, the wells were washed thrice with 200 μL PBS/T. A 100-μL diluted HRP-labeled antismouse IgG (1:500 in PBS/T with 1% BSA) was added per well. After incubation at room temperature for 30 min, the wells were washed five times with 200 μL PBS/T per well. Then, a 100 μL/well TMB substrate was added per well, and the plate was incubated at room temperature for 15 min. The stop solution

---

**Figure 2.** GA inhibits angiogenesis in vitro and in vivo. A, effects of GA on angiogenesis in the aortic ring assays. About 1-mm-long to 1.5-mm-long cleaned mice aortic rings were placed in the Matrigel-covered wells with 100 μL of Matrigel. After 4 d of incubation with 1.5 mL of ECGM with or without GA, images were taken with Olympus IX 70 invert microscope.

The number of microvessels was counted as described in the Materials and Methods. Columns, mean; bars, SE (n = 4; t test, P < 0.05). B, effects of GA on angiogenesis in vivo in Matrigel plug assay. Matrigel plugs (0.5 mL/plug) with neither GA nor VEGF, VEGF (4 ng/mL) but no GA, VEGF (4 ng/mL) and 0.1 μmol/L GA, and VEGF (4 ng/mL) and 0.2 μmol/L GA were injected s.c. in the midventral abdominal region of 5-week-old to 6-week-old C57BL/6 mice (five mice for each group). After 7 d, the Matrigel plugs were removed and fixed with formalin, and the 5-μm sections were stained with H&E staining. The number of erythrocyte-filled blood vessels in HPF (200×) was counted. Columns, mean; bars, SE (n = 4; t test, P < 0.005).
(100 µL/well) was added and mixed, followed by incubation at room temperature for 15 min. The plate was then detected at 405 nm with VERSAmax microplate reader (Molecular Devices), and the data (mean ± SE, n = 3) were repeated thrice.

**Western immunoblotting.** HUVECs pretreated with or without 4 nmol/L VEGF for 5 min were treated with or without GA for another 5 min. A 200-µg total cellular protein of each sample was immunoprecipitated with anti-c-Src, anti-FAK, and anti-AKT antibodies (Santa Cruz Biotech) and then subjected to the Western blotting. The pTyr antibody (Santa Cruz Biotech) was used for detecting c-Src phosphorylation, and pFAK397 antibody (Cell Signaling) was blotted for c-Src-associated FAK phosphorylation at multiple tyrosine residues. In addition, AKT phosphorylation was examined using pSer 473-AKT antibody (Cell Signaling). Anticleaved caspase-3 antibody (Santa Cruz Biotech) was used for detecting cleaved caspase-3 and poly(ADP) ribose polymerase (PARP) cleavage was detected by anti-PARP p85 fragment (Promega) in apoptosis assay.

**Statistical analysis.** The data (mean ± SE, n = 3) were repeated thrice for cell proliferation, apoptosis, migration, invasion, and aortic assays. Statistical significance of differences between control and sample groups was determined by t test. The minimal level of significance was P < 0.05.

**Results**

GA inhibits HUVEC migration, invasion, and tube formation. As cell migration is necessary for endothelial cells in angiogenesis and for cancer cells in tumor growth and metastasis (22, 23), we performed wound healing migration assays to determine the effects of GA (Fig. IA) on HUVEC migration and found 10 nmol/L GA strongly inhibited the migration of inactivated HUVECs (Fig. IB)). Cell invasion is a critical aspect of endothelial cells in angiogenesis (24), we performed transwell assays to evaluate the ability of inactivated HUVECs to pass through the membrane barrier of the transwell in the presence of GA. As shown in Fig. IC, 40 nmol/L GA inhibited almost all invasion activities of inactivated HUVECs, suggesting that GA significantly inhibited the invasion properties of endothelial cells at very low concentrations (nmol/L).

Although angiogenesis is a complex procedure of several kinds of cells, tube formation of endothelial cells is the key step (25). To further investigate the effect of GA on endothelial cell tube formation, we added HUVECs (4 × 10⁴) in 1 mL ECGM with different concentration of GA onto Matrigel layers. After 12 to 16 hours of incubation, the ability of endothelial cells forming tube-like structures was assessed with an inverted photomicroscope. Approximately, 50 nmol/L GA inhibited 50% tube formation of HUVEC cells on Matrigel assays and 100 nmol/L GA completely inhibited the tube formation ability of HUVECs on Matrigel (Fig. ID).
GA inhibits angiogenesis in vitro and in vivo. To examine the inhibitory effect of GA on angiogenesis, we performed aortic ring assays using isolated aortas from mice. The 1-mm-long to 1.5-mm-long aortic rings were put on Matrigel and covered by another Matrigel layer and ECGM with or without GA. After 4 days of incubation, the numbers of microvessel growth of the aortic rings were quantified and compared in the presence or absence of GA. We found that 10 nmol/L GA (higher concentration not shown) inhibited almost all new microvessel growth (Fig. 2A), suggesting that GA dramatically inhibited angiogenesis in vitro.

To further verify the inhibitory effect of GA on angiogenesis, we used Matrigel plug assay for antiangiogenesis effect of GA in vivo. We s.c. injected Matrigel (0.5 mL/plug) with or without VEGF, VEGF (4 ng/mL), VEGF (4 ng/mL) and 0.1 μmol/L GA, or 0.2 μmol/L GA in the midventral abdominal region of 5-week-old to 6-week-old C57BL/6 mice (five mice for each group). After 7 days, the mice were sacrificed and the Matrigel plugs were removed, sectioned, and H&E stained. As shown in Fig. 2B, 100 nmol/L GA inhibited VEGF-dependent angiogenesis whereas 200 nmol/L GA totally abolished angiogenesis in the Matrigel plug assays, indicating GA strongly inhibited angiogenesis in vivo. Based on the above analyses, we concluded that GA inhibited angiogenesis in vitro and in vivo using different angiogenesis assays.

GA inhibits tumor angiogenesis and tumor growth in vivo. Tumor angiogenesis provides oxygen, nutrients, and main routes for tumor growth and metastasis and acts as a rate-limiting step in tumor procedures (26). Metronomic chemotherapy is an effective method to suppress tumor growth for angiogenesis inhibitors with low dose of drug (10). To determine the effect of GA on tumor angiogenesis and tumor growth at low dosage, we used a xenograft mouse prostate tumor model. It has been shown that 4 mg/kg of GA at a frequency of one treatment every 2 days is a nontoxic dosage (27). We injected s.c. (2 × 10⁶ PC3 cells per mouse) into the mice. After the tumors had become established (~50 mm³), the mice were sacrificed and the tumors were removed. As shown in Fig. 3, at day 15 after injection of tumor cells, the average tumor size of control group was 1,144 ± 169 mm³, whereas that of GA-treated group was 169 ± 25.6 mm³. The average tumor weight of control group was 0.28 ± 0.08 g, whereas that of GA-treated group was 0.012 ± 0.0008 g (Fig. 3B), indicating that GA significantly inhibited tumor growth with metronomic chemotherapy.

To examine the inhibitory effect of GA on tumor angiogenesis, we stained the 5-μm tumor sections with specific blood vessel staining kit. The average vessel number in tumors of control group was 14 ± 2 (HPF), whereas that in GA-treated group was 1.8 ± 1.3 (HPF; Fig. 3C), indicating that GA significantly inhibited tumor angiogenesis and prevented prostate tumor growth. To evaluate the side effect or chemotoxicity of GA on mice normal growth, we recorded the mice body weights everyday. During the 15 days, the average body weight of control group decreased 1 ± 3.2 g, whereas that of GA-treated group increased 3.2 ± 0.9 g (Fig. 3D), indicating that 3 mg/kg/day GA for mice is not a toxic dosage or at least a low-toxic dosage. The observed mouse body weight decrease in control group is probably due to the tumor burden compared with the GA-treated group.

Figure 4. GA is more effective in activating apoptosis and inhibiting cell proliferation and migration in HUVECs than in PC3 cells. A and B, effects of GA on VEGF-induced migration of HUVECs and PC3 cells. B, columns, mean; bars, SE (n = 3; t test, P < 0.01). C and D, effects of GA on cell proliferation in HUVECs and PC3 cells. D, columns, mean; bars, SE (n = 3; t test, P < 0.01). IC₅₀ was identified as the GA concentration to inhibit 50% cell proliferation. E, GA activates apoptosis in HUVECs. HUVECs were treated with GA for 24 h, and whole-cell proteins were analyzed by Western blotting with anticleaved caspase-3 antibodies and anticleaved PARP p85 antibody.
GA is more effective in inhibiting cell migration and proliferation and activating apoptosis in HUVECs than in PC3 cancer cells. To better understand the inhibitory effects of GA on angiogenesis/tumor angiogenesis and tumor growth, it is important to explore whether GA has different effects on cell migration, proliferation, and apoptosis in endothelial and tumor cells. Considering that VEGFR2 is the primary receptor through which VEGFs regulate the angiogenic activities in both normal angiogenesis and tumor angiogenesis and that HUVECs express VEGFR2 (KDR/Flik-1) but PC3 cancer cells do not express VEGFR2 (28), we firstly examined the effects of GA on VEGF-dependent cell migration in HUVECs and PC3 cancer cells. We found 10 nmol/L GA strongly inhibited VEGF-dependent migration of HUVECs (Fig. 4A), whereas >100 nmol/L GA was required to inhibit VEGF-dependent migration for PC3 cancer cells (Fig. 4B), indicating that GA suppressed VEGF-induced cell migration for both endothelial cells and cancer cells and that endothelial cells were more sensitive to GA inhibition compared with PC3 cancer cells. Then we performed proliferation assays of HUVEC and PC3 cancer cells with GA. We found that 80 nmol/L GA inhibited 50% HUVEC cell proliferation (Fig. 4C), whereas >400 nmol/L GA were required to obtain the same inhibitory effect on PC3 cancer cells (Fig. 4D), indicating that GA was more effective in inhibiting proliferation of endothelial cells than that of PC3 cancer cells. Before we examined the effects of GA in activating apoptosis in HUVEC and PC3 cancer cells, we identified GA-promoted apoptosis in HUVECs with anticleaved caspase-3 and anticleaved PARP antibodies (Fig. 4E). Finally, we measured the apoptotic population of HUVEC and PC3 cancer cells treated with GA by flow cytometry analysis (fluorescence-activated cell sorting assays). As shown in Table 1, 80 nmol/L GA induced 40% HUVECs into apoptosis, whereas only 4% of PC3 cancer cells were induced into apoptosis by the same concentration of GA, indicating that GA was much more effective on endothelial cells than on PC3 cancer cells in promoting cell apoptosis. Taken together, the above different effects of GA on HUVECs and PC3 cancer cells suggest VEGFR2 is primarily, if not exclusively, expressed on endothelial cells. Thus, the inhibitory effects seen with PC-3 cell xenographs in mice (Fig. 3) are due to inhibition of angiogenesis and GA regulates the activity of VEGFR2.

**GA is an inhibitor of VEGFR2.** VEGFR2 is the primary receptor in VEGF signaling pathway that regulates endothelial cell proliferation, migration, differentiation, tube formation, and angiogenesis (14, 15). To understand the molecular mechanism of GA-mediated angiogenesis, we examined whether GA inhibits the activation of VEGFR2. We found GA dramatically inhibited the phosphorylation of VEGFR2 in the assays (Fig. 5A), suggesting that GA is a potential inhibitor of VEGFR2. To verify the inhibitory effect of GA on VEGFR2, we further examined the effects of different concentrations of GA on the specific activation of VEGFR2 using HTScan VEGFR2 kinase assay kit according to suggested methods (Cell Signaling Technology and PerkinElmer Life Sciences). We found that GA inhibited VEGFR2 kinase activity with the IC_{50} of 12 nmol/L (Fig. 5B), indicating GA is a potent VEGFR2 inhibitor.

**GA inhibits VEGFR2 signaling pathway.** VEGFR2 regulates cell migration and proliferation by regulating the activation of FAK and c-Src (29–31). To understand the inhibitory effects of GA on cell proliferation and migration, we examined the effects of GA on the phosphorylation of Src and FAK. As shown in Fig. 5C, GA significantly inhibited the phosphorylation of both c-Src and FAK but not affected the total protein expression of c-Src or FAK, suggesting that GA inhibited cell migration and proliferation by inhibiting VEGFR2 and its downstream protein kinase activities. The activation of c-Src with VEGF pretreatment (Fig. 5C, left) was stronger than that without VEGF treatment (Fig. 5C, right), which was consistent with VEGF-promoted c-Src activation by stimulating VEGFR2.

The phosphorylation of Y1175 of VEGFR2 mediates the activation of AKT to regulate cell proliferation (32). To further examine the downstream signaling pathways mediated by VEGFR2, we examined the activation of the serine/threonine kinase AKT (PKB). As shown in Fig. 5C, GA inhibited AKT phosphorylation (Fig. 5C), suggesting GA inhibited cell proliferation by regulating the activation of AKT signaling pathways.

### Discussion

We identified GA as a novel VEGFR2 inhibitor and comprehensively showed that GA inhibited angiogenesis and tumor progression. Our work focuses on inhibitory effects of GA on HUVEC proliferation, migration, invasion, and tube formation—four key characteristics of endothelial cells in angiogenesis. By directly blocking VEGFR2 phosphorylation and activation, GA suppressed the AKT signaling pathway and inhibited cellular proliferation. As a consequence of VEGFR2 inhibition by GA, the phosphorylation and activation of c-Src and FAK were blocked. Using nontoxic dosage of GA, we showed that GA can inhibit tumor angiogenesis and prostate tumor growth in SCID mouse models.

We showed that GA is a potent VEGFR2 inhibitor, which could provide a new mechanism of actions for xanthone family members (33). We observed GA was more effective in activating apoptosis and inhibiting migration and proliferation in HUVECs than that in PC3 cancer cells, which was in agreement with previous reports that HUVECs express VEGFR2 but PC3 cancer cells do not express it (28). GA also arrested the migration and proliferation of PC3 cancer cells at relatively high concentration, indicating that GA may not specifically inhibit VEGFR2 and GA may attenuate the actions of other receptor tyrosine kinases (RTK) at high doses. As compounds that act as RTK inhibitors always show inhibitory characteristics for multiple kinases (34), we will further investigate

---

**Table 1. GA activates apoptosis in HUVECs and PC3 cells**

<table>
<thead>
<tr>
<th></th>
<th>0 nmol/L GA</th>
<th>20 nmol/L GA</th>
<th>40 nmol/L GA</th>
<th>80 nmol/L GA</th>
<th>100 nmol/L GA</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC3</td>
<td>1 ± 0.3</td>
<td>1.6 ± 0.3</td>
<td>2.9 ± 0.4</td>
<td>4.3 ± 0.5</td>
<td>13.8 ± 3.6</td>
</tr>
<tr>
<td>HUVECs</td>
<td>2.3 ± 0.2</td>
<td>11.6 ± 1.1</td>
<td>14.4 ± 2.3</td>
<td>40 ± 3.6</td>
<td>57.6 ± 4.7</td>
</tr>
</tbody>
</table>
whether GA can inhibit other receptor tyrosine kinases. Interestingly, we also found that GA was more effective in inhibiting HUVEC migration (10 nmol/L) and invasion (40 nmol/L) than in inhibiting HUVEC proliferation (100 nmol/L) and activating apoptosis, suggesting that GA could inhibit endothelial cell migration and invasion before its reported effects on cell proliferation and apoptosis during angioprevention. These findings may provide a molecular mechanism for GA as a new antiangiogenesis candidate with low chemotoxicity during chemotherapy.

Phosphorylation of VEGFR2 at Tyr1175 is required for the activation of AKT and endothelial cell proliferation (17). We found that GA inhibited the activation of VEGFR2 and AKT, as well as cell proliferation. Phosphorylation of Tyr1175 of VEGFR2 is required for the binding site of TSAD, which mediates its substrate of c-Src and then regulates cell migration. VEGFR2 also directly regulates the phosphorylation of FAK and mediates cell migration (22, 35, 36). In this study, we show that GA inhibits the activation of FAK and c-Src and cell migration.

Although the relationship between anti-inflammation and angioprevention has not been fully understood, recent studies have reported several “classic” anti-inflammatory drugs lead to angioprevention (37), such as epigallocatechin gallate in green tea.

Figure 5. GA inhibits the activation of VEGFR2 and its downstream protein kinases. A, 1 nmol/L GA strongly inhibited VEGF-induced phosphorylation and activation of VEGFR2. After starvation in ECGM without serum for overnight, HUVECs were washed with 1 × PBS twice, followed by incubation in M199 medium. Then HUVECs were treated with 1 nmol/L of GA in the presence or absence of 4 nmol/L of VEGF for 5 min. VEGFR2 was immunoprecipitated using anti-VEGFR2 antibody. Anti–phosphorylated tyrosine antibody was used for the detection of phosphorylation of tyrosine residue of VEGFR2. B, inhibition of GA on VEGFR2 activation in a specific VEGFR2 inhibition assay. The calculated IC50 is 12 pmol/L, and the IC50 was identified as the concentration of GA to inhibit 50% of the activity of 100 ng VEGFR2. The data (mean ± SE, n = 3) repeat three times. C, GA suppressed VEGFR2-mediated protein kinase activation of c-Src, FAK, and AKT. HUVECs, pretreated with or without 4 nmol/L VEGF for 5 min, were treated with GA for another 5 min. D, diagram of signaling pathways for GA-mediated antiangiogenesis.
cell migration, invasion, and tube formation. Therefore, it is possible that GA regulates NF-κB signaling pathway by inhibiting the activation of VEGFR2 and TNFR2.

In summary, our studies show an axis of action by GA, e.g., GA functions as an inhibitor of VEGFR2 and its signaling pathway, leading to the inhibition of angiogenesis and tumorigenesis (Fig. 5D). We showed the previously unreported inhibition of GA on HUVEC cell proliferation, migration, and tube formation, as well as the antiangiogenesis activity of GA in vitro and in vivo. Our data suggest a new mechanism of action for the well-known xanthone family and their potential as antiangiogenesis and anticancer drugs.

Acknowledgments

Received 10/21/2007; revised 1/2/2008; accepted 1/8/2008.

Grant support: NIH National Cancer Institute grant 1R01CA106479 (M. Liu).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

References

Correction: Gambogic Acid Inhibits Angiogenesis and Tumor Growth

In the article on how gambogic acid inhibits angiogenesis and tumor growth in the March 15, 2008 issue of Cancer Research (1), there is an error in the labeling and printing of Fig. 5B. The corrected figure appears below.

Gambogic Acid Inhibits Angiogenesis and Prostate Tumor Growth by Suppressing Vascular Endothelial Growth Factor Receptor 2 Signaling

Tingfang Yi, Zhengfang Yi, Sung-Gook Cho, et al.


Updated version  Access the most recent version of this article at: http://cancerres.aacrjournals.org/content/68/6/1843

Cited articles  This article cites 42 articles, 17 of which you can access for free at: http://cancerres.aacrjournals.org/content/68/6/1843.full.html#ref-list-1

Citing articles  This article has been cited by 17 HighWire-hosted articles. Access the articles at: /content/68/6/1843.full.html#related-urls

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.