HIF-1. A substance YC-1 (3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole) inhibits HIF-1 and can be useful in cancer treatment: inhibits cell proliferation and is anti-angiogenic in hepatoma, carcinoma gástrico, neuroblastoma and renal carcinoma.

YC-1: A Potential Anticancer Drug Targeting Hypoxia-Inducible Factor 1

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Abstract

Background: Hypoxia-inducible factor 1 alpha (HIF-1α), a component of HIF-1, is expressed in human tumors and renders cells able to survive and grow under hypoxic (low-oxygen) conditions. YC-1, 3-(5′-hydroxymethyl-2′-furyl)-1-benzylindazole, an agent developed for circulatory disorders that inhibits platelet aggregation and vascular contraction, inhibits HIF-1 activity in vitro. We tested whether YC-1 inhibits HIF-1 and tumor growth in vivo. Methods: Hep3B hepatoma, NCI-H87 stomach carcinoma, Caki-1 renal carcinoma, SiHa cervical carcinoma, and SK-N-MC neuroblastoma cells were grown as xenografts in immunodeficient mice (69 mice total). After the tumors were 100–150 mm3, mice received daily intraperitoneal injections of vehicle or YC-1 (30 µg/g) for 2 weeks. HIF-1α protein levels and vascularity in tumors were assessed by immunohistochemistry, and the expression of HIF-1-inducible genes (vascular endothelial growth factor, ablase, and enolase) was assessed by reverse transcription–polymerase chain reaction. All statistical tests were two-sided. Results: Compared with tumors from vehicle-treated mice, tumors from YC-1-treated mice were statistically significantly smaller (P<.01 for all comparisons), expressed lower levels of HIF-1α (P<.01 for all comparisons), were less vascularized (P<.01 for all comparisons), and expressed lower levels of HIF-1-inducible genes, regardless of tumor type. Conclusions: The inhibition of HIF-1α activity in tumors from YC-1-treated mice is associated with blocked angiogenesis and an inhibition of tumor growth. YC-1 has the potential to become the first antiangiogenic anticancer agent to target HIF-1α.
Cell Culture
The Hep3B hepatoma, Caki-1 renal carcinoma, SiHa cervical carcinoma, and SK-N-MC neuroblastoma cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA). The NCI-H87 stomach carcinoma cell line was obtained from the Korean Cell Line Bank (Seoul, Korea). Hep3B cells were cultured in o-modified Eagle medium; Caki-1, SiHa, and SK-N-MC cells, in Dulbecco’s modified Eagle medium; and NCI-H87 cells, in RPMI-1640 medium. All culture media were supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin. 

Conditioned Media and VEGF Enzyme-Linked Immunosorbent Assay
Conditioned media and VEGF were collected from Hep3B cells in RPMI-1640 medium supplemented with 10% FBS, 1% L-glutamine, 1% nonessential amino acids, 1% sodium pyruvate, penicillin (100 U/mL), and streptomycin (100 µg/mL).

Xenografts of Human Tumors
Male nude BALB/cAnNCrj-nu/nu mice were purchased from Charles River Japan Inc. (Shin-Yokohama, Japan). The animals were housed in a pathogen-free room under controlled temperature and humidity. All animal procedures were performed according to the established procedures of the Seoul National University Laboratory Animal Maintenance Manual.

For the xenografts of human tumors, mice were injected subcutaneously with a volume of 0.1 mL containing 1 × 106 Hep3B cells. The mice were randomly assigned to either a control group or an experimental group. After the tumors reached an approximate volume of 100–150 mm3, the tumor-bearing mice in each group were randomly assigned to either a control group or an experimental group. The mice in the control group received daily intraperitoneal injections of YC-1 (30 µg/g) for 2 weeks after the Hep3B tumors had reached 100–150 mm³, after approximately 40 days. The mice in the control groups received daily intraperitoneal injections of DMSO.

For all mice, tumors were measured in two dimensions with calipers every 2 or 3 days, and the tumor volumes were calculated using the following formula: volume = x × y × z/2, where x is the width at the widest point of the tumor and y is the width perpendicular to a.

The results from individual mice were plotted as average tumor volume versus time.

Immunohistochemistry
For the immunohistochemistry of VEGF, platelet-derived growth factor (PDGF)-A, PDGF-B, and β-actin proteins in tumor tissue, the cells were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton-X100. Amino residues were blocked with 10% normal donkey serum in PBS. Primary antibodies were incubated overnight at 4 °C while specific for VEGF, platelet-derived growth factor (PDGF)-A, PDGF-B, and β-actin proteins in tumor tissue were detected using a mouse monoclonal anti-VEGF antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) at a dilution of 1:1000, a mouse monoclonal anti-PDGF-A antibody (Santa Cruz Biotechnology), a rabbit polyclonal anti-PDGF-B antibody (Santa Cruz Biotechnology) at a dilution of 1:1000, and a rabbit polyclonal anti-β-actin antibody (Santa Cruz Biotechnology) at a dilution of 1:2000. After 3 washes, the sections were incubated with a biotinylated secondary antibody and then with a peroxidase-conjugated streptavidin solution. Free peroxidase activity was detected with diaminobenzidine as the final chromogen.
immunostained sections were lightly counterstained with hematoxylin. For histologic assessment, HIF-1α-positive cells, CD31-positive microvessels, and necrosis were identified at magnifications of ×200, ×100, and ×40, respectively, and examined using a Sony XC-77 CCD camera and a Microcomputer Imaging Device model 4 (MCID-M4) image analysis system. The expression of HIF-1α and vessel density was measured by counting the numbers of immunopositive cells and vessel profiles (identified by CD31 staining) per square millimeter in each image. The extent of necrosis was measured by calculating the necrotic area per 6.25 mm². We analyzed 10 or more different sections per xenograft tumor.

NK Cell Activity

Splenic lymphocytes from 12 nude mice were used to determine the effect of YC-1 on NK cell activity in vitro and in vivo. Individual spleens were homogenized in PBS by passing tissues through steel mesh using a plunger and centrifuged over a Ficoll-Paque (Amersham Biosciences) gradient at 400g at room temperature for 30 minutes to isolate the lymphocyte population. The lymphocytes were removed and washed three times in PBS. NK cell activity in the total lymphocyte population was assessed using a 4-hour 51Cr-release assay with NK-sensitive YAC-1 cells as the target cell population. The YAC-1 cells were labeled with sodium chromate (Na251CrO4) at 0.25 µCi/mL for 1.5 hours at 37 °C in a humidified atmosphere containing 5% CO2, as described (21).

To examine the in vitro effect of YC-1 on NK cell activity, splenic lymphocytes (6.25 x 10⁴ to 5 x 10⁵) were incubated with YC-1 (0.1 to 50 µg/g) or DMSO for 24 hours and then incubated at the indicated effectors: target cell ratio with 1 x 10⁴ 51Cr-labeled YAC-1 cells in 96-well round-bottom plates at 37 °C in a humidified atmosphere containing 5% CO2. After 4 hours, the plates were centrifuged at 200g for 10 minutes, and 100-µL samples of medium were removed and counted for 1 minute in a gamma counter. Splenic lymphocytes taken from a single mouse were used in each experiment. Each assay was repeated three times, and the average value is the result from one experiment. Results are expressed as the ratio of the average values from four separate experiments and 95% confidence intervals (CIs).

To examine the in vivo effect of YC-1 on NK cell activity, mice received a daily intraperitoneal injection of DMSO (n = 4) or YC-1 (30 µg/g; n = 4) for 2 weeks. Splenic lymphocytes were isolated from each mouse and tested immediately for NK cell activity. The spontaneous release of 51Cr from YAC-1 cells was usually lower than 10% of the total 51Cr loaded. NK cell activity was calculated as follows: (experimental release minus spontaneous release)/(total release minus spontaneous release) x 100. Each assay was repeated three times, and the average value is the result from one experiment. Results are expressed as the ratio of the average values from four separate experiments and 95% CIs.

Statistical Analysis

All data were analyzed using Microsoft Excel 2000 software (Microsoft Corp., Redmond, WA). The Mann–Whitney U test (SPSS, version 10.0; Statistical Package for Social Sciences, Chicago, IL) was used to compare VEGF levels in culture media, the number of HIF-1α-positive cells, the number of vessels, NK cell activities, and tumor volumes (NCI-H87, SiHa, SK-N-MC, Caki-1) between the control and the YC-1 treated groups. Tumor volumes in the control and two YC-1-treated Hep3B groups were compared using an analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered statistically significant when P<.05. All statistical tests were two-sided.

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RESULTS

Effect of YC-1 on the HIF-1-Mediated Expressions of Hypoxia-Inducible Genes

Previously, we found that YC-1 treatment inhibits HIF-1α protein expression and decreases the mRNA levels of erythropoietin and VEGF in Hep3B cells cultured under hypoxic conditions. To investigate the inhibitory effect of YC-1 on HIF-1-mediated hypoxic responses, Hep3B cells were treated with YC-1 under hypoxic conditions. The HIF-1α protein level increased in cells cultured under these conditions for 4 hours without YC-1 but underwent a dose-dependent decrease in cells cultured with YC-1 (Fig. 1A, A). The expression of several HIF-1-regulated genes (VEGF, aldolase A, and enolase 1) showed a dose-dependent decrease in cells cultured with YC-1 for 16 hours, whereas the expression of β-actin mRNA was not affected (Fig. 1B, B). The HIF-1α mRNA level was also relatively unchanged in cells cultured with YC-1, suggesting that YC-1-mediated decrease in HIF-1α protein expression occurs at a post-transcriptional level.
We next examined whether the effects of YC-1 were specific to Hep3B cells by assessing the expression of HIF-1α protein and VEGF mRNA in other tumor cell lines (NCI-H87, SiHa, SK-N-MC, and Caki-1) cultured under hypoxic conditions in the absence or presence of YC-1. HIF-1α protein and VEGF mRNA were induced in all cell lines cultured under hypoxic conditions in the absence of YC-1 (Fig. 2). The levels of HIF-1α protein and VEGF mRNA were dose-dependently reduced in cells cultured under hypoxic conditions in the presence of YC-1 (Fig. 2). These results confirm that YC-1 inhibits the HIF-1-mediated induction of hypoxia-inducible genes, regardless of the tumor cell type.

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Fig. 2. The effect of YC-1 on the expression of hypoxia-inducible factor 1 alpha (HIF-1α) and the hypoxia-inducible gene vascular endothelial growth factor (VEGF) in cancer cells of different origin. A) NCI-H87 gastric carcinoma, SiHa cervical carcinoma, SK-N-MC neuroblastoma, and Caki-1 renal carcinoma cells were treated with the indicated concentrations of YC-1 5 minutes before being cultured under normoxic (N, 20% O2 v/v) or hypoxic (H, 1% O2 v/v) conditions for 4 hours. Levels of HIF-1α and β-actin proteins were analyzed by immunoblot analysis using a rabbit anti-HIF-1α antibody or a rabbit anti-β-actin antibody. Proteins were visualized by enhanced chemiluminescence. B) mRNAs for VEGF and β-actin were isolated from cells that had been treated with the indicated concentrations of YC-1 and cultured under normoxic or hypoxic conditions for 16 hours. mRNA expression was analyzed by semiquantitative reverse transcription–polymerase chain reaction.

Effects of YC-1 on Tumor Growth In Vivo

Because of the observed in vitro effects of YC-1, we investigated whether YC-1 inhibits angiogenesis in solid tumors by suppressing the activity of HIF-1 and whether YC-1 inhibits tumor growth in vivo. Mice injected with human tumor cells were treated daily with YC-1 for 2 weeks. Tumors in YC-1-treated mice were visibly smaller than those in vehicle-treated mice (Fig. 3). A). The change in tumor size was measured and plotted as average tumor size versus time (Fig. 3, A). Tumor growth was minimal in mice treated with YC-1 the day after the tumor cells were injected (the last day of the experiment: mean = 422 mm^3, 95% CI = 283 to 561 mm^3; P<.001 versus vehicle-treated group, mean = 1082 mm^3, 95% CI = 880 to 1284 mm^3) and was halted in mice treated with YC-1 after the tumors had become established (mean = 126 mm^3, 95% CI = 97 to 155 mm^3; P<.001 versus vehicle-treated group). NCI-H87 (Fig. 3), SiHa (Fig. 3), SK-N-MC (Fig. 3), and Caki-1 (Fig. 3) xenograft tumors were also statistically significantly smaller in mice treated with YC-1 than in mice treated with the vehicle (P<.01 for all comparisons). These results indicate that YC-1 effectively inhibits tumor growth in tumor-bearing mice.

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Fig. 3. The effect of YC-1 on the development of xenografted human tumors. Viable Hep3B hepatoma (A and B), NCI-H87 gastric carcinoma (C), SiHa cervical carcinoma (D), SK-N-MC neuroblastoma (E), or Caki-1 renal carcinoma (F) cells (5 × 10^6) were injected subcutaneously into the flanks of male nude mice. After the tumors reached 100–150 mm^3 in size (indicated by long arrow in B and short arrows in C–F), mice received an intraperitoneal injection of YC-1 (30 µg/g) or vehicle (dimethyl sulfoxide [DMSO]) daily for 2 weeks. Tumor size was measured over time. A) Hep3B xenografts on the flanks of mice treated with vehicle only (Control) or with YC-1 for 2 weeks. The arrows indicate Hep3B tumors on mouse flanks. B) Beginning 2 days after the injection of the Hep3B cells, some mice received injections of YC-1 daily for 2 weeks (indicated by a short arrow). Solid circles = vehicle, open circles = YC-1 (treatment of established tumors), and open triangles = YC-1" (treatment before established tumors). Each data point represents the mean (n = 12 for control; n = 6 for YC-1; n = 7 for YC-1"), and error bars show 95% confidence intervals. Statistical significance of differences in tumor sizes between the vehicle- and YC-1-treated groups for mice with Hep3B tumors (B) were compared using analysis of variance (ANOVA) and Duncan's multiple range tests. * denotes P<.001 relative to the control. Differences between tumor sizes in the vehicle- and YC-1-treated groups for mice with other tumors (C–F) were compared using a Mann–Whitney U test. Numbers beneath the error bars represent the P value of the difference relative to the control.

Effects of YC-1 on Angiogenesis, HIF-1α Protein, and VEGF Expression
To determine the mechanism by which YC-1 inhibits tumor growth, we examined Hep3B tumors morphologically and biochemically. H&E-stained tumor sections from vehicle-treated mice revealed well-developed blood vessels containing red blood cells and several mitotic figures (Fig. 4A). By contrast, tumor sections from YC-1-treated mice revealed frequent acinus formation without well-developed blood vessels (Fig. 4A).

**Fig. 4.** Histopathology and immunohistochemistry for Hep3B hepatoma tumors grown in nude mice. Viable Hep3B cells ($5 \times 10^6$) were injected subcutaneously into the flanks of male nude mice. After the tumors reached 100–150 mm$^3$ in size, mice received an intraperitoneal injection of YC-1 (30 µg/g) or vehicle (dimethyl sulfoxide [DMSO]) daily for 2 weeks. After the last treatment, the mice were euthanized and the tumors were removed, fixed with formalin, and embedded in paraffin. Tumor sections were cut from the paraffin blocks and stained with hematoxylin and eosin (A) or processed for immunohistochemical staining with an anti-CD31 antibody to detect endothelial cells (B) or with a rat anti-HIF-1α antibody for hypoxia-inducible factor 1 alpha (HIF-1α) (C). All immunostained sections were developed using the avidin–biotin–horseradish peroxidase method with diaminobenzidine as the chromagen. All sections were lightly counterstained with hematoxylin. Arrows indicate CD31-positive vessels in B and HIF-1α-positive cells in C. v = vessel; a = acinus; nu = nuclear staining; pn = perinuclear staining.

To determine whether the inhibitory effect of YC-1 on tumor growth is associated with the suppression of tumor angiogenesis, we examined the distribution of the endothelial marker CD31. Few CD31-immunopositive vessels were observed in tumor sections from YC-1-treated mice, whereas many vessels were observed in tumor sections from vehicle-treated mice (Fig. 4B).

Because HIF-1 is important in angiogenesis, we next assessed HIF-1α expression in tumor sections from vehicle- and YC-1-treated mice (Fig. 4C). Hep3B tumors from vehicle-treated mice showed HIF-1α protein in both the nucleus and perinuclear areas but only in relatively hypoxic regions away from blood vessels (Fig. 4C). By contrast, tumor sections from YC-1-treated mice showed no HIF-1α-immunoreactive cells (Fig. 4C).

We quantified the numbers of HIF-1α-positive cells and CD31-positive vessels in tumor sections from vehicle- and YC-1-treated mice (Fig. 5). Regardless of tumor cell origin, the expression of HIF-1α protein and blood vessel formation was statistically significantly lower in mice treated with YC-1 for 2 weeks than in vehicle-treated mice ($P < .01$ for all comparisons) (Fig. 5).

We also measured the extent of necrosis in Hep3B tumor sections stained with H&E. No statistically significant difference in the percentage of necrosis was found between tumors from vehicle-treated mice (40 lesions examined; mean = 16.3%, 95% CI = 12.2% to 20.4%) and those from YC-1-treated mice (six lesions examined; mean = 18.3%, 95% CI = 10.2% to 26.4%; $P = .17$). Similarly,
for the other tumor types, the differences in the extent of necrosis between tumors from vehicle- and YC-1-treated mice were not statistically significant (data not shown).

To confirm the effects of YC-1 on HIF-1α expression in Hep3B tumors, we isolated the HIF-1α protein by immunoprecipitation and immunoblotting. HIF-1α was detected by immunoprecipitation in tumor lysates incubated with anti-HIF-1α antibody, but not in those incubated with a preimmune serum (data not shown). The level of HIF-1α protein expression was markedly lower in YC-1-treated tumors than in vehicle-treated tumors (Fig. 6A). In addition, levels of VEGF protein and mRNA, and of aldolase and enolase mRNAs were also lower in YC-1-treated tumors than in vehicle-treated tumors (Fig. 6B). The decreased expression of VEGF, aldolase, and enolase may in turn account for the blocked angiogenesis and the growth retardation observed in YC-1-treated tumors.

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Fig. 6.
The effects of YC-1 on the expression of hypoxia-inducible factor 1 alpha (HIF-1α) and hypoxia-inducible genes in Hep3B hepatoma cell xenografts. Viable Hep3B cells (5 × 10^6) were injected subcutaneously into the flanks of male nude mice. After the tumors reached 100–150 mm^3 in size, mice received an intraperitoneal injection of YC-1 (30 µg/g) or vehicle (dimethyl sulfoxide [DMSO]) daily for 2 weeks. After the last treatment, the mice were euthanized, the tumors removed, and lysates prepared for immunoblotting (A) and mRNA analysis by semiquantitative reverse transcription–polymerase chain reaction (RT–PCR). A) Tumor lysates from vehicle-treated mice (C) and from YC-1-treated (YC-1) mice were assessed for HIF-1α, vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF)-A and PDGF-B, and β-actin protein levels by immunoblotting. B) The mRNA levels of VEGF, aldolase A, enolase 1, and β-actin in tumor lysates were measured by semiquantitative RT–PCR (18). The quality of the extracted RNAs was verified by identifying the 18S ribosomal RNA (rRNA) on a 1% denaturing agarose gel.

PDGF is another vasoactive factor that, like VEGF, promotes angiogenesis and growth in solid tumors. PDGF is stored in the α-granules of platelets, and its secretion is stimulated by platelet aggregation (22). Because YC-1 inhibits platelet aggregation, it is possible that some of the antiangiogenic effects of YC-1 are mediated by reduced levels of PDGF in tumors, although such an effect has not before been reported. Thus, to test the possibility that YC-1 inhibits PDGF-induced angiogenesis in tumors, we examined the levels of PDGF-A and PDGF-B protein in Hep3B tumors (Fig. 6A). No substantial differences in the levels of PDGF-A or PDGF-B were observed in tumors from vehicle- and YC-1-treated mice. This result suggests that the anti-platelet aggregation effect of YC-1 does not appear to affect tumor growth.

Effect of YC-1 on NK Cell Function
The athymic nude mouse, which has no thymus-dependent immunologic functions, is a useful model for assaying tumor growth potential in vivo. However, this mouse model has been shown to have thymus-independent NK cells, which are lymphoid cells with cytolytic activity capable of lysing tumors in the absence of previous stimulation (23). Thus, to rule out the possibility that YC-1 inhibits tumor growth by activating NK cells, we examined whether YC-1 affected the cytolytic activity of NK cells in vitro and in vivo. Splenic lymphocytes incubated with YC-1 in vitro had cytolytic activity against NK-cell sensitive YAC-1 cells that was comparable with that from splenic lymphocytes incubated without YC-1 (Fig. 7A). Moreover, splenic lymphocytes from mice treated with YC-1 for 2 weeks had cytolytic activity that was comparable with that from vehicle-treated mice (Fig. 7B).

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Fig. 7.
The effect of YC-1 on natural killer (NK) cell activity. A) Splenic lymphocytes (6.25 × 10^4 to 5 × 10^5), isolated from male nude mice, were incubated with YC-1 at various concentrations for 24 hours. The lymphocytes were then incubated at the indicated effector : target cell ratios with 51Cr-labeled YAC-1 cells (1 × 10^4) as described (21). After 4 hours, the amount of radioactivity in the culture supernatants was measured with a gamma counter. B) Male nude mice (n = 4 per group) received a daily intraperitoneal injection of vehicle (dimethyl sulfoxide [DMSO]) or YC-1 (30 µg/g) for 2 weeks. Splenic lymphocytes were then isolated and tested for NK cell
activity. Cytolytic activity was measured three times per experiment. The result is expressed as the mean of four separate experiments with 95% confidence intervals.

To examine whether there were differences in the number of NK cells that infiltrated the tumors in vivo, we quantified the number of NK cells in tumor sections by immunostaining with anti-asialo GM1 antibody (supplemental Fig. 1e, available at http://jncicancerspectrum.oupjournals.org/cgi/content/vo95/issue7/index.shtml). NK cells were observed in Hep3B tumor sections from vehicle- and YC-1-treated mice, although the difference in number was not statistically significant (vehicle-treated tumors [n = 6], mean = 8.8 per mm², 95% CI = 6.9 to 10.7 per mm²; YC-1-treated tumors [n = 6], mean = 8.4 per mm², 95% CI = 5.8 to 11.0 per mm², P = .7). These results suggest that YC-1 has no effect on NK cell function.

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DISCUSSION

Angiogenesis is essential for the growth and metastasis of solid tumors, and the inhibition of angiogenesis is emerging as a promising strategy for cancer treatment (24). Because of their role in angiogenesis, VEGF and its receptors have become major targets for antiangiogenic therapy. Indeed, antibodies and soluble proteins that interact with VEGF and its receptors have been investigated as an antiangiogenic therapy for solid tumors (25). Angiogenesis is often stimulated by hypoxic conditions such as those that can occur during tumor growth. YC-1 is a hypoxia-activated transcription factor that can regulate VEGF synthesis. HIF-1 activity is dependent upon the level of available HIF-1α, making this component another good antiangiogenic target (26). However, to date, no antitumor agent targeting HIF-1 has been reported. Here, we show that YC-1 has the potential to become the first antiangiogenic anticancer agent to target HIF-1α.

In addition to angiogenesis, changes in energy metabolism are another important adaptation process for cell survival under hypoxia. In hypoxic conditions, oxidative phosphorylation is impaired, and several glycolytic enzymes must be induced to maintain the basal level of adenosine 5′-triphosphate required for cell survival (27). Therefore, the inhibitory action of YC-1 on the expression of the aldolase and enolase genes in tumors probably inhibits cell survival under hypoxia and may promote cell death in hypoxic areas. However, no differences were found in the percentage of necrosis in vehicle- and YC-1-treated tumors, suggesting that the decreased expression of the glycolytic enzymes may not contribute substantially to tumor growth inhibition. We conclude that YC-1 appears to halt tumor growth by blocking angiogenesis and not by a direct cytotoxic effect on tumor cells. YC-1 was developed as an activator of soluble guanylyl cyclase (28). It increases the catalytic rate of the enzyme and sensitizes it to activation by nitric oxide or carbon monoxide (29). In vivo, YC-1 treatment inhibited platelet-rich thrombosis (15) and decreased mean arterial pressure (30), which were associated with increased cGMP levels in platelet and vascular smooth muscle cells. Thus, we anticipate that, at the dosage used for cancer chemotherapy (30 µg/g), YC-1 would result in increased bleeding time and hypotension. To develop YC-1 as a new anticancer agent, these untoward effects should be carefully evaluated. In our opinion, because these effects are clinically manageable, these potential disadvantages should not restrict the clinical use of YC-1 as an anticancer therapy. Moreover, YC-1 has merit as a cancer chemotherapy agent because of its low cytotoxicity. No serious toxicity was observed in any of the nude mice treated with YC-1 over a 2-week period (data not shown). Furthermore, YC-1 did not suppress the cytolytic activity of splenic lymphocytes in vitro or in vivo. Thus, we believe that YC-1 is worth investigating further for clinical applications in cancer therapy.

In summary, we tested whether YC-1 could target HIF-1 and inhibit tumor angiogenesis in vivo. We confirmed the inhibitory effects of YC-1 on the expression of HIF-1α and on the induction of VEGF, aldolase A, and enolase 1 in cancer cells cultured under hypoxic conditions. In vivo, treatment with YC-1 halted the growth of xenograft tumors originating from Hep3B, Caki-1, NCI-H87, SiHa, and SK-N-MC cells. Tumors from YC-1-treated mice showed fewer blood vessels and lower expression of HIF-1α protein and of HIF-1-regulated genes than tumors from vehicle-treated mice. These results suggest that YC-1 is an inhibitor of HIF-1 that halts tumor growth by blocking tumor angiogenesis and tumor adaptation to hypoxia.

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Footnotes

• E. J. Yeo and Y. S. Chun contributed equally to this work.
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