HS-1793, a resveratrol analogue, downregulates the expression of hypoxia-induced HIF-1 and VEGF and inhibits tumor growth of human breast cancer cells in a nude mouse xenograft model

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Abstract. A synthetic analogue of resveratrol, 4-(6-hydroxy-2-naphthyl)-1,3-benzenediol (HS-1793), with improved photosensitivity and stability profiles, has been recently reported to exert anticancer activity on various cancer cells. However, the molecular mechanism of action and in vivo efficacy of HS-1793 in breast cancer cells have not been fully investigated. In the present study, we evaluated the effect of HS-1793 on hypoxia-inducible factor-1α (HIF-1α), which drives angiogenesis and the growth of solid tumors, in addition to the in vivo therapeutic effects of HS-1793 on breast cancer cells. HS-1793 was found to inhibit hypoxia (1.0% oxygen)-induced HIF-1α expression at the protein level, and its inhibitory effect was more potent than that of resveratrol in MCF-7 and MDA-MB-231 breast cancer cells. Furthermore, HS-1793 reduced the secretion and mRNA expression of vascular endothelial growth factor (VEGF), a key mediator of HIF-1-driven angiogenesis, without affecting cell viability. To evaluate the anticancer effects of HS-1793 in vivo, triple-negative MDA-MB-231 breast cancer xenografts were established in nude mice. HS-1793 significantly suppressed the growth of breast cancer tumor xenografts, without any apparent toxicity. Additionally, decreases in Ki-67, a proliferation index marker, and CD31, a biomarker of microvessel density, were observed in the tumor tissue. Expression of HIF-1 and VEGF was also downregulated in xenograft tumors treated with HS-1793. These in vivo results reinforce the improved anticancer activity of HS-1793 when compared with that of resveratrol. Overall, the present study suggests that the synthetic resveratrol analogue HS-1793 is a potent antitumor agent that inhibits tumor growth via the regulation of HIF-1, and demonstrates significant therapeutic potential for solid cancers.

Introduction

Breast cancer is a prevalent type of cancer and in 2012, it was found to be the leading cause of cancer-related mortality in women worldwide (1). Clinically, breast cancer can be divided into distinct subtypes based on the expression of estrogen receptor (ER), progesterone receptor (PR), and amplification of HER-2/Neu, that have prognostic and therapeutic implications (2). Triple-negative breast cancer (TNBC) which is defined by the lack of ER, PR and HER-2 expression, accounts for ~15% of all breast carcinomas (3). In particular, patients with TNBC have a poor outcome compared to the other subtypes of breast cancer, with the 5-year survival rate being lower than 30% (4).

There have been significant advances in detection and chemotherapy, which provide the best prognosis for long-term survival and improve quality of life. However, ~70% of patients with breast cancer are inoperable due to tumor outgrowth or bone metastasis (5), possibly as a result of induced hypoxia (6). The hypoxic regions are easily found in most solid tumors due to the severe structural abnormality of tumor microvessels (7). Hypoxia plays a role as a negative prognostic and predictive factor owing to its multiple contributions to chemoresistance, radioresistance, angiogenesis, vasculogenesis, invasiveness, metastasis, resistance to cell death, altered metabolism and genomic instability (8). Thus, it is not surprising that hypoxia is associated with reduced survival in patient with several cancers (9). It has been reported that the hypoxic condition can govern TNBC progression (10), promoting adaptation through genes within the major hallmarks of cancer (11). TNBC is the breast cancer subtype most frequently associated with hypoxia.
and displays overexpression of hypoxia-inducible factor (HIF) target genes (12). Given the role in hypoxia and the activation of HIF-dependent gene networks is particularly robust in TNBC, targeting HIF directly might provide a new therapeutic option for patients with TNBC (13). There is no effective therapeutic agent readily available for TNBC at present. Therefore, more successful therapeutic strategies are required for breast cancer, such as TNBC, via the targeting of hypoxic conditions (14).

HIF is a crucial transcription factor that responds to hypoxic conditions. It transactivates a large number of genes involved in promoting angiogenesis, anaerobic metabolism and resistance to apoptosis. HIFs are heterodimers composed of one of three major oxygen-labile HIF-α subunits (HIF-1α, HIF-2α, or HIF-3α), and a constitutive HIF-1β subunit, which together form the HIF-1, HIF-2 and HIF-3 transcriptional complexes, respectively (15). Under aerobic conditions, HIF-1/2α is hydroxylated by prolyl hydroxylases (PHDs) at two conserved proline residues in the oxygen-dependent degradation domain (ODD). The hydroxylation of HIF-1/2α facilitates binding of the von Hippel-Lindau protein (pVHL) to HIF-1/2α ODD, which causes poly-ubiquitination and proteasomal degradation of HIF-1/2α (16). However, under hypoxic conditions, hydroxylation does not occur and HIF-1/2α are stabilized and accumulate. HIFs then bind to a conserved DNA sequence known as the hypoxia response elements, and activate the transcription of a variety of hypoxia-responsive genes. The most potent proangiogenic growth factor, vascular endothelial growth factor (VEGF), is one of the HIF-1α-regulated genes and mediates hypoxia-driven angiogenesis. As a result of dysregulated and rapid cell proliferation, which is a characteristic of cancer cells and functionally abnormal blood vessels that form in solid tumors, the environment around cancer cells changes from normoxia (~21% O₂) to hypoxia (~1% O₂) (17,18). HIFs mediate the adaptation of cancer cells to an explicit hypoxic microenvironment. This mediation leads to VEGF expression, followed by the stimulation of angiogenesis, and thereby, increased O₂ delivery. By repeating this process, hypoxic cancer cells acquire invasive and metastatic properties, as well as resistance to cancer therapy, which together constitute the lethal cancer phenotype. Given these factors, compounds that can inhibit HIF-1 may have the potential for use as anticancer agents.

Resveratrol (3,4,5-trihydroxy-trans-stilbene; Fig. 1A), a polyphenol derived from grapes and peanuts, has been shown to possess a wide range of health benefits, including cardioprotective, antioxidant, anti-inflammatory and anti-aging effects (19). Intense efforts over the past decades have indicated that resveratrol exhibits chemopreventive and therapeutic effects against a wide range of cancers (20). In spite of resveratrol’s great anticancer potential, its utility as a therapeutic anticancer agent is limited by its relatively low bioavailability, photosensitiveness and metabolic instability. Thus, numerous approaches are being undertaken to overcome these limitations and to obtain synthetic analogues superior to resveratrol.

HS-1793 [4-(6-hydroxy-2-naphthyl)-1,3-benzenediol; Fig. 1B], a novel synthetic resveratrol analogue, has been shown to exert stronger antitumor effects than those of resveratrol in a variety of cancer cell lines (21-25). Furthermore, it induced the modulation of tumor-derived T lymphocytes, especially in its suppressive role on the Treg cell population (26). It exhibits apoptogenic activity in a wide range of cancer cells, including breast (21,22,27), prostate (24), colon (23,28), leukemia (23) and renal carcinoma cells (25). The way in which this resveratrol analogue exerts its antiproliferative effects has not been fully elucidated. HS-1793 has been shown to induce G2/M cell cycle arrest (27), downregulate Bcl-2 and Bcl-xL expression (23-25,27), activate caspase pathways (27), induce endoplasmic reticulum stress-mediated apoptosis and inactivate Akt (28,29). We have previously shown that this resveratrol analogue can inhibit hypoxia-induced HIF-1α and VEGF expression in PC-3 prostate cancer cells via inhibition of phosphorylation of PI3K and Akt (30), and cause cell cycle arrest and apoptotic cell death in MCF-7 (hormone-dependent, wild-type p53) and MDA-MB-231 (TNBC, mutated p53) breast cancer cells (27). However, most of the studies investigating HS-1793’s anticancer potential have been carried out in vitro. Only a limited number of animal studies have been conducted to reveal its anticancer activities.

In the present study, therefore, we used resveratrol and its synthetic analogue, HS-1793, to investigate and compare their effects on the expression of HIF-1 and VEGF in MCF-7 and MDA-MB-231 breast cancer cells in vitro and further anticancer effects in vivo with triple-negative MDA-MB-231 breast cancer xenografts in nude mice.

Materials and methods

Chemicals. Resveratrol and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), trypan blue stain and antibody against β-actin were purchased from Sigma-Aldrich (St. Louis, MO, USA). HS-1793 was synthesized and supplied by Professor Honguk Suh (Department of Chemistry, Pusan National University, Busan, Korea). A 100 mM solution of resveratrol or HS-1793 was prepared in ethanol and stored in small aliquots at -20°C. The stock solution was diluted, when required, in cell culture medium. The maximum concentration of ethanol did not exceed 0.1% (v/v) in the treatment range, at which it did not influence cell growth. Anti HIF-1α was purchased from BD Transduction Laboratories (San Jose, CA, USA). Antibodies against VEGF and histone H1 were obtained from Santa Cruz Biotechnology (Dallas, TX, USA), Matrigel was purchased from BD Biosciences (San Jose, CA, USA).

Cell culture. The cancer cell lines used in the present study included human breast carcinoma (MCF-7 and MDA-MB-231) with normal human breast epithelial cell line (MCF-10A) serving as control. MCF-7 and MDA-MB-231

Figure 1. Chemical structures of resveratrol (3,4,5-trihydroxy-trans-stilbene) and HS-1793 [4-(6-hydroxy-2-naphthyl)-1,3-benzenediol].
were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). MCF-10A cells were generously provided from Dr Robert J. Pauley (The Barbara Ann Karmanos Cancer Institute, Detroit, MI, USA). MCF-7 and MDA-MB-231 cells were cultured in Dulbecco's modified Eagle's medium (DMEM; HyClone Laboratories, Inc., Logan, UT, USA) containing 10% heat-inactivated fetal bovine serum (FBS; HyClone Laboratories) and 1% antibiotic-antimycotic solution (HyClone Laboratories) at 37°C in a humidified atmosphere with 5% CO2, not exceeding passage number 20. MCF-10A cells were maintained in DMEM/F-12 (1:1) with 5% horse serum (Thermo Fisher Scientific, Waltham, MA, USA) in 37°C incubator supplied with 5% CO2.

**Hyposia experiments.** Experiments to investigate the effects of hypoxia were carried out in a hypoxia chamber in an anaerobic system (Thermo Fisher Scientific, Marietta, OH, USA) as previously described (30). Hypoxic conditions were designated as 1% O2 and 5% CO2 with the temperature maintained at 37°C. Normoxia was defined as the conditions in a standard CO2 incubator (21% O2 and 5% CO2). For hypoxia experiments, MCF-7 and MDA-MB-231 cells were grown to 50% confluency in a standard CO2 incubator at 37°C. Twenty-four hours prior to experiments, aliquots of cell culture media were placed in normoxic and hypoxic chambers to allow equilibration to the corresponding conditions. Immediately before each experiment, cell culture media were withdrawn from MCF-7 and MDA-MB-231 cells and replaced with equilibrated media.

**MTT assay and growth inhibition.** Cell viability was determined by a colorimetric MTT assay as previously described (31). Briefly, cells were seeded onto 6-well plates at a density of 2x10^5 cells/well and allowed to adhere and grow overnight. Cells were then treated with increasing concentrations of resveratrol, HS-1793, or ethanol vehicle for 24 h in normoxic condition or for 4 h in hypoxic condition. Fresh medium with MTT was added to the wells, and the plate was incubated at 37°C for 2 h. The medium was discarded, the formazan crystals were dissolved in dimethyl sulfoxide, and the absorbance at 540 nm was measured using an ELISA plate reader (Thermo Fisher Scientific, Vantaa, Finland). The ethanol vehicle-treated cells served as the indicator of 100% cell viability. Percentage of cell viability was calculated using the following calculation formula: Cell viability (%) = (OD sample/OD control) x 100%. IC50 value (concentration of resveratrol or HS-1793 that reduce 50% cell viability compared to ethanol vehicle-treated control cell) was determined from the graph of viability (%) vs. resveratrol or HS-1793 concentration ranging between 100 and 12.5 µM by 2-fold serial dilution. All cell lines were assayed for three biological replicates each with triplicates.

**Western blot analysis.** Cells were homogenized in protein lysis buffer, and the debris was removed by centrifugation at 12,000 rpm for 10 min at 4°C. The nuclear and cytosolic fractions from tumor tissue were prepared as previously described (32). The protein concentrations in all samples were determined by protein assay reagents (Bio-Rad Laboratories, Hercules, CA, USA). Equal quantities of proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis. They were then transferred onto polyvinylidene fluoride membranes, and blocked with 5% non-fat dried milk for 1 h at 37°C. The membranes were probed with specific primary antibodies overnight at 4°C, and then incubated with the corresponding secondary antibodies for 1 h at 37°C. The specific protein bands were visualized with an ECL detection system (GE Healthcare, Piscataway, NJ, USA).

**VEGF ELISA.** To analyze VEGF expression quantitatively, MCF-7 and MDA-MB-231 cells were pretreated with resveratrol, HS-179, or vehicle for 30 min. The treatment was then removed and replaced with fresh media, which were pre-conditioned in normoxic or hypoxic conditions. Cells were incubated in the presence or absence of resveratrol or HS-1793 at corresponding conditions for 24 h. The supernatants in the wells were collected, cleared by centrifugation and stored at -20°C. ELISA was performed using the human VEGF Quantikine kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's suggested protocol.

**RNA extraction and RT-PCR.** Total RNA isolated from breast cancer cells using a RNeasy Mini kit reagent (Qiagen, Jena, Germany), was reverse transcribed using a Bioneer RT/PCR PreMix in the presence of oligo dT (Bioneer Corp., Daejeon, Korea). The resulting complementary DNA was amplified with the following sets of oligonucleotide primers: VEGF (sense, 5'-AGGAGGGCGAATCCTACGC-3' and antisense, 5'-CAAGGCGCCACAGGATTTTC-3'), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; sense, 5'-CGGAGTGACCGATTTCAGG-3' and antisense, 5'-AGCCCTTC TCCATGTTGTTAGACGAC-3'). GAPDH served as an internal control. PCR products were analyzed by electrophoresis on a 1.5% agarose gel (Bio Basic, Inc., Markham, ON, Canada) in the presence of ethidium bromide, and were visualized with a UV transilluminator (MultiImage™ Light Cabinet; Alpha Innotech Corp., San Leandro, CA, USA).

**Animal studies.** The animal protocol used in the present study was reviewed and approved by the Pusan National University-Institutional Animal Care and Use Committee (PNU-IACUC, Busan, Korea) in terms of ethical procedures and scientific care (approval number, PNU-2015-0318). Five-week-old female BALB/c nude mice (Japan SLC, Inc., Hamamatsu, Japan) were used for in vivo experiments. The animals were maintained in constant, specific pathogen-free laboratory conditions for a 12 h light/dark cycle. They were given water and fed standard mouse chow ad libitum. For injections, MDA-MB-231 cells were trypsinized and counted using trypan blue to identify viable cells. Animals were injected with 1x10^6 MDA-MB-231 cells [in 100 µl of phosphate buffered saline (PBS) and Matrigel, 1:1] in the right flank and allowed to form xenografts. When the average tumor volume reached 40 mm^3, mice were randomly assigned to one vehicle and three treatment groups (6 mice per group): i) vehicle; ii) HS-1793 (5 mg/kg); iii) HS-1793 (10 mg/kg); and iv) resveratrol (20 mg/kg). HS-1793 and resveratrol were dissolved in PBS containing 0.1% v/v dimethyl sulfoxide (DMSO) and administered intraperitoneally twice a week. Tumor diameters were determined with a caliper, and the tumor volume was calculated using a standard formula: tumor
volume \((\text{mm}^3) = L_1 \times (L_2)^2 \times 0.5236\), where \(L_1\) is the long diameter and \(L_2\) is the short diameter. Toxicity was assessed by survival, activity and changes in body weight. At the completion of 4 weeks of treatment, the mice were euthanized and tumor samples were dissected out, weighed, fixed in formalin, and processed to determine the expression of target proteins as described in the sections on immunohistochemical analysis and western blotting.

**Immunohistochemical analysis of tumors.** Tumor tissues were fixed in 10% v/v neutral buffered formalin, embedded in paraffin, sectioned to 5 µm and mounted on slides. The sections were blocked with normal goat serum and incubated with the following antibodies; anti-CD31 (Abcam, Cambridge, MA, USA) and anti-Ki-67 (Abcam). Stained slides were visualized with an Axiosvert 100 microscope (Zeiss Carl, Göttingen, Germany) and the images were captured at a x200 magnification.

**Statistical analyses.** Results were expressed as the mean ± standard deviation (SD) of three separate experiments and analyzed using the Student's t-test. Means were considered significantly different at \(P<0.05\) or \(P<0.01\).

**Results**

**HS-1793 suppresses proliferation of MCF-7 and MDA-MB-231 cells.** We examined the effects of resveratrol or HS-1793 on the viability of MCF-7, MDA-MB-231 and MCF-10A cells cultured in normoxic condition for 24 h. Table I summarized the IC\(_{50}\) value of resveratrol and HS-1793 on all the tested cells. The IC\(_{50}\) values in MCF-7, MDA-MB-231 and MCF-10A cells treated with resveratrol were 88.2±4.7, 90.6±2.9 and >100 µM, respectively. The IC\(_{50}\) values in MCF-7 and MDA-MB-231 cells treated with HS-1793 were 26.3±3.2, 48.2±4.2 and >100 µM, respectively. Therefore, HS-1793 treatment exhibited 3.3- and 1.9-fold more anti-proliferative effects against MCF-7 and MDA-MB-231 cells than resveratrol. However, no significant differences were observed between resveratrol and HS-1793 against the non-malignant normal MCF-10A cells.

**HS-1793 inhibits hypoxia-induced HIF-1α protein in MCF-7 and MDA-MB-231 cells.** To examine the effects of resveratrol and HS-1793 on HIF-1α expression, we first exposed breast cancer cells to hypoxic conditions and measured the HIF-1α protein level to determine the optimum conditions. As shown in Fig. 2, hypoxia induced the expression of HIF-1α in MCF-7 and MDA-MB-231 cells in a time-dependent manner. Unlike MCF-7 cells, in MDA-MB-231 cells the expression started at 2 h, reached a maximum at 4 h, and decreased at 8 h (Fig. 2B). Since both breast cancer cell lines exhibited the highest expression of HIF-1α at 2-4 h, we used the 4 h time-point in subsequent experiments.

**Decrease of HIF-1α protein levels by HS-1793 is unrelated to cell death.** To investigate whether resveratrol and...
HS-1793-induced cell death was responsible for the suppression of HIF-1α accumulation, the cell viabilities in normoxic and hypoxic conditions were determined using the MTT assay. When both cells were treated with various concentrations of resveratrol and HS-1793 for 4 h in hypoxic conditions, no significant reductions in the viability was observed at any concentration (Fig. 3). These results suggest that the decrease in HIF-1α under hypoxic conditions may not be due to cell death.

**HS-1793 downregulates hypoxia-induced VEGF expression in breast cancer cells.** VEGF is one of the downstream target genes of HIF-1α; therefore, its level increases under hypoxic conditions, and it plays a crucial role in tumor angiogenesis (33). Therefore, we investigated whether HS-1793 inhibits VEGF under hypoxic conditions. In order to do this, we first performed ELISA to determine whether HS-1793 and resveratrol affect VEGF levels in breast cancer cells. As expected, hypoxia caused a rise in the VEGF level when compared...
with normoxia (Fig. 4A and B). However, the expression levels of VEGF were decreased in a concentration-dependent manner following treatment with resveratrol and HS-1793 treatments (Fig. 4A and B). Moreover, HS-1793 reduced the VEGF level to a greater extent than resveratrol in both cell types. These results showed that HS-1793 is more effective than resveratrol at inhibiting the production of VEGF in both cancer cell lines. In addition, HS-1793 treatment at 50 µM concentration more efficiently reduced the expression levels of VEGF in MDA-MB-231 cells than in MCF-7 cells.

**HS-1793 suppresses hypoxia-induced mRNA expression of VEGF at the transcriptional level.**

In order to determine whether HS-1793 induces a reduction of VEGF through suppressing the expression of VEGF, we determined its mRNA levels under hypoxic conditions using reverse transcription-polymerase chain reaction (RT-PCR). Breast cancer cells were pretreated with various concentrations of resveratrol or HS-1793 and incubated for 4 h under hypoxic conditions, and the mRNA levels were measured. As shown in Fig. 4C and D, HS-1793 downregulated the expression of VEGF mRNA, with the more marked results observed in MDA-MB-231 cells. In MCF-7 cells, the effect was observed at a concentration of 12.5 µM HS-1793, a concentration at which resveratrol had no significant effects on the mRNA expression of VEGF (Fig. 4C). In addition, we saw the suppression of VEGF mRNA by resveratrol only at the highest concentration (50 µM) used in this study. We also found that resveratrol slightly inhibited the mRNA expression of VEGF in MDA-MB-231 cells, whereas HS-1793 highly effective at inhibiting VEGF mRNA expression in a concentration-dependent manner (Fig. 4D). Neither resveratrol nor HS-1793 had any effect on the mRNA expression of HIF-1α under these experimental conditions (data not shown).

Therefore, these results indicate that the inhibition of VEGF expression by HS-1793 occurs at the transcriptional level. In summary of *in vitro* experiments, HS-1793 treatments showed more efficient downregulation of HIF-1α and VEGF expression levels in MDA-MB-231 cells than in MCF-7 cells, we decided to use TNBC MDA-MB-231 cells for further *in vivo* xenograft experiment.

**HS-1793 effectively inhibits the growth of human breast xenografts.**

To explore the therapeutic effects of HS-1793 as a potentially clinically useful agent, we compared the *in vivo* efficacy of resveratrol and its synthetic analogue, HS-1793, in TNBC MDA-MB-231 tumor-bearing mice. As shown in Fig. 5A, tumor growth was rapid in the vehicle-treated control group, whereas HS-1793 significantly inhibited MDA-MB-231 xenograft tumor growth in a dose-dependent manner. Notably, the tumor-inhibitory effects of low-dose HS-1793 (5 mg/kg twice a week) and a 4-fold higher dose (20 mg/kg twice a week) of the parent agent, resveratrol, were similar, although no statistical significance was observed (Fig. 5A, C and D). HS-1793 (5 mg/kg twice a week) showed 2-fold higher maximum growth inhibition when compared to resveratrol (Fig. 5A and D), indicating that the *in vivo* efficacy of HS-1793 was superior to that of resveratrol. In addition, there were no adverse side-effects, such as weight loss, ulcerations, or general decreases in well-being in the drug-treated mice in comparison to the vehicle-treated control group during the experimental period. This indicates the non-toxicity of both resveratrol and HS-1793 (Fig. 5B).
To shed light on the mechanism by which HS-1793 inhibited tumor growth in nude mice, we examined the presence of proliferation markers in tumor tissues from vehicle- and HS-1793-treated groups. Ki-67 (a proliferation marker) expression was significantly lower in the HS-1793-treated group than in the vehicle-control group (Fig. 6A). As shown in Fig. 6A (right panel), HS-1793 was more effective than resveratrol in downregulating Ki-67 levels. The results of proliferation index were summarized in Fig. 6B. We also examined the effects of HS-1793 and resveratrol on tumor-associated angiogenesis, as this process is critical for tumor survival and proliferation (34). Change in angiogenesis was validated using immunohistochemistry of CD31 expression in the sections of tumors. Results showed that as compared to vehicle-treated and resveratrol-treated tumors, the expression of CD31 was significantly lower in tumors treated with HS-1793 (Fig. 6C). This result suggests relatively hampered angiogenesis in HS-1793-treated tumors, which may be contributing in the slower tumor growth in this group of mice.

HS-1793 inhibits HIF-1 and VEGF expression in xenograft tumors from mice. Up to this point, our results indicated that HS-1793 can inhibit the proliferation and vessel formation which linked to tumor development. We next sought to determine the effects of resveratrol and HS-1793 treatment on the expression of HIF-1α and its-regulated gene product VEGF in tumor tissue. As shown in Fig. 7, the tumor tissues from vehicle-treated mice groups expressed HIF-1α and VEGF. Treatments of resveratrol (20 mg/kg) and HS-1793 (5 and 10 mg/kg) significantly downregulated HIF-1α and VEGF expression in MDA-MB-231 xenograft tumors from at least three different mice. The results were confirmed by western blot analysis. The expression levels of HIF-1α and VEGF were reduced in HS-1793-treated groups compared to vehicle-treated control. These findings suggest that HS-1793 may exert its anti-tumor effects not only by inhibiting tumor proliferation but also by suppressing angiogenesis, which could significantly contribute to the inhibition of tumor growth in vivo.
10 mg/kg) successfully suppressed the expression of HIF-1α and VEGF in tumor tissues. The results also indicated that the tumor tissues expressed VEGF (Fig. 7A). However, HS-1793 is more effective than resveratrol in suppressing the expression of HIF-1α and VEGF in vivo (Fig. 7B).

Discussion

Numerous studies have suggested that the transcription factor HIF-1α is a crucial mediator of the hypoxic response, which plays a role in triggering tumor metastasis and developing chemoresistance in cancer cells. HIF-1α overexpression is closely associated with the unfavorable prognosis and increased mortality in cancer patients (35,36). Thus, new agents that target this transcription factor have gained attention. The goal of the present study was to determine whether the novel resveratrol analogue HS-1793 could inhibit HIF-1α, which is closely linked with cancer cell proliferation, invasion and angiogenesis.

Our results showed that HS-1793 reduced the protein level of HIF-1α without affecting its mRNA level, therefore, suggesting that its action occurs at the post-transcriptional level. We found that HS-1793 activates the ubiquitin-proteasome pathway, which is responsible for HIF-1α protein degradation (data not shown). This is evidenced by the fact that the 26S proteasome-specific protease inhibitor, MG132, is able to delay the degradation of HIF-1α in the presence of HS-1793. This is further supported by our previous report that showed HS-1793 promoted the degradation of HIF-1α via the ubiquitin-proteasome pathway in prostate cancer cells (30). However, a recent study has documented that the autophagy-lysosome pathway involves degradation of the HIF-1α protein (37), thus, HS-1793 could induce autophagy in cancer cells (unpublished data). The precise mechanism by which HS-1793 regulates HIF-1α needs to be elucidated in future studies.

This study demonstrated that HS-1793 decreased hypoxia-induced mRNA expression and secretion of VEGF in breast cancer cells, which is in agreement with our previous finding in prostate cancer cells (30). VEGF is known to be a major signaling molecule involved in tumor angiogenesis, and is regulated by HIF-1α (38). Therefore, the observed inhibition of HIF-1α may have accounted for the downregulation of VEGF by HS-1793. We also found that HS-1793 decreased angiogenesis in vivo, as indicated by the inhibition of CD31, a marker for microvessel density and the suppression of VEGF. The downregulation of the levels of these proteins suggests that this resveratrol analogue possesses anti-angiogenic potential.

Accumulating evidence indicates that resveratrol requires relatively high doses and frequent injections to exhibit its tumor growth inhibitory effect. This is due to its poor bioavailability, as a result of its low intestinal uptake and short initial half-life (39-42). Furthermore, there has been controversy about the antitumor effects of resveratrol on mammary tumors, with early studies showing that HS-1793 exerted a considerable effect on the in vivo growth of an FM3A breast tumor in C3H/He mice (26). The same study also showed the chemopreventive effect of HS-1793 (26). Jeong et al (46) showed that HS-1793-administration increased the number of interferon (IFN)-γ-secreting cells in splenocytes, which lead to the switch-off of M-2 polarized tumor-associated macrophages with immunosuppressive and tumor progressive properties, which likely contributes the antitumor effect of HS-1793.

In conclusion, we provide novel evidence that HS-1793 exhibits its anticancer activity, at least in part, by modulating HIF-1α and its regulating gene, VEGF. In our xenograft mouse study with TNBC MDA-MB-231 cells, which bear an aggressive phenotype, HS-1793 not only inhibited tumor growth, but also suppressed microvessel formation, which strongly correlated with the inhibition of cell proliferation, and the decrease in angiogenesis. These findings provide a rationale for further investigation into this novel resveratrol analogue for chemoprevention and/or treatment in human breast cancer.

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