Triphenylethylene-Coumarin Hybrid TCH-5c Suppresses Tumorigenic Progression in Breast Cancer Mainly Through the Inhibition of Angiogenesis

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Abstract: Background: Coumarins are a wide group of naturally occurring compounds which exhibit a wide range of biological properties such as anti-cancer activities. Here, we characterized the biological functions of three Triphenylethylene-Coumarin Hybrids (TCHs) both in cell culture and nude mouse model.

Methods: Cell proliferation assay was performed in the cell cultures of both EA.hy926 endothelial cell and breast cancer cell lines treated with different concentrations of compound TCH-10b, TCH-5a and TCH-5c. Flowcytometry assay and Western blotting were used to further investigate the effect and mechanism of TCH-5c on EA.hy926 cell proliferation and cell cycle. The effects of TCH-5c on endothelial cell migration and angiogenesis were determined using cytoskeleton staining, migration assay and tube formation assay. Inhibition of breast cancer cell line derived VEGF by TCH-5c was shown through ELISA and the use of conditioned media. SK-BR-3 xenograft mouse model was established to further study the anti-tumorigenic role of compound TCH-5c in vivo.

Results: We found that compound TCH-5c has inhibitory effects on both vascular endothelial cells and breast cancer cell lines. Compound TCH-5c inhibited proliferation, resulted in cell death, increased p21 protein expression to induce G0/G1 arrest and changed endothelial cell cytoskeleton organization and migration in EA.hy926 endothelial cells. Compound TCH-5c also inhibited breast cancer cell line derived VEGF secretion, decreased breast cancer cell-induced endothelial cell tube formation in vitro and suppressed SK-BR-3 breast cancer cell-initiated tumor formation in vivo.

Conclusion: Our study demonstrates that the coumarin derivative TCH-5c exerts its anti-cancer effects by 1. inhibiting endothelial cell proliferation, migration. 2. suppressing tube formation and angiogenesis induced by breast cancer cells in vitro and in vivo. Our results have potential implications in developing new approaches against breast cancer.

Keywords: Breast neoplasms, angiogenesis inhibitors, vascular endothelial growth factor, coumarin, TCH-5c, western blotting.

1. INTRODUCTION

Breast cancer remains a major health problem in women worldwide owing to its increasing incidence and mortality. Current therapies in the treatment of breast cancer are limited [1, 2]. There is an urgent need to find new cures against this deadly disease. One potential novel remedy to treat breast cancer is to cut off blood supply to the tumor cells. Solid tumors, including breast cancer, require blood vessels for growth. The formation of new vessels from pre-existing ones, called angiogenesis, is a necessary step for cancer growth and progression [3, 4]. The imbalance between pro- and anti-angiogenic signals in tumor microenvironment causes primitive vasculature expanding into a new complex network, which contributes to poor prognosis in breast cancer [5]. Anti-angiogenic therapies could destroy the tumor vasculature, leading to the death of breast cancer cells [6, 7]. Thus, it is of great importance to study breast cancer angiogenesis and test compounds which have anti-angiogenic properties in vitro and in vivo.

Coumarins are a wide group of naturally occurring compounds, which exhibit a wide range of biological properties and serve as a significant source of inspiration for the new anticancer agents [8-10]. Extensive studies have been carried out on the design and synthesis of coumarin derivatives with improved anticancer activity [8, 11, 12]. In our previous studies, we designed and synthesized a series of novel Triphenylethylene-Coumarin Hybrids (TCHs) containing different amounts of amino side chains for the anti-tumor drug discovery [13]. The results showed that such triphenylethylene-coumarin hybrids exhibited a broad-spectrum and good anti-proliferative activity and had significant interactions with Ct-DNA by the intercalative mode of binding.

In order to explore the mechanism on their antitumor activities in detail, herein, we examined the anti-proliferative activities and...
anti-angiogenesis effects of three compounds (TCH-10b, -5a, and -5c) in SK-Br-3 Estrogen Receptor (ER)-negative and MCF-7 ER-positive breast cancer cell lines and endothelial cell lines. TCH-5c was also investigated for its roles in tumorigenic progression in a breast cancer xenograft model.

2. MATERIALS AND METHODS

2.1. Chemicals, Antibodies and Cell Lines

TCHs (TCH-10b, TCH-5a and TCH-5c) were synthesized in our facility according to the protocol reported previously [13]. The structures of all the compounds were determined by Nuclear Magnetic Resonance (NMR), Mass Spectrometry (MS), and elemental analysis, as has previously been reported (Fig. 1A). Antibodies were bought from commercial sources that are listed below: p21 (sc-397, Santa Cruz, CA, USA), cyclin D1 (2261-1, Epitomics, California, USA), cyclin B1 (1495-1, Epitomics, California, USA), β-actin (sc-47778, Santa Cruz, USA), goat anti-rabbit IgG-HRP (sc-2004, Santa Cruz, USA), goat anti-mouse IgG-HRP (sc-2005, Santa Cruz, USA), goat anti-rabbit IgG-HRP (sc-2004, Santa Cruz, USA). Human umbilical vein cell line EA.hy926 and breast cancer cell line SK-BR-3 and MCF-7 were purchased from the Cell Resource Center, Shanghai Academy of Life Sciences, Shanghai, China. EA.hy926, SK-BR-3 and MCF7 cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% FBS (Gibco, Shanghai, China). EA.hy926 endothelial cells were harvested by trypsinization, planted onto the Matrigel at 5×10^3 cells per well and incubated for 6 additional hours at 37°C. The tube-like structures were observed by microscopy and the total lengths were quantified in five randomly selected microscopic fields per well using Image-Pro Plus software 6.0 (Media Cybernetics, Inc., Rockville, USA).

2.2. Conditioned Cell Culture Media Collection and Protein Detection by Western Blotting or ELISA

For collecting the conditioned media, SK-BR-3 or MCF7 cells were seeded and incubated for 24 hours in culture media. Then, the media was removed, and the cells were treated with DMSO (0.1%) or TCH-5c (5μM) for an additional 24 hours in serum-free DMEM. Conditioned media (DMEM-CM and TCH-5c-CM) were collected then, the conditioned media were filtered by 0.45μm filters (Millipore, Massachusetts, USA) and normalized to the cell number by serum-free DMEM. For protein detection, whole-cell lysates or subcellular extracts were prepared and subjected to Western blot as described [14]. VEGF ELISA Kit (Neobioscience Technology, China) was used to quantify Vascular Endothelial Growth Factor (VEGF) in conditioned media or mouse serum.

2.3. Measurement of Cytotoxicity, Cell Proliferation and Migration

Cytotoxicity Detection (LDH) kit (Roche Applied Science, Mannheim, Germany) was used to evaluate cell cytotoxicity. Absorbance was determined at 490nm. A commercially available Cell Titer 96® AQueous One Solution Cell Proliferation Assay Kit (Promega, Madison, USA) was used to estimate cell proliferation as described [14]. Values at OD490 were collected using a Biotek Epoch Spectrophotometer (Bio Tek Instruments, Inc., Winooski, USA). For cell migration study, cells were seeded in six-well plates at 10^6 cells/well and then treated with DMSO (0.1%) or TCH-5c (2.5μM) for 24 hours. Then, the cells were scraped to make a ‘wound’ for cell migration and cultured in DMEM with 1% FBS for an additional 6 or 24 hours before being fixed and stained with 0.5% crystal violet. The ‘wound’ was observed under a light microscope. The migration activity of cells was exhibited as the wound healing area in each field and shown as the percentage of the control group. Next, transwell migration assay was performed using Transwell® Permeable Supports (Corning Incorporated, Corning, USA) with 8-μm pore size as described [14]. Cells were seeded (1×10^5 per well) in the upper chamber of a Transwell plate in DMEM with 0.5% FBS. DMEM with 10% FBS was added to the lower chambers to induce cell migration. Migrated cells on the lower surface were fixed and stained with 0.5% crystal violet and counted using a light microscope. The experiment was repeated independently 3 times.

2.4. Flow Cytometry

For the flow cytometry analysis, the cells were stained with Propidium Iodide (PI) and run on a flow cytometer. The cell cycle profiles were determined using BD Cell Quest™ Pro Software (Becton, Dickinson and Company, Franklin Lakes, USA). The experiment was repeated independently 3 times.

2.5. Actin Cytoskeleton Staining and Tube Formation Assay

For the analysis of actin cytoskeleton, cells were fixed and permeabilized with 0.1% Triton X-100. The cells were then incubated with TRITC-phalloidin in the dark. Cell nuclei were visualized with 4,6-diamidino-2-phenylindole (DAPI) and observed under a fluorescence Microscope (Olympus, Japan). For tube formation, 12-well plates were pre-coated with 10 mg/mL chilled Matrigel (Corning, Bedford, USA) and solidified at 37°C for 30 minutes. EA.hy926 endothelial cells were harvested by trypsinization, planted onto the Matrigel coat at 5×10^5 cells per well and incubated for 6 additional hours at 37°C. The tube-like structures were observed by microscopy and the total lengths were quantified in five randomly selected microscopic fields per well using Image-Pro Plus software 6.0 (Media Cybernetics, Inc., Rockville, USA).

2.6. Xenograft in Nude Mice

For the in vivo xenograft experiments, female BALB/c-nu mice, 4-6 weeks old, were purchased from the Beijing HFK Bioscience Co., Ltd (Beijing, China). All of the mice were bred and housed in a specific pathogen-free environment at the Animal Research Center in our university. All the animal experiments were approved by the Animal Research Ethics Committee of the authors’ institution. Nude mice were injected subcutaneously with a cell suspension of 0.2 ml in PBS containing 5×10^6 SK-BR-3 cells into the right mammary fat pad. TCH-5c 3mg/kg/day (freshly dissolved in 0.25% DMSO), was administered by oral gavage daily for two weeks starting from the next day after SK-BR-3 cell injection. Mice in the control group received an equal volume of 0.25% DMSO (0.2 ml/day) in the same manner. Each experimental group consisted of 7 mice. After tumor establishment, the palpable xenograft nodules were measured for the longest diameter (L) and the shortest diameter (W) every two days using a caliper. The tumor volumes were calculated using the formula: V = L × W^2 × 0.5. Mice were sacrificed at day 14 after injection. The xenograft tumors were dissected, weighted and photographed.

2.7. Immunohistochemical Staining

Xenografts from nude mice were fixed, sliced and subjected to Immunohistochemical (IHC) staining as described previously [14]. The slides were incubated with the antibodies against p21 (dilution: 1:200, Proteintech, Chicago, USA), VEGF (dilution: 1:200, Proteintech, Chicago, USA) and CD31 (dilution: 1:200, Novusbio, CO, USA) overnight at 4°C followed by incubation with HRP-conjugated secondary antibody kit.

2.8. Statistical Analysis

All quantitative data are presented as mean±SEM for three or more independent experiments. Student’s T-test or one-way ANOVA were performed as appropriate. For growth curve assay, two-way ANOVA was performed, followed by Bonferroni posttests. p<0.05 was considered as statistically significant.

3. RESULTS

3.1. TCH-5c Inhibits Proliferation and Induces Cell Death in EA-Hy926 Endothelial Cells

To investigate whether the three TCHs (TCH-10b, TCH-5a and TCH-5c) have an effect on proliferation in endothelial cells,
human EA.hy926 endothelial cells were treated with 0, 1, 5, 10μM of TCHs (TCH-10b, TCH-5a and TCH-5c) for 48 hours. Cell proliferation was measured afterward. Proliferating activity significantly reduced in 5 and 10μM of TCH-5c treated cells (Fig. 1B). TCH-10b and TCH-5a did not have a significant effect on the growth of EA.hy926 cells. Therefore, we focused on TCH-5c in subsequent experiments. Cytotoxicity analysis was performed to further investigate the anti-proliferation and pro-death activity of compound TCH-5c in EA.hy926 endothelial cells. Treatment of EA.hy926 cells with different concentrations of compound TCH-5c resulted in a dose-dependent cell death (Fig. 1C).

3.2. Compound TCH-5c Induced G0/G1 Arrest in EA.hy926 Endothelial Cells

To further investigate the effect and mechanism of TCH-5c on EA.hy926 cell proliferation and cell cycle, EA.hy926 cells were treated with 5 or 10μM of compound TCH-5c for 48 hours and FACS cell-cycle analysis was performed. Treatment of EA.hy926 cells with TCH-5c resulted in a remarkable increase in G0/G1 phase cells and a corresponding decrease in S and G2/M phase cells in a dose-dependent manner (Fig. 2A). Moreover, compared with cells treated with DMSO, TCH-5c treatment for 48h significantly increased p21 protein level and decreased cyclin B1 level in EA.hy926 cells (Fig. 2B). Increased expression of p21 and reduced cyclin B1 level at least in part explains the G0/G1 arrest of EA.hy926 cells induced by compound TCH-5c.

3.3. Compound TCH-5c Changes Cytoskeleton Organization and Inhibits Migration and Tube Formation of EA.hy926 Endothelial Cells

To evaluate the role of TCH-5c on EA.hy926 cytoskeleton organization and motility, we performed phalloidin staining and migration assay. EA.hy926 cells were treated with 5μM of TCH-5c for 48 hours. Then, cells were stained with TRITC-phalloidin for
Fig. (2). Compound **TCH-5c** promoted p21 expression and G0/G1 arrest in EA-hy926 endothelial cells. (A) EA-hy926 cells were treated with different concentration of triphenylethylene-coumarin hybrid derivatives **TCH-5c** for 48 hours. The cells were fixed, stained with PI and the cell cycle distribution was analyzed by FACS analysis. (B) EA-hy926 endothelial cells were treated with 5μM of **TCH-5c** for 48 hours. Crude proteins were extracted from the cells and subjected to Western blot analysis with antibodies against p21, cyclin B1 and cyclin D1. β-actin was used as a control to ensure equal protein loading. **Left**, blots from a representative experiment. **Right**, densitometry; results were normalized to β-actin. The bars represent the means± S.E. from three independent experiments. *, *p*<0.05 vs. DMSO-treated group.

Fig. (3) contd....
Fig. (3). Compound TCH-5c regulates EA.hy926 endothelial cell cytoskeleton organization and migration. (A) EA.hy926 endothelial cells were treated with DMSO or TCH-5c (5μM) for 24 hours and then, fixed and stained for F-actin with TRITC-phalloidin. Photographs of Phalloidin (red) and DAPI (purple) are presented; magnification ×400. (B) EA.hy926 cells were treated with DMSO or TCH-5c (2.5μM) for 24 hours and wound healing assay was performed. Migration of EA.hy926 cells was photographed under a light microscope (Magnification, ×100). One representative experiment is shown (left); three independent experiments are summarized (right). *, p<0.05 vs. DMSO group. (C) Transwell chamber assay of stimulatory effect of TCH-5c on cell migration. Cells were pre-treated with TCH-5c (2.5μM) for 24 hours and then seeded in transwell chambers for 10 hours and the number of migrated cells, which were observed using a microscope at ×100 magnification, was determined. (D) EA.hy926 endothelial cells were pre-treated with DMSO or TCH-5c (5μM) for 24 hours and, subsequently, seeded on 24-well plates (2.5×10^5 cells/well) precoated with Matrigel for 6 hours. Representative photographs of tube formation are shown (magnification ×100). Tube length was quantitated using Image-Pro Plus software (n=5 per group). *, p<0.05 vs. DMSO group.

Fig. (4). Compound TCH-5c inhibits breast cancer cell induced EA.hy926 endothelial cell tube formation. (A) MCF7 and SK-BR-3 breast cancer cells were treated with different concentration of TCHs for 48 hours and cell proliferation assay was performed using the CellTiter 96® Aqueous One Solution Cell Proliferation Assay Kit. Absorbance was measured at 490nm. *, p<0.05 vs. control group. (B) SK-BR-3 or MCF7 breast cancer cells were seeded in 24-well plates at 1×10^5 cells/well. After 24 hours, media was removed, washed in PBS, and treated with DMSO or TCH-5c (5μM) for an additional 24 hours in serum-free DMEM. Then, conditioned media (DMSO-CM and TCH-5c-CM) were collected, respectively. VEGF concentration in conditioned media was analyzed by ELISA using human VEGF ELISA kit (Neobioscience Technology, China). Absorbance was measured at 450nm. *, p<0.05 vs. DMSO-CM group. (C) SK-BR-3 or MCF7 breast cancer cells were pre-treated with DMSO or TCH-5c (5μM) and conditioned media were collected and used to stimulate EA.hy926 endothelial cells for 24 hours. Subsequently, pretreated EA.hy926 cells were seeded on 24-well plates (2.5×10^3 cells/well) precoated with Matrigel for 6 hours. Representative photographs of tube formation are shown (magnification ×100). Tube length was quantitated using Image-Pro Plus software (n=5 per group). *, p<0.05 vs. DMSO-CM group.
Fig. (5). TCH-5c suppresses SK-BR-3 breast cancer cell-induced tumorigenicity in vivo. (A) SK-BR-3 breast cancer cells were subcutaneously injected into the right mammary fat pad of nude mice, TCH-5c was administered orally from the next day to 2 weeks after injection. Weight of tumor bearing mice was monitored every two days after subcutaneous injection. (B) Tumor volume was monitored every two days after subcutaneous injection, and tumor growth curve is shown. Tumor volume was calculated by the formula: \( V = \frac{1}{2} \times a \times b^2 \). A two-way ANOVA and Bonferroni post-tests were done for the growth curve. * \( p < 0.05 \) vs. DMSO group. (C) Mice were sacrificed, photographed, and tumors were harvested from mice 14 days after subcutaneous injection. (D) Volume of tumors were measured by the formula: \( V = \frac{1}{2} \times a \times b^2 \). *, \( p < 0.05 \) vs. DMSO group. (E) Tumor weights were measured. *, \( p < 0.05 \) vs. DMSO group. (F) VEGF concentration in mouse serum was analyzed by ELISA using VEGF ELISA kit. Absorbance was measured at 450 nm. *, \( p < 0.05 \) vs. DMSO group. (G) Xenograft tumors were fixed and sliced. IHC staining was performed using anti-VEGF, anti-p21 and anti-CD31 antibodies. Magnification 100×.

F-actin detection. DMSO treated EA.hy926 cells showed actin-rich protrusions. Compared with DMSO-treated cells, treatment of 2.5 \( \mu \)M of TCH-5c in EA.hy926 cells resulted in a strong reduction in microtubule extension to the cell periphery (Fig. 3A), indicating that TCH-5c affects the mobility of EA.hy926 cells. Subsequently, wound healing assay and transwell migration assay showed that TCH-5c administration significantly inhibited migrating ability of EA.hy926 cells (Fig. 3B and 3C). Collectively, these results suggest that TCH-5c treatment changes cytoskeleton organization and suppresses migration in EA.hy926 endothelial cells. To further investi-
gate the effect of TCH-5c administration on tube forming ability of endothelial cells, EA.hy926 cells were pre-treated with compound TCH-5c for 24 hours and, subsequently, plated on Matrigel for 6 hours. Tube formation were evaluated by measuring tube length in each group. As shown in Fig. (3D), treatment with compound TCH-5c significantly decreased EA.hy926 cell tube formation in vitro.

3.4. Compound TCH-5c Inhibits Both Breast Cancer Cell Proliferation and Breast Cancer Cell-Induced Angiogenesis

Angiogenesis is one of the most important processes during tumor growth and metastasis. Secretion of pro-angiogenesis factor by tumor cells and interaction between endothelial cells and tumor cells play vital roles in angiogenesis. We first investigated whether the three TCHs (TCH-10b, TCH-5a and TCH-5c) have an effect on proliferation in breast cancer cells. Human breast cancer SK-BR-3 and MCF7 cells were treated with 0, 5, 10, 25μM of TCHs (TCH-10b, TCH-5a and TCH-5c) for 48 hours. Cell proliferation was measured afterwards. Proliferating activity significantly reduced in 5, 10 and 25μM of TCH-5c treated SK-BR3 and MCF7 cells (Fig. 4A). Secretion of VEGF from TCH-5c-treated breast cancer cell SK-BR-3 and MCF7 was measured to determine the roles of TCH-5c in anti-angiogenesis signaling in breast cancer cells. TCH-5c treatment down-regulated VEGF secretion into cell media significantly in SK-BR-3 and MCF7 cells (Fig. 4A). Next, the conditioned media DMSO-CM and TCH-5c-CM, collected from SK-BR-3 or MCF7 cell cultures were used to treat EA.hy926 cells for 24 hours. The cells were then used for tube formation analysis. Tube length of EA.hy926 cells cultured in the condition media collected from TCH-5c treated SK-BR-3 and MCF7 breast cancer cells was significantly shorter than those in the control cells (Fig. 4C). In essence, these results suggest that compound TCH-5c inhibits breast cancer cell proliferation and suppresses breast cancer cell-induced angiogenesis in vitro.

3.5. TCH-5c Inhibits Breast Cancer Tumorigenicity In Vivo

According to the proliferation and VEGF secretion assays (Fig. 4A and B), SK-BR-3 cells responded well to TCH-5c treatment. To further study the anti-tumorigenic role of compound TCH-5c, SK-BR-3 xenograft model in nude mice was established, which is a model with deficient immune response and incompletely eliminated inflammatory rejection [15]. SK-BR-3 breast cancer cells were injected into the right mammary fat pad of nude mice by subcutaneous injection. TCH-5c was administered by oral gavage daily from the next day to 2 weeks after cell injection. Tumor growth was carefully monitored and size of tumor formation was measured every two days. The weight of tumor bearing mice and tumor growth curve are shown in Fig. (5A and 5B). Our data show that the speed of tumorigenic growth was much slower in TCH-5c-treated group than the control group (Fig. 5B), while there was no significant difference in general status and weight of tumor-bearing mice between the two groups (Fig. 5A). Animals were sacrificed at day 14 and xenograft tumors were harvested, photographed and weighed (Fig. 5C). Tumor volumes and weight from TCH-5c-treated group were significantly smaller than in the DMSO-treated group (Fig. 5D and 5E). Serum VEGF levels were also tested in DMSO or TCH-5c-treated mouse (Fig. 5F). Results indicated that serum VEGF levels from TCH-5c-treated group were significantly lower than in the DMSO-treated group. Moreover, IHC staining was performed to investigate VEGF, p21 and CD31 expression in xenograft tumors. The results showed that TCH-5c-treatment leading to a lower VEGF level and higher p21 level in xenograft tumors (Fig. 5G). CD31 staining showed limited angiogenesis in TCH-5c-treated group compared with the control group (Fig. 5G). Taken together, these results demonstrate the anti-tumorigenicity and anti-

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Fig. (6). Schematic model describing TCH-5c-mediated inhibition of angiogenesis and tumor progression in breast cancer.
angiogenesis effects of compound TCH-5c in SK-BR-3 breast cancer-bearing nude mice.

4. DISCUSSION

A large number of studies have suggested that tumors induce angiogenesis from the preexisting vasculature which is necessary for solid tumor growth [16, 17]. In our present study, several novel TCHs possessing different numbers of amino side chains were reported to have anti-proliferation activity in cancer cell lines [13]. In the present study, we sought to study the effects of three compounds TCH-10b, TCH-5a and TCH-5c in breast cancer angiogenesis and tumor growth. According to the results, TCH-5c exhibits high anti-proliferation activity and low cytotoxicity in EA.hy926 endothelial cells. Furthermore, TCH-5c demonstrated the ability to disrupt cytoskeleton organization and inhibit migration in EA.hy926 cells. Moreover, endothelial cell proliferation, motility and morphology were observed to critically affect angiogenic tube formation [18]. Next, we performed in vitro tube formation assay in the present study and further demonstrated the anti-angiogenic tube formation function of TCH-5c in EA.hy926 cells. We also found that TCH5c increased cyclin-dependent kinase inhibitor p21 protein expression and decreased cell cycle-promoting factor cyclin B1 level to induce cell cycle arrest and inhibit proliferation in EA.hy926 endothelial cells.

Among the factors influencing the functions of endothelial cells, VEGF is a predominant activator of endothelial cell functions in new blood vessel formation (angiogenesis) during development [19, 20]. Published studies indicate that breast cancer cells can secrete VEGF to promote new blood vessel growth [21]. In order to identify the role of TCH-5c in anti-angiogenic signaling in breast cancer cells, VEGF production and secretion in TCH-5c treated breast cancer cells were examined. Our results indicated that TCH-5c suppressed VEGF secretion from both SK-BR-3 ER-negative cells and MCF-7 ER-positive cells. Angiogenesis is a multistep process which plays an essential role in tumorigenic progression. Previous data have shown that tumors induce sprouting from the surrounding vasculature. We focused on the function of TCH-5c in mammary tumorigenesis in the present study and demonstrated that TCH-5c decreased breast cancer cell line derived VEGF secretion, leading to direct inhibition of breast cancer cell-induced endothelial cell tube formation and migration in vitro and profound suppression of SK-BR-3 breast cancer cell-initiated tumor formation in vivo. In the nude mouse cancer model, we also found that TCH-5c treatment reduced VEGF level in mouse serum, implying that TCH-5c inhibits tumor progression by decreasing VEGF induced angiogenesis. The combined inhibitory effects of TCH-5c on vascular endothelial cells and breast cancer cells are the inhibition of angiogenesis and eventual regression of tumor due to lack of blood supply (Fig. 6). Our in vivo experimental results have offered strong evidence that TCH-5c works in the complicated microenvironment in vitro to reduce tumor size and retard tumor progression.

CONCLUSION

Vascular endothelial cells are major components of the vascular wall and play vital roles in vascular formation, remodeling, and angiogenesis [14, 19, 22]. Vascular endothelial cell and tumor cells play vital roles in the tumor microenvironment and cancer progression. Coumarins exhibit a wide range of pharmacological activities, which include anti-proliferative, anti-microbial, anticancer, anti-inflammatory, anti-oxidant, anti-convulsant, and antihypertensive activities [8, 23, 24]. Here, we have demonstrated the novel roles of compound TCH-5c in inhibiting breast cancer cell-derived angiogenesis and tumorigenic progression in vitro and in vivo by inhibiting breast cancer cell proliferation, VEGF secretion and endothelial cell tube formation. Our study has potential implications for new approaches to design therapies directed against breast cancer angiogenesis.

LIST OF ABBREVIATIONS

TCH = Triphenylethylene-Coumarin Hybrids
NMR = Nuclear Magnetic Resonance
MS = Mass Spectrometry
PI = Propidium Iodide
DAPI = 4',6-Diamidino-2-Phenylindole
VEGF = Vascular Endothelial Growth Factor

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal experiments were approved by the Animal Research Ethics Committee of the Hebei University, China

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. The procedures of experiments involving animals were in accordance with the standards set forth in the eighth edition of “Guide for the Care and Use of Laboratory Animals”.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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