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Research article

Asiaticoside inhibits breast cancer progression and tumor angiogenesis via YAP1/VEGFA signal pathway

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ABSTRACT

Objective: Breast cancer poses a major health risk to millions of females globally. Asiaticoside (AC) is a naturally occurring compound derived from *Centella asiatica*, a widely used medicinal plant in the oriental countries and has potential antitumor properties. The primary aim of this study was to investigate the anti-cancer effects of synthesized AC at the cellular level and assess its ability to inhibit tumor growth and angiogenesis in breast cancer.
Methods: The proliferative capacities of MCF-7 and MDA-MB-231 cells were determined using CCK-8 assay. To analyze invasion and migration, Transwell assays were conducted on the same cell lines. Additionally, apoptosis was analyzed *in vitro* using flow cytometry. Real-time RT-PCR was used to examine mRNA expression, and Western-blotting assay was employed to examine protein expression. Subcutaneous injection of MDA-MB-231 cells into female BALB/c nude mice was followed by treatment with AC to study its anti-tumor effects *in vivo*.
Results: AC treatment reduced cell proliferation and triggered apoptosis in MCF-7 and MDA-MB-231 cells. The invasive and pro-angiogenesis ability were also impaired upon AC treatment. AC administration also impeded the tumor growth and tumor-associated angiogenesis of MDA-MB-231 cells in nude mice, which was accompanied by the decreased levels of YAP1 and VEGFA.
Conclusion: Taken together, our results demonstrated the anti-cancer activity of AC in breast cancer. AC is able to suppress the malignancy of breast cancercells via YAP1/VEGFA signal pathway.

1. Introduction

Breast cancer is the leading cause of mortality worldwide and is a significant contributor to cancer-related fatalities in women, with a rising morbidity rate annually [1]. The process of angiogenesis plays a crucial role in the progression and spread of breast cancer [2].

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Therapies targeting angiogenesis have demonstrated positive outcomes in the treatment of numerous types of cancers [3]. Despite the advancement, these therapeutic choices often lead to overall toxicity, resistance to treatment, and the development of a population of drug-resistant cancer stem cells [4]. There remains a significant demand for effective anti-angiogenic medications for breast cancer treatment without adverse effects.

Investigations into the natural compounds present in plants have yielded promising findings for the development of novel pharmaceuticals for various targets, including anti-cancer and anti-angiogenic activities [5,6]. For instance, rutin is a plant pigment present in certain fruits and vegetables, which was reported to inhibit breast cancer cell growth *in vitro* and *in vivo* by blocking the signaling from epidermal growth factor receptor [7]. Bioactive compound curcumin was found to suppress the tumor growth of MDA-MB-231 cells in the nude mice through down-regulating cell cycle related genes and inducing apoptosis [8]. Thymoquinone, a natural compound purified from *Nigella sativa,* could reduce chemokine receptor type 4 (CXCR4) expression and hinder the metastasis of breast cancer cells [9]. Moreover, many natural products were found to synergize with existing treatment to overcome drug resistance [10]. In such a case, tetrandrine (an alkaloid derived from *Stephania tetrandra*) sensitizes drug-resistant breast cancer cells to the chemotherapeutic agent paclitaxel [11]. Thus, natural compounds from medicinal plants are valuable resources for discovering potential drug repurposing.

Asiaticoside (AC) is a phytomolecule isolated from *Centella asiatica*, appearing as white needle-shaped crystals [12]. This compound has been demonstrated with broad-spectrum anti-tumor activities. AC has been reported to possess anti-proliferation effect in colon cancer [13]. Furthermore, AC treatment could reverse chemo-resistance and promote apoptotic cell death in hepatocellular Carcinoma [14]. In multiple myeloma, AC also exhibits anti-cancer effect through autophagy induction [15]. Of note, previous evidence indicated that AC could induce tumoricidal effect in breast cancer cells through apoptosis induction [16]. Nevertheless, the molecular mechanism underlying the anti-tumor activity of AC in breast cancer is unclear. The potential impact of AC on tumor-associated angiogenesis has not yet been explored. In this study, we investigated the anti-cancer activity of synthesized AC on breast cancer cell lines and assessed its ability to inhibit tumor growth and angiogenesis *in vivo*. Our data demonstrated the tumoricidal effect of AC and the inhibition on tumor-associated angiogenesis in breast cancer, which is likely to be linked to its ability to repress YAP1/VEGFA signal pathway.

2. Materials and methods

2.1. Cell culture and treatment

MCF-7 and MDA-MB-231 human breast cancer cells were sourced from the American Type Culture Collection (MA, USA) and cultured in Dulbecco's modified Eagle's medium (Gibco, CA, USA) that was supplemented with 10 % fetal bovine serum (FBS) and penicillin/streptomycin (100 U/mL) at 37 °C in 95 % humidified environment with 5 % CO^2. The aforementioned cell lines (5×10^5/mL) were seeded into six-well plates and allowed to incubate for 24 h before the treatment with AC at varying concentrations (0.1, 0.5, or 2 μM, MedChemExpress, Shanghai, China) or 1 % DMSO (control). After 48 h, the cells in the medium were collected as the cancer-conditioned medium, with the adherent cells being detached by 0.05 % trypsin and collected by centrifugation at 1000 rpm for 5 min for further analysis. IBS003031 (a YAP1 agonist, 10 μM, ProbeChem, CA, USA) and recombinant VEGFA (20 ng/ml, Sino Biological, Shanghai, China) were applied together with AC for 48 h to examine the effect of YAP1/VEGFA signal upon AC treatment.

2.2. Colony formation

Briefly, 1×10^3 cells were plated in the 6-cm culture dish and cultured to form colonies for a period of two weeks. Following PBS rinsing, cells were fixed in 4 % paraformaldehyde and stained for 15 min with 0.4 % crystal violet. The number of colonies containing more than 10 cells was quantified, and the values from replicated wells were averaged.

2.3. Cell proliferation, apoptosis and cell cycle analysis

The growth ability of cells was assessed using a cell counting kit-8 (Beyotime, Beijing, China) according to specific protocols. Cells treated with various experimental conditions and the control samples were seeded in 96-well plates with complete medium (100 μl) for different time intervals (Corning, NY, USA). Following this, 20 μL of CCK-8 solution was added to each well and the plates were further incubated for 2 h at 37 °C before measuring the absorbance at 450 nm using a microplate reader. For apoptosis analysis, an Annexin V-FITC/PI apoptosis detection kit (BD Biosciences, CA, USA) was utilized to stain both the control and AC-treated MCF-7 and MDA-MB-231 cells. The cells underwent a 15-min incubation at room temperature in the dark, and the apoptotic events was immediately quantified using the BD FACSCalibur™ Flow Cytometer (BD Biosciences). The DNA content detection kit (Soleibao, Beijing, China) was used to detect cell cycle distribution based on the manufacturer's instructions.

2.4. Transwell assays

After the treatment with either DMSO (control) or AC, the dead cells in the culture medium were discarded, and the collected live cells were quantified using trypan blue staining (Beyotime). The cells were harvested in suspension with serum-free medium and a total of 5×10^4 live cells in 200 μl medium were seeded into each well of the top Transwell chambers (pore size, 8 μm; BD Biosciences) with or without Matrigel coating (BD Biosciences). The chamber coated with matrigel was used for invasion assay. Then, 600 μL of

complete medium containing 10 % fetal bovine serum was added to the bottom chambers. Following 24-h incubation periods at 37 °C with 5 % CO_2 for migration and invasion assays respectively, cells on the lower surface of transwell membrane were fixed with 4 % paraformaldehyde for 30 min and stained for an additional 15 min with 0.1 % crystal violet. Subsequently, the migrating and invading cell numbers were counted udner a light microscope at × 200 magnification (Olympus Corporation).

2.5. Caspase-3 activity determination

The caspase-3 activity colorimetric assay (ab39401, Abcam, Cambridge, UK) was used in this study to assess caspase-3 activity according to specified procedures. To create each reaction well, 50 μL of sample was added in each sample well and 50 μL of 2x Reaction Buffer was added into the background wells. Subsequently, each sample received 50 μL of 2x Reaction Buffer containing 10 mM DTT, followed by the addition of 5 μL of the 4 mM DEVD-p-NA substrate. The resulting mixture was then incubated at 37 °C for 60 min. Absorbance at OD 400 nm was measured using a microplate reader.

2.6. Western blot

The protein extract was quantified using the bicinchoninic acid kit (Thermo Fisher Scientific, CA, USA) following specific instructions. Next, protein separation was achieved through SDS-PAGE electrophoresis, followed by transfer onto polyvinylidene fluoride membranes (Millipore, MA, USA). Subsequently, the membranes were blocked with 5 % BSA in TBST buffer, and then incubated overnight at 4 °C with primary antibodies against anti-cleaved caspase 3 (1: 1400, ab2302, Abcam), anti-pan caspase 3 (1:1000, ab90437, Abcam), anti-YAP1 (1: 1500, ab244980, Abcam), anti-VEGFA (1: 1200, ab39250, Abcam), and anti-GAPDH (1: 2000, ab245355, Abcam). After rinsing with TBST buffer for 10 min, the membranes were subjected to a 2-h incubation with a horseradish peroxidase (HRP)-labeled secondary antibody (1: 5000, ab205718, Abcam) at room temperature. Following three washes with TBST buffer, the Chemi DocXRS + Imaging System from Bio-Rad, CA, USA, was used for signal detection.

2.7. Tube formation assay

Human umbilical vein endothelial cells (HUVECs) (4×10^3/well) were seeded onto 96-well plates coated with 50 μL/well Matrigel (60 μL, BD Bioscience). HUVECs were maintained in Dulbecco's modified Eagle's Medium supplemented with 10 % fetal bovine serum at 37 °C for 8 h. One culture condition with the addition of tumor-condition medium (1: 1 in volume) was included to assess the impact on the tube formation. After incubation for 12 h the cell culture was washed with PBS, and the formation of capillary-like structures was observed under a microscope at × 200 magnification, followed by measurement of branch length using the Image J software (NIH, MA, USA).

2.8. Quantitative real-time polymerase chain reaction (RT-PCR)

The mRNA levels of YAP1 and VEGFA were examined in MCF-7 and MDA-MB-231 cells under varying conditions by real-time RT-PCR. Specifically, total cellular RNA extraction was carried out utilizing the RNeasy RNA Isolation Kit (QIAGEN, Germany), followed by RNA purity evaluation via spectrophotometry. Subsequently, reverse transcription into complementary DNA (cDNA) was conducted using the PrimeScript II 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) with 10 ng of total RNA from each sample. qRT-PCR analysis was completed with SYBR Green PCR Master Mix (Thermo Fisher Scientific) utilizing primers specific to YAP1 and VEGFA. The quantification of their expression levels was determined employing the $2-\Delta\Delta Ct$ method, while GAPDH was employed as an internal control. Primer sequences were as follows: 5′- CGCTCTTCAACGCCGTCA-3′ (F), 5′-AGTACTGGCCTGTCGGGAGT-3′ (R) for YAP1; 5′- AGGGCAGAATCATCACGAAGT-3′ (forward [F]), 5′- AGGGTCTCGATTGGATGGCA-3′ (reverse [R]) for VEGFA; and GAPDH: 5′-GGAGCGAGATCCCTCCAAAAT-3′ (F), 5′-GGCTGTTGTCATACTTCTCATGG -3′ (R).

2.9. Xenograft mouse model of breast cancer

The female BALB/c nude mice (weighing 13–15 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. and housed in a pathogen-free environment (20–26 °C, 40–70 % humidity, and a 12-h light/dark cycle). The animals were allowed with free access to water and food. Our experimental procedures were approved by the Animal Ethics Committee at Animal Ethics Committee of General Hospital of Eastern Theater Command (IACUC-2020031703). A xenograft animal model was created by subcutaneously injecting MDA-MB-231 cells (2×10^6 cells per animal) into the nude mice. Once the tumors reached a 200 mm^3, the mice were randomly assigned to either the control group or the AC treatment group (each group containing $n = 6$ mice). The mice were treated with intraperitoneal injection of either the vehicle (control) or AC (10 mg/kg body weight, administered thrice a week for 3 weeks). Tumor volume was measured using a vernier caliper with the formula: Tumor volume V (mm3) = $\pi/6$ (Length) × $(\text{Width})^2$. The mice were euthanized by CO_2 inhalation and cervical dislocation after 4 weeks of drug intervention, followed by the tumor tissue excision, and immunohistochemical staining with anti-Ki-67 (1:500, ab15580, Abcam) or anti-CD31 (1: 600, ab212712, Abcam) antibody.

2.10. Statistical analysis

SPSS26.0 (IBM, MA, USA) was employed for statistical analysis. Data were displayed in the format of mean ± standard deviation

(SD). Student's t-test was performed to compare the data from two groups, while one-way or two-way ANOVA plus Bonferoni multiple comparisons test was carried out for comparing multiple groups. Significance levels were set at $p < 0.05$, 0.01, and 0.001.

3. Results

3.1. The impact of AC on the proliferation, migration, and invasion of MCF-7 and MDA-MB-231 cells

To assess the potential anti-cancer effects of AC on human breast cancer, we investigated its impact on the growth, migration, and invasion of MCF7 and MDA-MB-231 cells. After 72 h of treatment, we observed that cell proliferation of both cell lines showed a dose-dependent decrease with the increasing concentrations of AC (0.1, 0.5, or 2 μM) (Fig. 1A). Furthermore, the number of colonies formed in each group of AC treatment showed a substantial reduction compared to the control (Fig. 1B). Through the Transwell assays we also

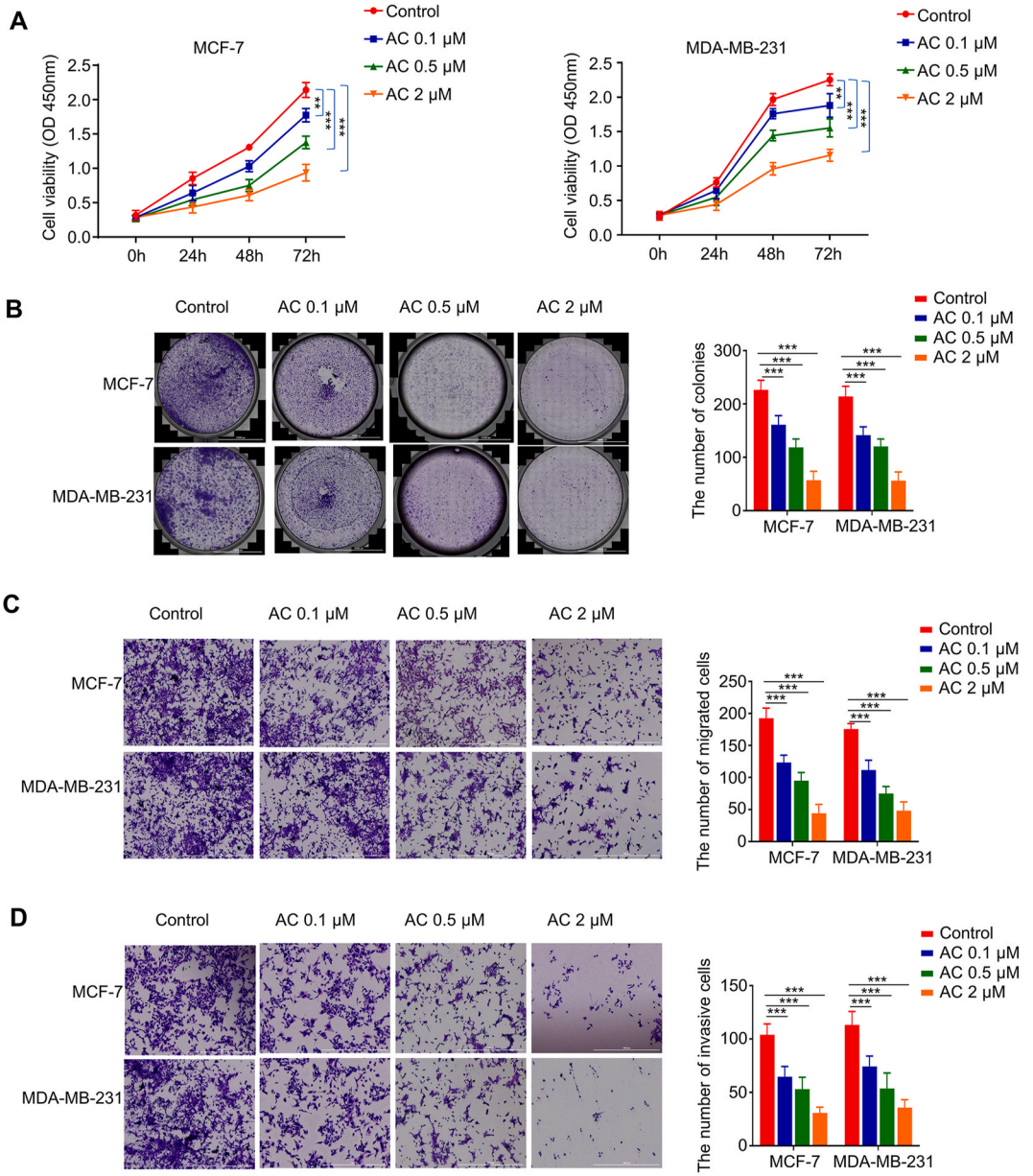

Fig. 1. Effects of AC on proliferation, migration, and invasion of MCF-7 and MDA-MB-231 cells. (A) Cell growth assay in MCF-7 and MDA-MB-231 cells treated with different doses of AC (0, 0.1, 0.5, or 2 μM). (B) Colony formation in MCF-7 and MDA-MB-231 cells treated with different doses of AC (0, 0.1, 0.5, or 2 μM). (C–D) Transwell migration assay (24 h) in MCF-7 and MDA-MB-231 cells treated with different doses of AC (0, 0.1, 0.5, or 2 μM). N = 3 independent experiments. Statistics: A: two-way ANOVA; B–D: one-way ANOVA. $^{**}p < 0.01$, $^{***}p < 0.001$.

observed a dose-dependent reduction in the number of cells that migrated and invaded in response to AC treatment, indicating the ability of AC to impede migration and invasion in breast cancer cells (Fig. 1C and D).

3.2. The pro-apoptotic effects of AC in breast cancer cells

To assess the impact of AC treatment on cell death, we first measured intracellular caspase-3 activity in control and AC (2 μM) treated MCF-7 and MDA-MB-231 cells. There was a significant elevation of caspase 3 activity upon AC treatment (Fig. 2A). Consistently, flow cytometric analysis revealed an increased number of apoptotic cells in the AC-treated group compared to the control (Fig. 2B). We also showed the increased levels of cleaved caspase 3 and Bax after AC treatment, while the levels of anti-apoptotic protein Bcl-2 were decreased (Fig. 2C, Fig. S1). Furthermore, the analysis of cell cycle distribution suggested the arrest of MCF-7 and MDA-MB-231 cells in G2/M phase after AC treatment (Fig. 2D). Collectively, our data demonstrate the antiproliferative and pro-apoptotic effect of AC in breast cancer cells.

3.3. AC treatment impairs the pro-angiogenic capacity of tumor cells on HUVECs

Next, the culture supernatants of control- and AC (2 μM)-treated MCF-7 and MDA-MB-231 cells as the conditioned medium to cultivate HUVECs. CCK8 cell growth assay showed that the conditioned medium from AC (1 and 2 μM)-treated MCF-7 and MDA-MB-231 cells decreased cell proliferation in HUVECs, compared to the medium from control group of tumor cells (Fig. 3A). There was also

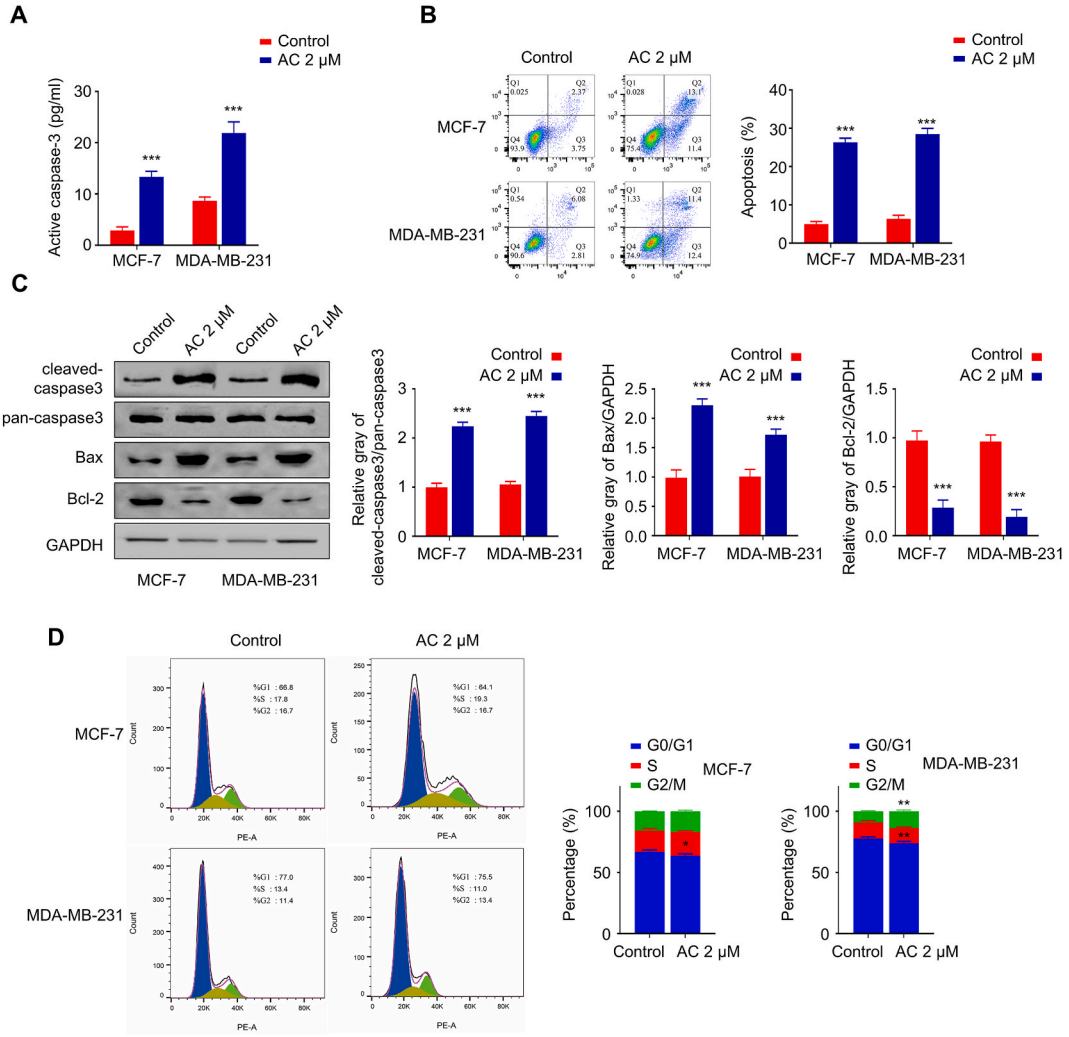

Fig. 2. The pro-apoptotic effects of AC in breast cancer cells. (A) The caspase-3 activity measurement in control (DMSO) and AC-treated cells. (B) Flow cytometry analysis of apoptotic events in control and AC-treated cells. (C) Western blots of caspase 3 cleavage, Bax and Bcl-2 in control and AC-treated cells. (D) Cell cycle analysis in control and AC-treated cells. N = 3 independent experiments. Cells were treated with chemicals for 48 h. Statistics: student's *t*-test. $^{**}p < 0.01$, $^{***}p < 0.001$.

an impaired tube formation (manifested as the reduced branching length of formed tubes) in HUVECs after the culturing with the conditioned medium from AC (1 and 2 μM)-treated cells (Fig. 3B). Transwell assay showed that the culturing with the conditioned medium from AC (1 and 2 μM) suppressed the migration of HUVESC (Fig. 3C). However, the direct treatment with AC did not affect the proliferation, tube formation and migration of HUVECs (Fig. S2). These results, overall, AC treatment impairs the pro-angiogenic capacity of tumor cells on HUVECs.

3.4. AC regulates the YAP1/VEGFA signaling axis within MCF-7 and MDA-MB-231 cells

We next investigated the effect of AC on yes-associated protein 1 (YAP1) and vascular endothelial growth factor A (VEGFA) in MCF-7 and MDA-MB-231 cells. YAP1 is a transcription factor which is involved in the development, metastasis, and poor survival of breast cancer patients, and VEGFA is a key pro-angiogenic factor in cancer metastasis [17,18]. RT-qPCR and Western blot analysis demonstrated that AC treatment could reduce YAP1 and VEGFA expression in MCF-7 and MDA-MB-231 cells (Fig. 4A and B, Fig. S3). We next included IBS003031 (a YAP1 agonist) and recombinant VEGFA (rVEGFA) in the AC treatment group to examine whether the activation of YAP1 and VEGFA signaling could show rescue effect. Both IBS003031 and rVEGFA could promote the cell growth, and augment cell invasion and migration in MCF-7 and MDA-MB-231 cells with AC treatment (Fig. 4C–E). Besides, the activation of YAP1 and VEGFA signaling also suppressed apoptosis induced by AC treatment (Fig. 4F). Thus, AC treatment suppresses the malignancy of MCF-7 and MDA-MB-231 cells through YAP1/VEGFA signaling axis.

3.5. AC inhibits tumorigenesis and angiogenesis in nude mice by dampening the YAP1/VEGFA signaling axis

To further corroborate the anti-cancer effect of AC on tumorigenesis as well as angiogenesis of breast cancer cells, nude mice were

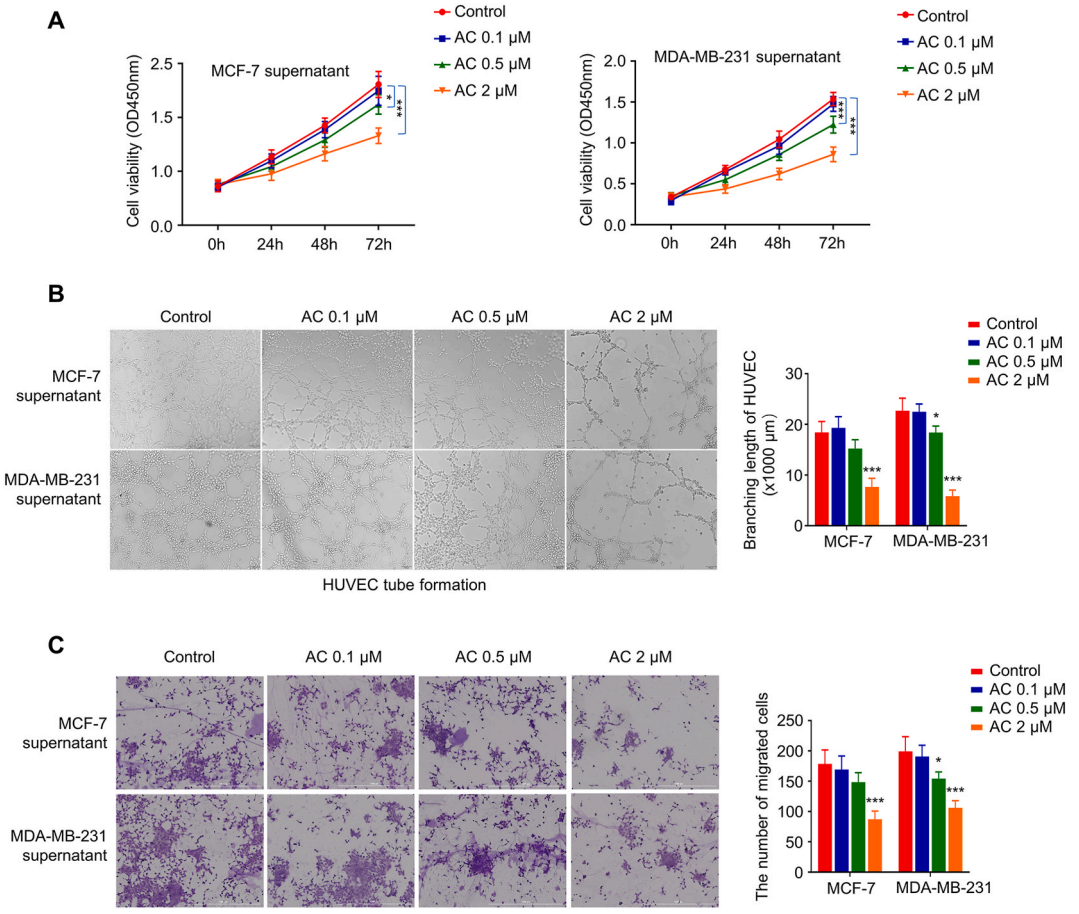

Fig. 3. AC treatment impairs the pro-angiogenic capacity of tumor cells on HUVECs. The culture supernatants of control- and AC (0.1, 0.5, 2 μM, 48 h)-treated breast cancer cells were collected for incubating HUVEC cells. (A) CCK8 assay shows a decreased cell proliferation in AC-conditioned medium-treated HUVECs. (B) Tube formation assay (12 h-incubation) reveals a decrease in the branching length of formed tubes in AC-conditioned medium-treated HUVECs. (C) Transwell assay (24-h incubation) shows the impaired cell migration in AC-conditioned medium-treated HUVECs. N = 3 independent experiments. Statistics: A: two-way ANOVA; B–C: one-way ANOVA. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

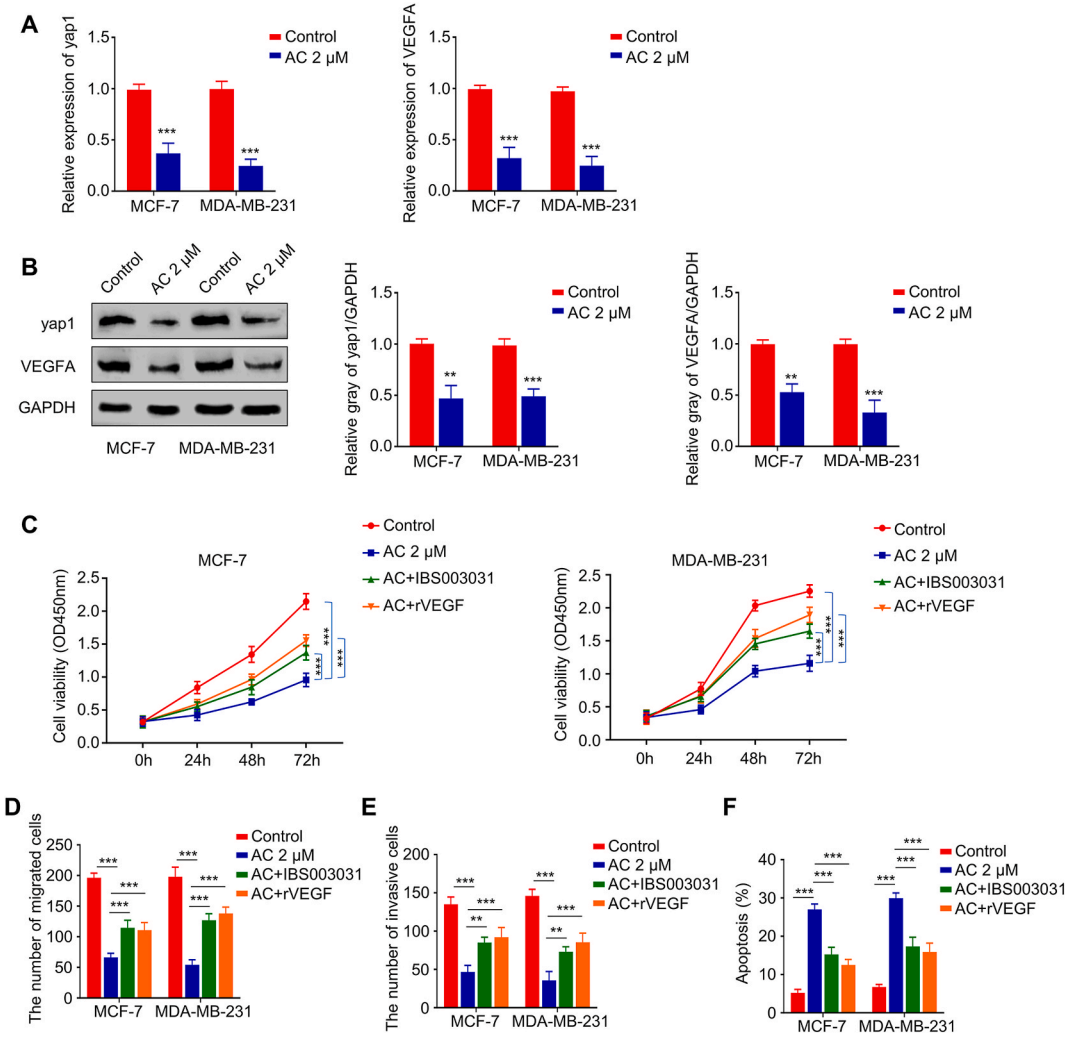

Fig. 4. AC regulates the YAP1/VEGFA signaling axis within breast cancer cells. (A) AC (2 μM) treatment decreases YAP1 and VEGFA mRNA level, as detected by RT-qPCR. (B) Western blots shows that AC treatment decreases the protein levels of YAP1 and VEGFA. (C–F) MCF-7 and MDA-MB-231 cells were treated with AC (2 μM), AC + IBS003031 (a YAP1 agonist, 10 μM) and AC + recombinant VEGFA (20 ng/ml). (C) Cell proliferation analysis by CCK-8 assay; (D) Transwell migration assay; (E) Transwell invasion assay; and (F) Apoptosis detection in above experimental groups. N = 3 independent experiments. Cells were treated with chemicals for 48 h. Transwell invasion and migration assay were performed for 24 h using the cells after chemical treatment. Statistics: A–B: student's *t*-test; C: two-way ANOVA; D–F: one-way ANOVA. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

injected with MDA-MB-231 cells (Fig. 5A). After AC (10 mg/kg) treatment, the tumor growth and weight were largely reduced (Fig. 5B and C). The mRNA and protein expressions of Yap1 and VEGFA were significantly diminished by AC administration in the tumor samples (Fig. 5D–E, Fig. S4). Notably, the expression of Ki-67 (proliferation marker) and CD31 (endothelial marker) in the AC treatment group were dramatically reduced in comparison with control group, indicating that AC treatment inhibits tumorigenesis and angiogenesis (Fig. 5F).

4. Discussion

Breast cancer shows a greater reliance on angiogenesis compared to other forms of cancer [19]. The targeting of angiogenesis presents a potential avenue for treating breast cancer [20]. Various plant-derived compounds displaying potential anticancer properties have been identified, presenting valuable leads for drug development [21], AC, extracted from *Centella asiatica* - a plant traditionally used for medicinal purpose, has shown promise in the management of cancer [13–16]. This research illustrated the ability of AC to reduce cell proliferation, promote apoptosis, and hinder angiogenesis of breast cancer cells *in vitro* and *in vivo*.

Neo-angiogenesis is essential for the continuous development and the spread of breast cancer [19,20]. Here,we showed that AC could suppress the pro-angiogenic effect of breast cancer cell conditioned medium on HUVECs. This effect may be due to the

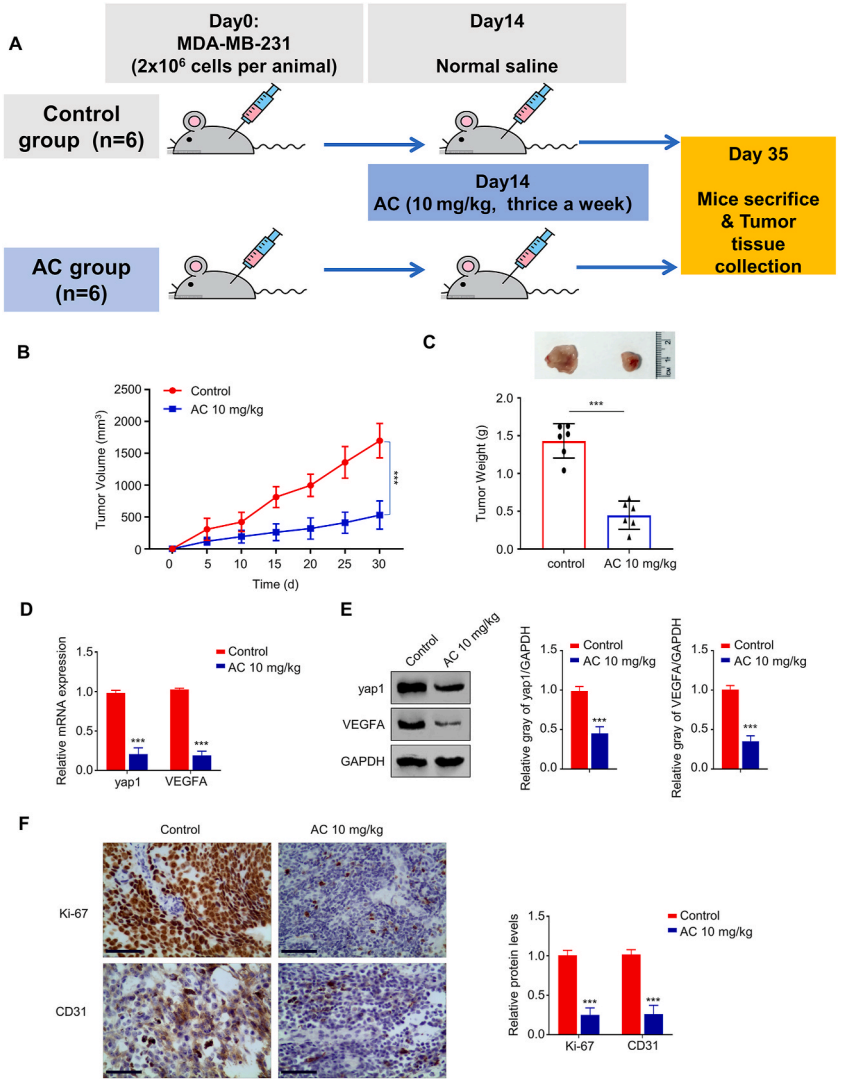

Fig. 5. AC inhibits tumorigenesis and angiogenesis in nude mice by dampening the YAP1/VEGFA signaling axis. (A) The schematics of animal experimental protocol. (B) Tumor volume (mm^3) in the control and AC treatment groups. (C) The mean tumor weight (mg) in the control and AC treatment groups. (D) RT-qPCR analysis of YAP1 and VEGFA in the tumor tissues of control and AC treatment groups. (E) Western blots detection of YAP1 and VEGFA in the control and AC treatment groups. (F) Ki-67 and CD31 immunostaining in the control and AC treatment groups. N = 6 animals in each group. Statistics: A: two-way ANOVA; B–F: student's *t*-test.***$p < 0.001$.

observation that AC treatment reduced VEGFA expression in breast cancer cells. VEGFA is a key pro-angiogenic factor and its expression level is linked to the poor prognosis in different cancer patients [22–24]. Our data suggest that AC could target VEGFA-dependent angiogenesis in breast cancer. In consistence with our finding, a previous study showed that AC treatment suppresses angiogenesis in thyroid cancer cells by inhibiting hypoxia-inducible factor 1 [25].

We further showed that AC treatment impaired the malignant features of breast cancer cells including cell growth, migration and invasion. In different cancer types, AC seems to excert anti-proliferation effect through targeting different signaling pathway. AC was reported to inhibit cell proliferation of colon cancer cells through dampening the NF-κB signaling [13]. In gastric cancer, AC can induce endoplasmic reticulum stress to arrest cell growth. This effect was demonstrated to be mediated by the up-regulation of miR-635 and the down-regulation of high mobility group AT-Hook 1 (HMGA1) [26]. Of note, AC treatment could also sensitize drug-resistant cancer cells to chemotherapeutic agents [14,15,27]. Therefore, further study should be conducted to investigate whether AC could synergize with existing chemotherapy to boost the tumoricidal effect.

YAP1 is the canonical transcription factor involved in the Hippo signaling pathway, which regulates cell proliferation and organ size [28]. In normal conditions, YAP1 is inactivated due to the activation of Hippo pathway; but in cancer cells, YAP1 signaling can become deregulated, leading to uncontrolled cell growth and tumor formation. Both mutations in the Hippo pathway and amplification of the YAP1 gene could lead to YAP1 activation [29,30]. When YAP1 become over-activated in cancer cells, it can promote cell

growth, survival, migration and invasion [31–34]. YAP1 also protect cells from apoptosis induction, and down-regulation of YAP1 could sensitize cancer cells to apoptotic cell death [35,36]. In line with these previous evidence, we showed that AC treatment suppressed cell growth and triggered apoptosis in breast cancer cells, which is accompanied by YAP1 down-regulation. The application of YAP1 agonist could reverse the effect of AC treatment. Thus, our data suggest that AC treatment may undermine the malignancy of breast cancer cells through diminishing YAP1 expression. Interestingly, YAP1 was found to promote angiogenesis in retinal microvascular endothelial cells [37], and YAP1 activation induces VEGFA expression in renal cell carcinoma [38]. Therefore, these studies and our data suggest that the anti-angiogenic effect of AC treatment may be mediated through the suppression of YAP1 signaling.

To summarize, the current research demonstrated that AC suppressed cell growth, migration, invasion, and angiogenesis in breast cancer cells. Treatment with AC also hindered tumor growth and blood vessel formation in the mouse model. Our data also highlight YAP1/VEGFA as the cellular targets of the anticancer activity of AC treatment. Future work is needed to study the potential synergistic effect of AC with existing anti-cancer therapies.

Ethics approval

Animal experimental procedure gained approval from Animal Ethics Committee of General Hospital of Eastern Theater Command (IACUC-2020031703). The experimental protocol was performed in accordance with the relevant guidelines and regulations of the Basel Declaration. The study is reported in accordance with ARRIVE guidelines (https://arriveguidelines.org).

Data availability statement

The data in the article is available upon e-mail request from the corresponding author.

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CRediT authorship contribution statement

Mengmeng Guo: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Yu Ying:** Writing – original draft, Data curation, Conceptualization. **Yun Chen:** Writing – original draft, Methodology, Data curation, Conceptualization. **Xian Miao:** Writing – original draft, Methodology. **Zhenghong Yu:** Writing – original draft, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e37169.

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