ORIGINAL PAPER

Astragaloside IV inhibits cell proliferation and metastasis of breast cancer via promoting the long noncoding RNA TRHDE-AS1

Shufang Hu[1] · Weihong Zheng[2] · Li Jin[3]

Abstract

Astragaloside IV (ASV), which has several pharmacological abilities, shows potential therapeutic effects on certain cancers by regulating the expression of long noncoding RNA (lncRNA). However, the anticancer role that ASV plays by regulating lncRNAs in breast cancer remains unknown. In this study, we first demonstrated that the lncRNA of TRHDE antisense RNA 1 (TRHDE-AS1) was downregulated in breast cancer tissues and cells. Low TRHDE-AS1 expression is associated with poor outcomes in patients with breast cancer and potentially contributes to the aggressive tumor biology of breast cancer. Furthermore, ASV significantly increased TRHDE-AS1 expression in a dose- and time-dependent manner in breast cancer cells. By upregulating TRHDE-AS1, ASV repressed breast cancer cell growth and metastasis both in vitro and in vivo. Taken together, our data indicated that TRHDE-AS1 participates in the anticancer role of ASV in breast cancer, which provides evidence for the application of ASV for breast cancer therapy.

Keywords Astragaloside IV · lncRNA · TRHDE-AS1 · Breast cancer · Proliferation

Introduction

Breast cancer is the most commonly diagnosed type of cancer among females. Globally, there are approximately 2.1 million newly diagnosed female breast cancer cases every year [1, 2]. Although major advancements have been made in surgery and chemoradiotherapy, poor prognosis, and acquired resistance to chemotherapeutic agents affect the clinical therapeutic effects. This disease is categorized into intrinsic subtypes according to the expression of its estrogen, progesterone, and HER2 receptors (ER, PR, and HER2, respectively): luminal A: [ER]+/[PR]+/[HER2]-; luminal B: [ER]+/[PR]+/[HER2]+; Her 2 phenotype: [ER]−/[PR]−/[HER2]+; and triple-negative phenotype: [ER]−/[PR]−/[HER2]- [3]. Because of the heterogeneity of cancer cells, matching a particular patient with appropriate treatment based on breast cancer biomarkers remains challenging [4]. Thus, developing new therapeutic strategies against breast cancer is particularly important.

Astragaloside IV (ASV), one of the active ingredients of the Chinese traditional herb Astragalus membranaceus, has several pharmacological activities including anticancer, immunoregulatory, antioxidant, neuroprotective, and cardio-protective abilities, etc. [5–9]. Recent studies have clarified that ASV shows potential therapeutic effects on a variety of diseases by regulating the expression of long noncoding RNA (lncRNA). Du et al. [9] found that ASV protected cardiomyocytes against hypoxia by down-regulating the lncRNA of GAS5. Li et al. [10] reported an inhibitory effect of ASV on the malignant biological behavior of hepatocellular carcinoma cells by suppressing the expression of the lncRNA of ATB. Qian et al. [11] demonstrated that ASV significantly alleviated idiopathic pulmonary fibrosis by increasing lncRNA sirt1 AS expression. Moreover, ASV has

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been verified to perform inhibitory roles in breast cancer through different pathways. Wang et al.[12] reported that ASV executed breast cancer cells through the regulation of Nrf2 via the PI3K/AKT/mTOR signaling pathway. Xue's group found that ASV inhibited breast cancer cell invasion by suppressing Vav3-mediated Rac1/MAPK signaling [13]. ASV was also shown to enhance the taxol chemosensitivity of breast cancer via caveolin-1-targeting oxidant damage [14]. However, the correlation between ASV and lncRNA expression has not yet been reported.

The lncRNA of TRHDE antisense RNA 1 (TRHDE-AS1), which is located at chromosome 12q21.1, has been reported to be aberrantly expressed in osteosarcoma samples and lung cancer tissues [15, 16]. Here, we report that TRHDE-AS1 acted as an anti-oncogene lncRNA in breast cancer. The overexpression of TRHDE-AS1 inhibited cell proliferation and cell growth and regulated the cell migration and invasion of breast cancer cells. Moreover, we demonstrated that ASV could up-regulate the expression of TRHDE-AS1 and inhibit the malignant biological behavior of breast cancer cells through TRHDE-AS1 both in vitro and in vivo.

Materials and methods

Cell culture and treatments

The human breast epithelial MCF-10A cell line and the human breast cancer cell lines MCF-7, MDA-MB-231, and MDA-MB-468 were obtained from the American Type Culture Collection (ATCC, VA, USA). The MCF-10A cells were cultured in Dulbecco's modified Eagle medium (DMEM)/F12 plus with 5% horse serum, 1% penicillin and streptomycin (Gibco, MD), 20 ng/mL EGF, 0.5 μg/mL hydrocortisone, 100 ng/mL cholera toxin, and 10 μg/mL insulin (Sigma-Aldrich, China). The MCF-7, MDA-MB-231, and MDA-MB-468 cells were cultured in DMEM plus with 10% fetal bovine serum (FBS, Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin with 5% CO_2 at 37 °C. ASV was purchased from MedChemExpressCo. Ltd. (Shanghai, China); it was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 mg/mL and then stored at − 20 °C for further dilution.

Bioinformatics analysis

Breast cancer gene expression arrays with survival data were obtained from the Cancer Genome Atlas (TCGA) dataset. We used project GEPIA (gepia.cancer-pku.cn) to compare TRHDE-AS1 expression in breast cancer tissues with that in normal breast tissues. We used R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl) to assess the

overall survival (OS) of breast cancer patients according to TRHDE-AS1 expression. OS was defined as the period from the date of the pathological diagnosis to death. We used the TANRIC website to evaluate TRHDE-AS1 mRNA levels in the different molecular subtypes of breast cancer in TCGA datasets.

Cell transfection

To construct the TRHDE-AS1 expression plasmid, the cDNA encoding TRHDE-AS1 was amplified using PCR and then subcloned into the pcDNA3.1 vector (Invitrogen, USA). The plasmids were sequenced in Genscript Co., Ltd. (Nanjing, China). Next, pcDNA3.1-TRHDE-AS1 and empty vectors were transfected into breast cancer cells using Lipofectamine 2000(Invitrogen, USA), and the cells were selected with G418 to obtain TRHDE-AS1 stably overexpressed breast cancer cell lines. siRNAs targeting TRHDE-AS1 and negative control siRNAs were synthesized by Biomics Biotechnologies Co., Ltd. (Nantong, China). The following siRNA strands were used: sense siTRHDE-1: 5′- GAUGACAAGUGAGAAAGAATT-3′, antisense siTRHDE-1:5′-UUCUUUCUCACUUGUCAU CTT;sense siTRHDE-2: 5′-GCUAAAGAAGAUGAUUAU ATT, antisense siTRHDE-2: UAUAAUCAUCUUCUUUAG CTT; sense siNC: 5′-UUCUCCGAACGUGUCACGUTT-3′, antisense siNC: 5′-ACGUGACACGUUCGGAGAATT-3′. The siRNAs were transfected into cells using Lipofectamine 2000.

Quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from the cells using TRIzol Reagent (Invitrogen) and was then used to perform reverse transcription with the HiFi-MMLV cDNA kit (Beijing ComWin Biotech Co., Ltd., Beijing, China). qRT-PCR was performed with the UltraSYBR Mixture on the StepOnePlus Real-time PCR system (Applied Biosystems, Foster City, CA, USA). The following primers were used for qRT-PCR: TRHDE-AS1 forward, 5′-TTCAACAGACTACAACCGTAC-3′ and reverse, 5′-AGGCGAACTGGTGTAATA-3′ [12]; β-actin forward, 5′-GGCACCACACCTTCTACAAT-3′ and reverse, 5′-GTGGTGGTGAAGCTGTAGCC-3′). β-actin was used as an endogenous control, and the fold-change of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method.

Western blot

Proteins from the tissues or cells were extracted usingRIPA Lysis Buffer (Beyotime Biotechnology, China) and then separated by 12% SDS-PAGE. After being transferred onto a polyvinylidene difluoride (PVDF) membrane, the membrane was blocked with 1% bovine serum albumin at 4 °C

overnight. Then, the membranes were incubated with primary antibodies for MMP-2, MMP-9 (40994, 13667, Cell Signaling Technology, USA), PCNA, and β-actin (10205–2-AP, 20536–1-AP, Proteintech group, USA) at RT for 2 h. After being washed, the membranes were further incubated with HRP-labeled secondary antibodies and then image exposure was performed.

CCK-8 assay

The cells in each group were seeded into 96-well plates at a density of 5×10^3 cells/well. After 24 h of incubation, the cells were further incubated with CCK-8 solution (Beyotime Biotechnology, China) for 2 h. A SpectraMaxM3 microplate reader (Molecular Devices, USA) was used to measure the absorbance at 450 nm.

EdU analysis

5-Ethynyl-2′-deoxyuridine (EdU) was added into the cell culture medium at a final concentration of 10 mM, and the medium was sequentially used to culture the cells for 2 h. Then, the fluorescence was observed using a Nikon ECLIPSE Ti-S microscope (Nikon, Japan).

Colony formation assay

The cells were placed into 6-well plates at a density of 500 cells/well and cultured for 14 days. Afterwards, 0.1% crystal violet (Yuanye Biological Technology, Shanghai, China) was added to the plates to stain the cell colonies. Finally, the colonies were photographed, and the number of colonies was counted manually.

Wound healing and transwell assays

For the wound healing assay, the cell layers were scratched using a 20-μL pipette tip to create wounded gaps when they reached 90% confluence in the 6-well plates; the plates were then washed twice with PBS to remove the floating cells. After 12 h, the edge of the scratch was photographed, and the results were recorded as the relative migration distance. For the transwell migration assay, a cell suspension (1×10^5 cells/mL) was seeded to the upper chamber (Corning, Corning, NY, USA) of the inserts (0.2 mL/well), and DMEM containing 20% FBS was added to the lower chamber. After culturing, the cells that migrated to the bottom of the membrane were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The invasion assay was performed in the same way as the migration assay except that the membrane was coated with matrigel (BD Biosciences, Bedford, MA, USA).

Enzyme-linked immunosorbent assay (ELISA)

The conditioned medium of cells in each group was collected. The supernatant was centrifuged at a low temperature at 12 000 rpm for 15 min, and the supernatant was collected and stored at –80 °C. The operation was performed according to the instructions of ELISA kit. The MMP-2 (EK0459, Boster Biological Technology, China) and MMP-9 (EK0465, Boster Biological Technology, China) secretion levels in each treatment group were detected.

Xenograft mouse model

The animal experiments were conducted in accordance with the protocols of the Animal Ethics Committee of Zhejiang University. BALB/c nude mice (male, 5 weeks old, 16–20 g) were purchased and maintained in the laboratory animal research center of Zhejiang University. MDA-MB-231- and TRHDE-AS1-silenced MDA-MB-231 cells were collected at a concentration of 1×10^7 cells in PBS and subcutaneously implanted into the flanks of the BALB/c nude mice. After 14 days, the mice were assigned to different groups ($n = 6$ in each group) and were treated with either DMSO or ASV (20 mg/kg) by intraperitoneal injection every 3 days. Tumor volume was calculated using the formula $V = W2 \times L \times 0.5$. The mice were killed, and the tumors were harvested for the follow-up experiments.

Tail vein metastasis model

MDA-MB-231- or TRHDE-AS1-silenced MDA-MB-231cells (4×10^5) were injected into the tail veins of the mice. Two days after tumor cell injection, the ASV treatment groups were intraperitoneally injected with ASV (20 mg/kg) every 3 days. Six weeks post injection, the mice were killed, and the lungs were removed and paraffin embedded. Consecutive Sects. (4 μm) were sliced and stained with hematoxylin and eosin.

Statistical analysis

The data are presented as the means ± standard error. Significant differences between two groups were assessed using t tests, and significant differences among multiple groups were assessed using a one-way analysis of variance. $P < 0.05$ was considered statistically significant. Survival curves were constructed with the Kaplan–Meier method.

Results

The expression analysis of TRHDE-AS1 in the online database

TCGA was employed to examine the association between TRHDE-AS1 expression levels and clinical outcomes of patients with breast cancer. The results showed that TRHDE-AS1 expression was distinctly downregulated in breast cancer tissues and that the lower expression group had a poorer OS rate (Fig. 1a and b, $P < 0.05$). We also noted that TRHDE-AS1 expression levels were downregulated in all molecular subtypes of breast cancer (basal-like, luminal A and luminal B, and HER2 overexpressed) (Fig. 1c, $P < 0.01$). Moreover, the expression of TRHDE-AS1 in ER- and PR-negative samples was lower significantly than that in ER- and PR-positive samples (Fig. 1d and e). Collectively, these data suggest that low TRHDE-AS1 expression is associated with poor outcomes in patients with breast cancer and potentially contributes to the aggressive tumor biology of breast cancer.

Overexpression of TRHDE-AS1 inhibits the proliferation and metastasis of breast cancer cells

We also compared TRHDE-AS1 expression levels in the human breast cell line MCF-10A and in the human breast cancer cell lines MCF-7, MDA-MB-231, and MDA-MB-468; we found that the TRHDE-AS1 levels were much higher in MCF-10A cells than in breast cancer cells (Fig. 2a). Then, to investigate the role of TRHDE-AS1 in breast cancer, we constructed TRHDE-AS1 overexpression cell lines. Overexpressed TRHDE-AS1 mRNA levels were verified in MCF-7 and MDA-MB-231 cells by qRT-PCR (Fig. 2b). A CCK-8 assay was employed to evaluate the effects of TRHDE-AS1 on cell proliferation, and the results showed that the growth of overexpressing MCF-7 and MDA-MB-231 cells was markedly suppressed compared with the control cells (Fig. 2c). An EdU analysis revealed that TRHDE-AS1 overexpression dramatically reduced the proportion of positive breast cancer cells (Fig. 2d). Moreover, a colony formation assay showed that TRHDE-AS1 overexpression significantly inhibited the clonogenic capacity of MCF-7 and MDA-MB-231 cells (Fig. 2e). All the above results verified the inhibitory effect of TRHDE-AS1
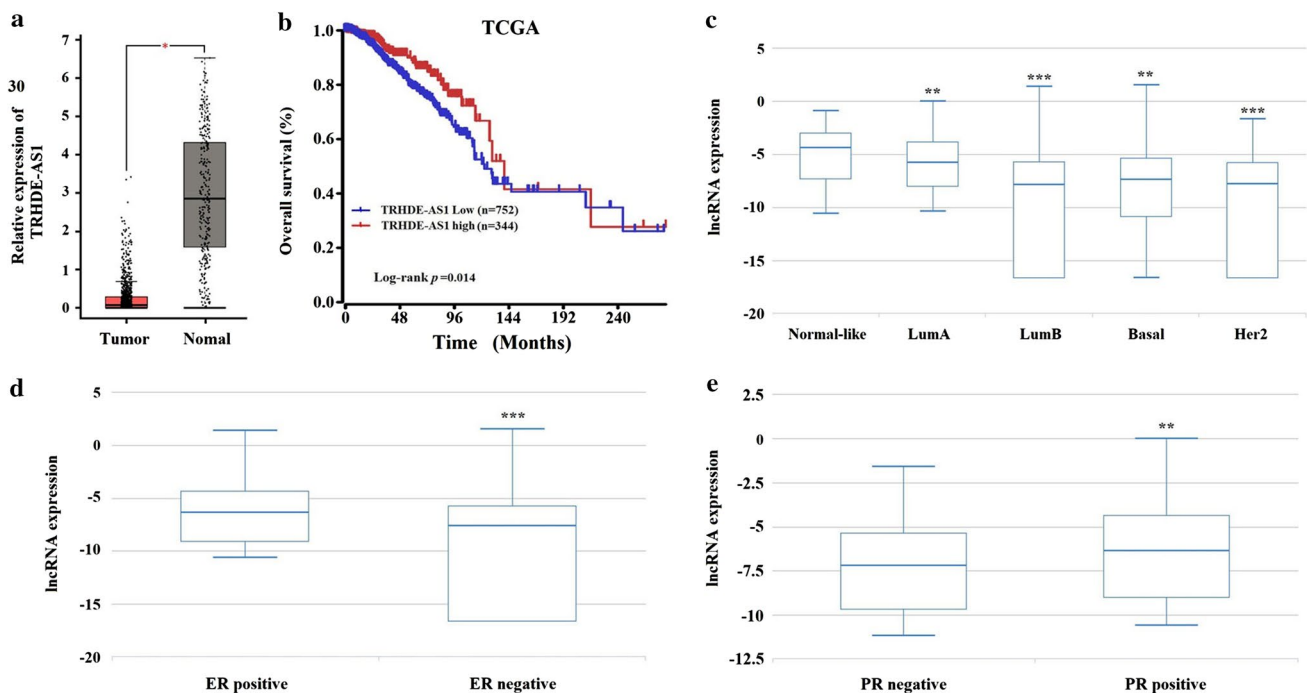

Fig. 1 The expression analysis of TRHDE-AS1 in an online database. **a** TRHDE-AS1 expression in normal and breast cancer tissue. The data were obtained from TCGA and analyzed using GEPIA (gepia.cancer-pku.cn). **b** Kaplan–Meier plots of patient outcomes based on TRHDE-AS1 expression. The plots were generated using R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl). **c** The expression of TRHDE-AS1 in five pathological types of breast cancer in the TANRIC database. **d** The expression of TRHDE-AS1 in ER-positive and ER-negative breast cancer samples from the TANRIC database. **e** The expression of TRHDE-AS1 in PR-positive and PR-negative breast cancer samples from the TANRIC database. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$
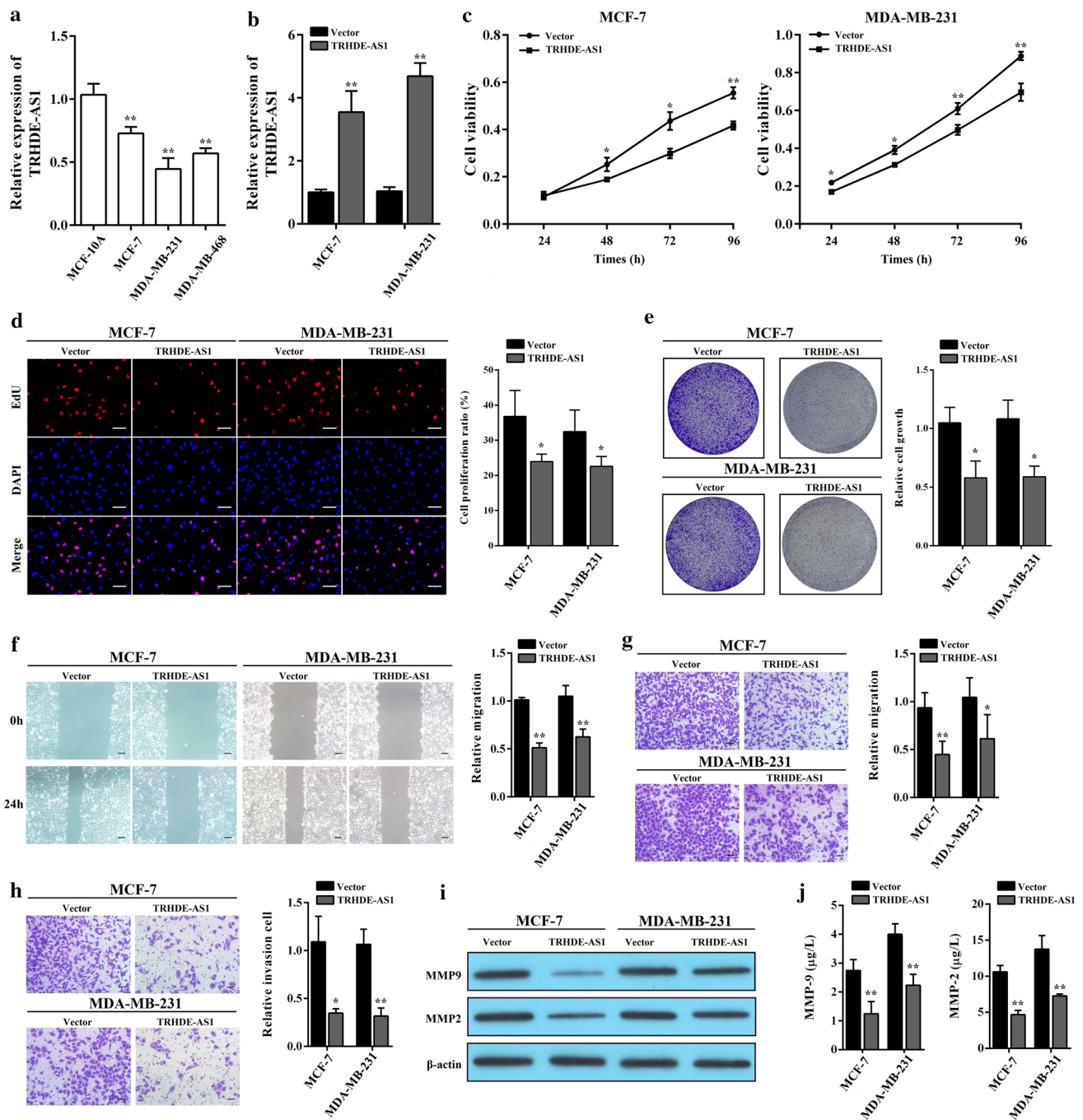
Fig. 2 The effect of TRHDE-AS1 on the proliferation and metastasis of breast cancer cells. **a** TRHDE-AS1 expression in the normal human mammary epithelial cell line and breast cancer cell lines. **b** The relative expression of TRHDE-AS1 in breast cancer cells transfected with pcDNA3.1-TRHDE-AS1 and empty vectors. **c** CCK-8 and **d** EdU assays examined the proliferation ability. The proliferative cells were labeled with red fluorescence, and DAPI was used to label the cell nucleus, scale bar = 100 μm **e** Colony formation assays examined the colony formation abilities. **f** Wound healing and **g** transwell migration assays examined the migration ability, scale bar = 500 μm **h** The transwell invasion assay examined the invasion ability, scale bar = 500 μm **i** Western blot was performed to detect the protein levels of MMP9 and MMP2. **j** ELISA was performed to detect the secretion of MMP9 and MMP2. *$P < 0.05$, **$P < 0.01$

on breast cancer cell proliferation. Afterwards, wound healing and transwell assays were utilized to detect the migration and invasion of TRHDE-AS1 overexpressed cells. The results of the wound healing and transwell migration

assays showed that the migration of both MCF-7 and MDA-MB-231 cells was inhibited after TRHDE-AS1 overexpression (Fig. 2f and g). The transwell invasion assay indicated that the number of invading TRHDE-AS1 overexpressed

MCF-7 and MDA-MB-231 cells was notably decreased (Fig. 2h). In addition, the western blot analysis revealed that TRHDE-AS1 overexpression significantly reduced the expressions of MMP-2 and MMP-9 in breast cancer cells (Fig. 2i), the results of ELISA showed that TRHDE-AS1 overexpression significantly reduced the secretion of MMP-2 and MMP-9 in cell culture medium (Fig. 2j). Taken together, these data suggest that TRHDE-AS1 acts as an anti-onco-gene in breast cancer.

ASV promotes TRHDE-AS1 expression in breast cancer cells

To investigate the effects of ASV on the expression of TRHDE-AS1 in breast cancer cells, MCF-7 and MDA-MB-231 cells were treated with 20, 40, or 80 µg/mL ASV for 48 h. As shown in Fig. 3a, ASV increased TRHDE-AS1 expression in both MCF-7 and MDA-MB-231 cells in a dose-dependent manner. We next treated MCF-7 and MDA-MB-231 cells with 80 µg/mL ASV for different lengths of times. The results showed that ASV increased TRHDE-AS1 expression in both MCF-7 and MDA-MB-231 cells in a time-dependent manner (Fig. 3b). Overall, these data
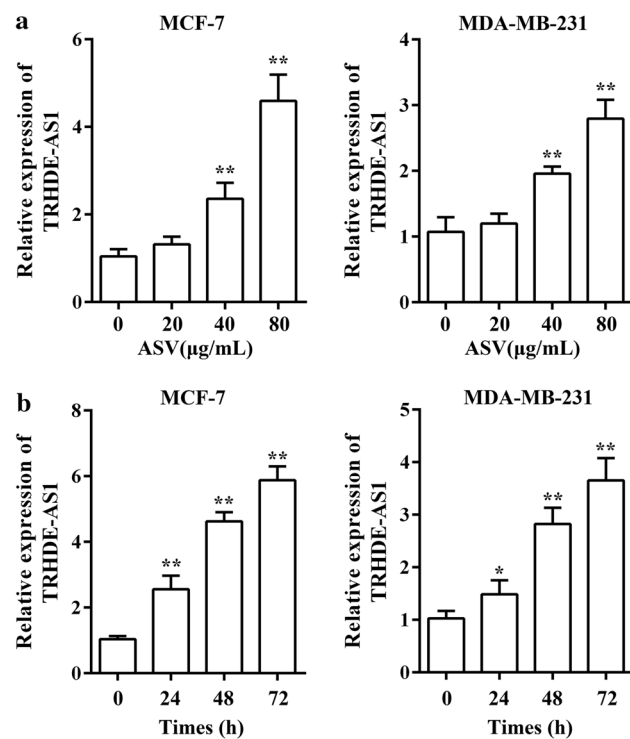

Fig. 3 ASV promotes the expression of TRHDE-AS1 in breast cancer cells. **a** qRT-PCR examined the TRHDE-AS1 expression in breast cancer cells treated with a dose series of ASV (0, 20, 40, and 80 µg/mL) for 48 h. **b** qRT-PCR examined the TRHDE-AS1 expression in breast cancer cells treated with 80 µg/mL ASV for 0, 24, 48, or 72 h. *$P < 0.05$, **$P < 0.01$

demonstrated that ASV dose- and time-dependently promoted TRHDE-AS1 expression in breast cancer cells.

ASV inhibits the proliferation of breast cancer cells via regulating TRHDE-AS1 expression

In Fig. 4a, the expression level of TRHDE-AS1 in cells transfected with siRNAs against TRHDE-AS1 (siTRHDE-1 and siTRHDE-2) was markedly lower than that in cells transfected with si-NC. Then, transfected cells were treated with or without ASV for 48 h. The results showed that the down-regulated TRHDE-AS1 levels were significantly increased by ASV treatment both in siTRHDE-1 and siTRHDE-2 transfected cells. Next, the CCK-8 assay, the EdU analysis, and the colony formation assay were performed to determine the effect of ASV on breast cancer cells. The data demonstrated that 40 and 80 µg/mL ASV significantly repressed the growth rate and clonogenic capacity of MCF-7 and MDA-MB-231 cells (Fig. 4b–d). Furthermore, downregulated TRHDE-AS1 expression reversed the repressive roles of ASV on breast cancer cell proliferation, indicating that ASV inhibited the proliferation of breast cancer cells via regulating TRHDE-AS1 expression.

ASV inhibits the metastasis of breast cancer cells by regulating TRHDE-AS1 expression

To explore whether ASV influenced the metastasis of breast cancer cells, we treated MCF-7 and MDA-MB-231 cells with 40 or 80 µg/mL ASV for 48 h and performed wound healing and transwell assays. The wound healing assay demonstrated that the migration of MCF-7 and MDA-MB-231 cells was remarkably inhibited, which was in agreement with the data from the transwell migration assay (Fig. 5a and b). In addition, the migration of MCF-7 and MDA-MB-231 cells was impaired following TRHDE-AS1 silencing (Fig. 5a and b). The transwell invasion assay showed that ASV treatment significantly suppressed the invasion capacity of breast cancer cells; however, this suppression was reversed by TRHDE-AS1 silencing (Fig. 5c). Furthermore, the reduction in MMP-2 and MMP-9 expression and secretion caused by ASV was increased with TRHDE-AS1 silencing in MCF-7 and MDA-MB-231 cells (Fig. 5d and e). Therefore, our data demonstrated that ASV inhibited the metastasis of breast cancer cells by regulating the expression of TRHDE-AS1.

ASV treatment inhibits breast cancer growth and metastasis in vivo

We further examined how ASV affects breast cancer proliferation and metastasis through the regulation of TRHDE-AS1 expression. We subcutaneously implanted MDA-MB-231 and TRHDE-AS1 silencing
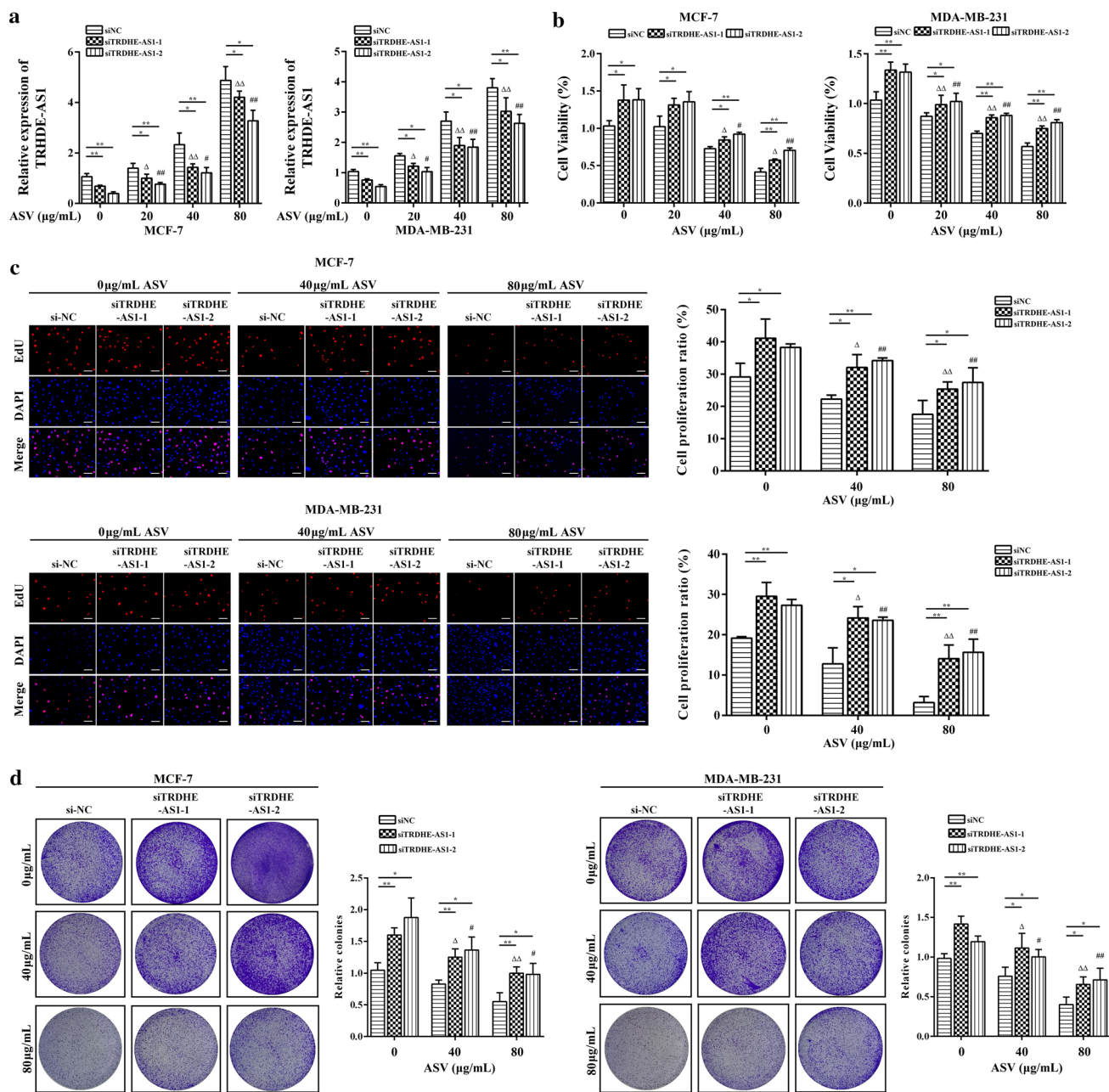
Fig. 4 ASV represses breast cancer cell proliferation via upregulating TRHDE-AS1. **a** The relative expression of TRHDE-AS1 in breast cancer cells transfected with TRHDE-AS1 siRNA and the negative control with or without ASV. **b** CCK-8 and **c** EdU assays were employed to detect cell proliferation after treatment with or without ASV for 48 h, scale bar = 100 μm **d** Colony formation assays examined the colony formation abilities of cells after treatment with or without ASV for 48 h, scale bar = 500 μm. si-NC vs. si-TRHDE-AS1: *$P < 0.05$, **$P < 0.01$; untreated vs. ASV treated si-TRHDE-AS1: $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, untreated vs. ASV treated si-TRHDE-AS2: $^{\#}P < 0.05$, $^{\#\#}P < 0.01$

MDA-MB-231cells into the flanks of BALB/c nude mice. The assessment of tumor volume at various times after implantation and intraperitoneal ASV treatment showed that compared with the drug untreated group, ASV treatment significantly inhibited tumor growth in both the si-NC and si-TRHDE-AS1 groups; however, the inhibitory effect of ASV on tumor growth was restored by TRHDE-AS1 silencing (Fig. 6a–c). A tail vein metastasis model was used to examine the effects of ASV and TRHDE-AS1 on metastasis in breast cancer cells. As shown in Fig. 6d, the HE staining of lung sections of the mice indicated that ASV treatment markedly reduced the formation of lung nodules in mice, but TRHDE-AS1 silencing restored this change. Furthermore, the western
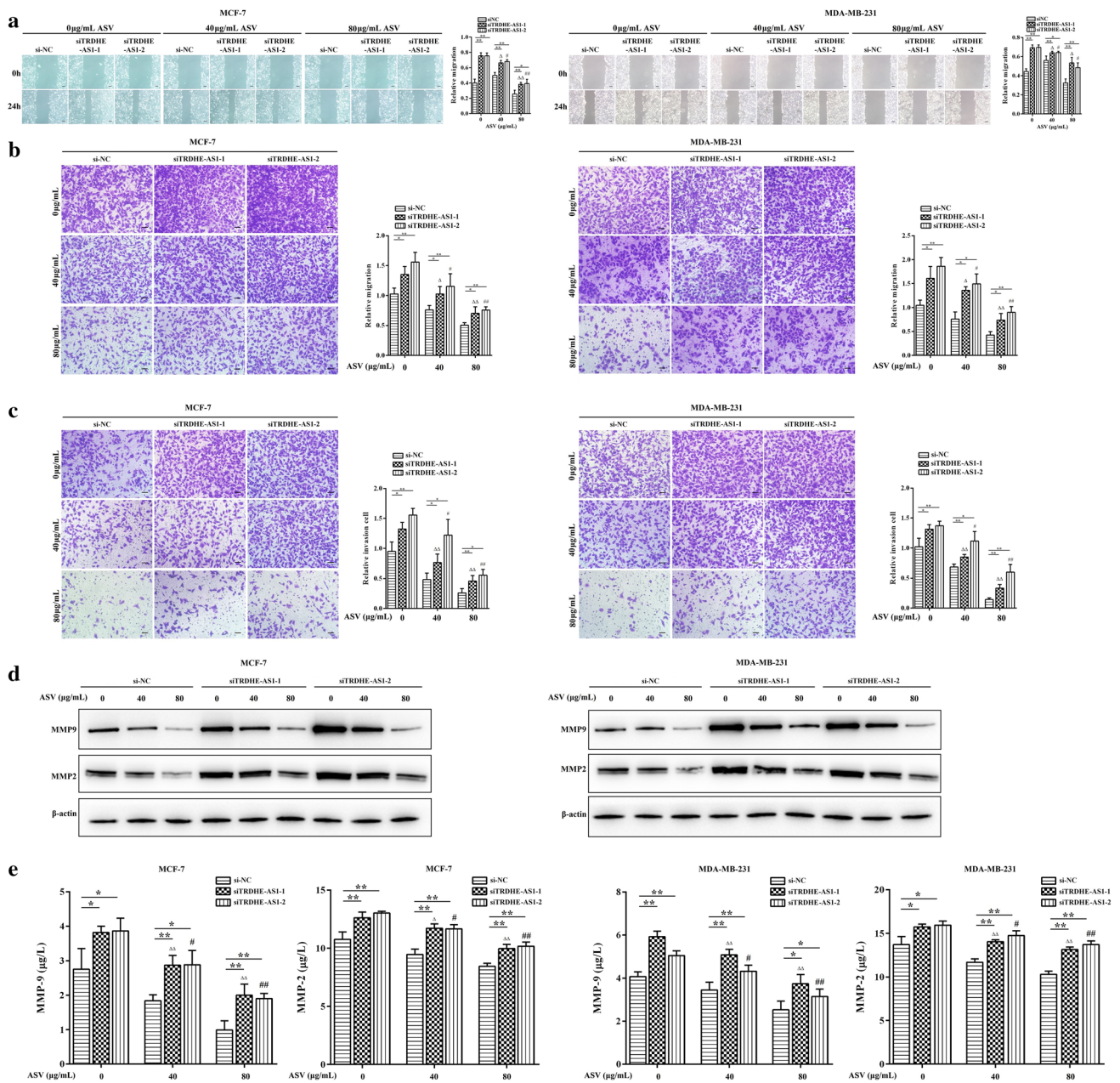
Fig. 5 ASV represses breast cancer cell metastasis via upregulating TRHDE-AS1. **a** Wound healing and **b** the transwell migration assay were employed to examine cell migration after treatment with or without ASV for 48 h, scale bar = 500 μm. **c** The transwell invasion assay was employed to examine cell invasion after treatment with or without ASV for 48 h, scale bar = 500 μm. **d** Western blot was performed to detect the protein levels of MMP9 and MMP2 after treatment with or without ASV for 48 h. **e** ELISA was performed to detect the secretion of MMP9 and MMP2 in the cell culture medium after treatment with or without ASV for 48 h. si-NC vs. si-TRHDE-AS1: *$P < 0.05$, **$P < 0.01$; untreated vs. ASV treated si-TRHDE-AS1: $^\Delta P < 0.05$, $^{\Delta\Delta}P < 0.01$, untreated vs. ASV treated si-TRHDE-AS2: $^\#P < 0.05$, $^{\#\#}P < 0.01$

blot analysis of tumor tissues showed that ASV treatment significantly inhibited the expression of the cell proliferation marker PCNA and the metastasis-associated proteins MMP-2 and MMP-9; conversely, TRHDE-AS1 silencing restored the expression of these proteins (Fig. 6e). Hence, these results clarified that ASV inhibited breast

cancer growth and metastasis in vivo, and ASV performed an inhibitory function by repressing TRHDE-AS1 expression.
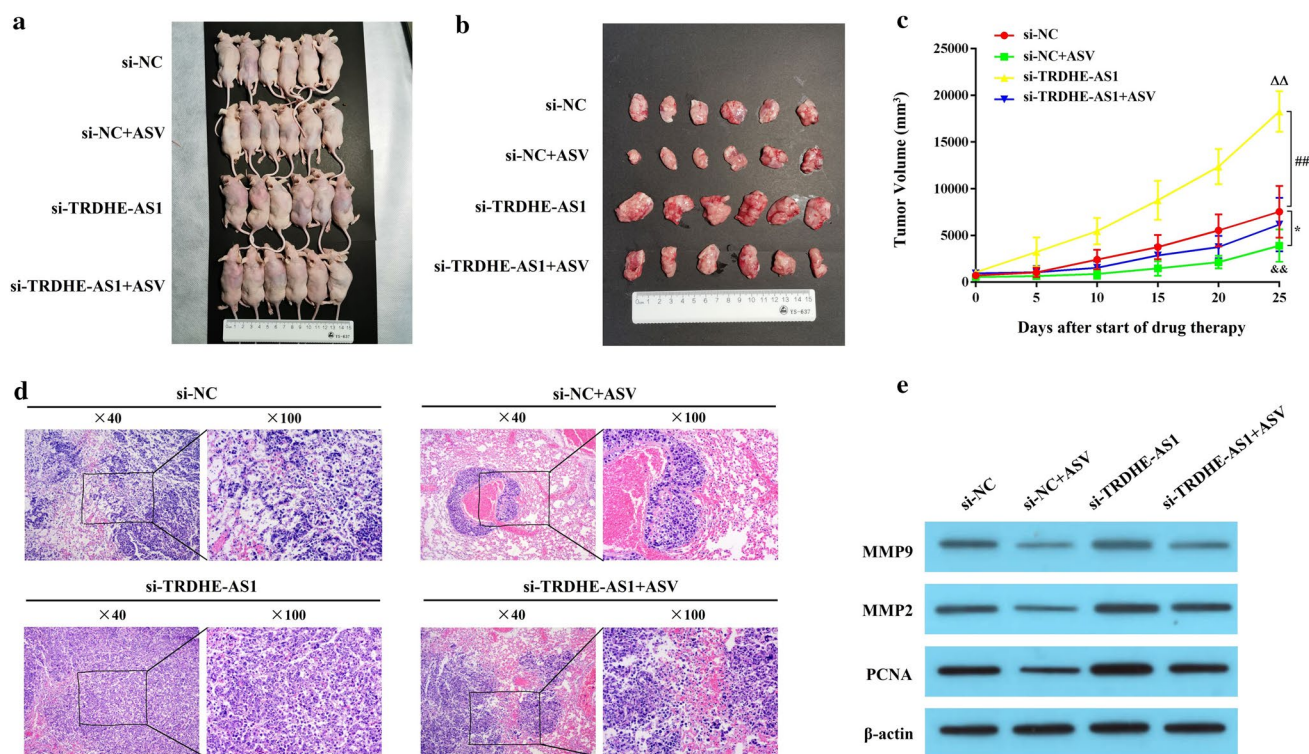
Fig. 6 ASV treatment inhibited tumor growth and metastasis in vivo. **a** Representative BALB/c nude mice and **b** tumors from each treatment group ($n=6$ per group). **c** Tumor growth curves for each subcutaneously implanted group of cells.) Representative histological photomicrographs of lung tissue sections from each treatment group ($n=6$ per group) stained with H&E to show the metastasis of the breast cancer cells. **e** Western blot analysis of MMP9, MMP2, and PCNA expression in tumor tissues. si-NC vs.si-TRDHE-AS1 group: [#]$P<0.05$, [##]$P<0.01$; si-NC vs. si-NC ASV treated group: [*]$P<0.05$, [**]$P<0.01$; si-TRDHE-AS1 vs. si-TRDHE-AS1 ASV treated group: [Δ]$P<0.05$, [ΔΔ]$P<0.01$; si-NC ASV treated vs. si-TRDHE-AS1 ASV treated group: [&]$P<0.05$, [&&]$P<0.01$

Discussion

Advances in sequencing technologies have resulted in the discovery of a range of noncoding RNA (ncRNA) species. These ncRNAs form 'junk' transcriptional products that play regulatory roles in several cellular processes including chromatin remodeling, transcription, post-transcriptional modifications, and signal transduction [17]. LncRNAs, which are greater than 200 nucleotides in length, are a common type of ncRNAs that are not as highly conserved as microRNAs (miRNAs) and circular RNA (circRNAs) [17, 18]. Regardless, lncRNAs display a conserved structure, which indicates their robust involvement in various biological functions [19]. Multiple studies have reported that lncRNAs participate in tumorigenesis through the regulation of gene expression in tumor cells by acting as miRNA sponges [20, 21], guiding chromatin modifying complexes [22, 23], interacting with hnRNPs [24], and affecting alternative splicing or translation [25, 26]. Aberrant expressions of lncRNA also play an important role in the development of breast cancer, but the functions and mechanisms of TRHDE-AS1 in breast cancer have not been explored until now. In this study, we first focused on TRHDE-AS1 expression in breast cancer. Through a bioinformatics analysis, we confirmed that TRHDE-AS1 levels were significantly lower in breast tissues and low TRHDE-AS1 expression was dramatically associated with shorter OS in breast cancer patients, which is in agreement with the data of Pang et al. and Yang et al. [27–29]. We also demonstrated the low TRHDE-AS1 expression in breast cancer cell lines by qRT-PCR. Moreover, our findings provided evidence that the overexpression of TRHDE-AS1 could inhibit the proliferation and metastasis of breast cancer cells. Therefore, our study is the first to clarify the role of TRHDE-AS1 in the development of breast cancer and indicate its potential as a biomarker.

Traditional Chinese medicine has been practiced for more than 2500 years for the clinical prevention and treatment of various diseases, and herbal medicine is the most important part of this type of medicine [30]. The combined treatment of breast cancer with Chinese herbal compounds has demonstrated clinical effectiveness [31, 32]. The Chinese herb A. membranaceus contains many compounds with complex chemical profiles, including saponins, polysaccharides, flavonoids, phytosterols, fatty acids, etc. [10]; therefore, the extracts of A. membranaceus have significant anticancer activities via complex mechanisms [33]. ASV is the main

component of Astragalus saponins and has proved to exert antitumor effects on breast cancer and enhance the chemosensitivity of the breast cancer cells. Here, our data first indicated that lncRNA also acts a critical mediator of the anticancer activities of ASV in breast cancer. We found that ASV distinctly promoted TRHDE-AS1 expression levels in breast cancer cells in both dose- and time-dependent manners. Furthermore, the downregulation of TRHDE-AS1 was reported to inhibit the progression of lung cancer. In this study, we demonstrated that ASV suppressed the proliferation and metastasis of breast cancer cells while the knockdown of TRHDE-AS1 partially reversed the inhibitory roles of ASV on breast cancer cells. Furthermore, by establishing a xenograft mouse model and a tail vein metastasis model, we found that the growth and metastasis of breast cancer in mice, which was repressed by ASV, was also restored by TRHDE-AS1 silencing.

In summary, we first demonstrated that TRHDE-AS1 participated in the progression of breast cancer and was activated by ASV in breast cancer cells. ASV suppressed the proliferation and metastasis of breast cancer cells by upregulating TRHDE-AS1 expression both in vitro and in vivo. Our data provide evidence for the application of ASV in breast cancer therapy. In the future, we will continue to research the relationship between ASV and TRHDE-AS1 and the pathways through which they play a role in the development of breast cancer.

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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