Harmine suppresses the malignant phenotypes and PI3K activity in breast cancer

Pengmei Yao[a], Penghua Yao[b], Xiaoxia Ku[b] and Jing Yang[c]

Breast cancer remains a serious threaten to the women's health, discovery of potent treatment would help to improve the outcomes of breast cancer patients. Harmine extracted from *Peganum harmala L*, has been reported to exert tumor suppressive activity in several malignancies. Our objective was to demonstrate the effects of harmine on the malignant phenotypes of breast cancer cells. Breast cancer cell lines (MDA-MB-231, SKBR3, and MCF-7) and human normal breast cell line MCF-10A were employed in the present study. The MTT and colony formation assays were applied to the detection of cell viability and proliferation. Wound healing and transwell assays were performed to evaluate the alterations of cell migration and invasion after harmine treatment. Flow cytometry was applied to assess the effect of harmine in inducing cell apoptosis. Furthermore, western blotting assay was used to detect the biomarkers of epithelial-mesenchymal transition and phosphatidylinositol 3 kinase (PI3K) signaling pathway. The tumorigenesis ability was detected by subcutaneous implantation. Harmine dose-dependently suppressed the viability and proliferative capacity of breast cancer cells. Flow cytometry showed that harmine induced apoptosis in MCF-7 and MDA-MB-231 cells. In addition, harmine effectively inhibited the migration and invasion abilities of breast cancer cells. Western blotting indicated harmine significantly promoted E-cadherin and PTEN expression, while suppressed N-cadherin, vimentin, PI3K, p-mTOR, and AKT levels. Interfering the PTEN expression by siRNA partly rescued the activity of PI3K signaling pathway. Moreover, harmine injection also suppressed the tumorigenesis of breast cancer cells. Our results suggested that Hermine could suppress multiple malignant phenotypes and inhibit PI3K signaling, which supports that harmine might be a potential tumor-suppressive natural compound against breast cancer.

Anti-Cancer Drugs 2023, **34**:373–383

Keywords: breast cancer, epithelial–mesenchymal transition, harmine, migration, invasion, phosphatidylinositol 3 kinase/protein kinase B

[a]Department of Breast Surgery, The First Affiliated Hospital of Jinzhou Medical University, [b]Medical College of Jinzhou Medical University and [c]Department of Otolaryngology, Shengjing Hospital, China Medical University, Shenyang, Liaoning, China

Correspondence to Dr. Jing Yang, PhD, Department of Otolaryngology, Shengjing Hospital, China Medical University, Shenyang, Liaoning 121000, P.R. China
E-mail: yangjing7694@126.com

Received 14 May 2022 **Revised form accepted** 12 August 2022.

Introduction

As one of the most common cancers, breast cancer possesses a high mortality, which seriously endangers the health of women worldwide [1,2]. As reported, approximately 1.7 million breast cancer patients are diagnosed, and over 500 000 patients died from breast cancer worldwide annually [1,3]. Thanks to the development of early screening and diagnostic technologies, the mortality of breast cancer have declined in developed areas such as North America and European Union in recent years [4]. Meanwhile, its incidence and mortality remain high in most developing areas like Africa and South America [1,2]. At present, surgery is the mainstream therapy for early-stage breast cancer, and the following systemic therapy helps improve prognoses. However, metastatic breast cancer is usually considered incurable, and the treatment purpose is to prolong survival and improve life quality [1,5]. Therefore, discovering effective drugs to inhibit breast cancer progression and metastasis is significant for the development of breast cancer therapy.

Harmine is a sort of β-carboline alkaloid extracted from *Peganum harmala* L, a traditional Chinese herb used to relieve cough and rheumatism [6]. According to modern pharmacological studies, *P. harmala* L has been proven to possess therapeutic effects against malignant tumors, and harmine maybe the key active component for its anti-cancer effect [7]. It is reported that harmine inhibits the progression of various malignant tumors, such as breast cancer [8], lung cancer [9], gastric cancer [10] and liver cancer [11]. Hamsa *et al*. demonstrated that harmine could act as a strong angiogenesis inhibitor by suppressing key factors such as vascular endothelial growth factor and matrix metalloproteinases [12]. However, the mechanism of the anti-tumor effects of harmine remains not fully revealed.

Epithelial–mesenchymal transition (EMT) is a crucial event in involved in multiple biological processes such as individual growth, wound healing, and organism

development etc [13–15]. In addition, EMT also promotes the invasion and metastasis of malignant tumor cells, characterized by polarity disappearance, the destruction of the cytoskeleton, and the recombination of mesenchymal-like features [16,17]. Furthermore, the EMT process is often accompanied by changes in the expression levels of multiple biomarkers, such as the overexpression of mesenchymal markers, N-cadherin, and vimentin, as well as the downregulated epithelial markers, E-cadherin [18,19]. The dysregulation in these biomarkers would trigger a series of changes in cell behaviors, which are closely related to metastasis breast cancer.

In this study, several phenotypic and molecular experiments were performed to detect the impact of harmine on the proliferation, invasion, and EMT process, and evaluated the alteration of phosphatidylinositol 3 kinase (PI3K) signaling pathway in breast cancer cells. The purpose of our work was to evaluate the tumor suppressive effects of harmine against breast cancer cells and to reveal its impact on cell signaling.

Material and methods

Cell culture

The breast cancer cell lines (MDA-MB-231, SKBR3, and MCF-7) and human normal breast cell line MCF-10A were obtained from the Institute of Shanghai Biochemistry and Cell Biology (Shanghai, China). And the cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) contained with 10% fetal bovine serum (FBS; HyClone Laboratories, Utah, USA). All the cells were maintained in a humidified incubator with 5% CO_2 at 37°C.

MTT assays for cell viability

The MTT assay was applied to detect cell viability. The MDA-MB-231 and MCF-7 cells were seeded into 96-well plates, respectively, at a density of 1×10^4 cells per well. After incubation for 24 h, cells were treated with a gradient concentration of harmine (0, 40, 80, 120, 160, 200, and 240 μM), and then cultivated for another 48 h or 72 h. After treatment, the harmine-containing medium was replaced with DMEM supplemented with MTT (0.50 mg/mL). Cells were then incubated at 37°C for 4 h. And then, the solution was removed and replaced with 150 μL of dimethyl sulphoxide (DMSO). Finally, the absorbances at 490 nm in each well were detected by Multiskan Ascent plate reader (Thermo, Waltham, Massachusetts, USA).

Wound healing assay for cell migration

Wound healing assay was performed to evaluate the cell migration. The MDA-MB-231 and MCF-7 cells were seeded into 24-well plates, respectively, and then scraped with the 20-μL pipette tips. After being washed with PBS, the scratches were photographed under a microscope (Olympus, Tokyo, Japan). Then, cells were cultured in FBS-free medium containing a gradient concentration of Harmine (0, 50, 100, and 150 μM) for 24 h. Followingly, the scratches were observed again at 100X magnification field. The migration distances and wound healing rate were measured and analyzed by Image J software (NIH, Bethesda, Maryland, USA).

Transwell assay

The effect of Harmine in suppressing the invasion of breast cancer cells was quantified via transwell assay. After being treated with harmine (0, 50, 100, and 150 μM) for 24 h, cells were harvested and resuspended in DMEM without serum. Cells (1×10^4/well) were re-seeded into each upper chamber pre-coated with 50 μL 1:1 mixture of Matrigel and DMEM, while the DMEM containing 20% FBS was added into each lower chamber. Followed by culturing in incubator at 37°C for 48 h, the non-invaded cells on the membrane upper surface were wiped, and the invaded cells were fixed by paraformaldehyde, followed by staining with 0.1% crystal violet for 10 min. After being washed with PBS, the results of the invasion were photographed under a microscope.

Protein extraction and western blot

After harmine treatment, cells were collected and lysed by RIPA lysis buffer (Beyotime, Nantong, China) containing 1% PMSF. And then the protein concentration was assessed by the bicinchoninic acid kit (Keygen, Nanjing, China). Equal quality of protein samples was separated by 10% SDS-PAGE, followed by transfer onto PVDF membranes using an electro-transfer system (Bio-Rad, Hercules, California, USA). After being blocked in 5% skim milk for 1 h at room temperature, the membranes were incubated with different primary antibodies at 4°C overnight. After being washed with TBST solution three times, the membranes were incubated with peroxidase-conjugated secondary antibodies (cat. no. ab6721; 1:3000; Abcam, Cambridge, UK) for 0.5 h at 37°C. Finally, the blots were detected using Chemiluminescent ECL assay kit (Advasta, Menlo Park, California, USA) with the Bio-Rad Gel imaging system. In this research, GAPDH was employed as the internal reference for different protein expressions, and Image J software was used to quantify protein expression. The primary antibodies used are listed below: anti-E-cadherin (cat. no. ab40772; 1:1000; Abcam), anti-N-cadherin (cat. no. ab18203; 1:1500; Abcam), anti-vimentin (cat. no. ab92547; 1:1000; Abcam), anti-PI3K(cat. no. ab32089; 1:1500; Abcam), anti-phosphatase and tension homologue deleted from chromosome 10 (PTEN, cat. no. ab267787; 1:1500; Abcam), anti-protein kinase B (AKT, cat. no. 10176-2-AP;1:1500; Proteintech) anti-p-AKT-s473 (cat. no.66444-1-Ig; 1:1500; Proteintech, Wuhan, China), and anti-GAPDH (cat. no. ab16891; 1:2000; Abcam).

Colony formation assay

To check the effect of harmine in breast cancer cells, a soft agar colony formation assay was applied in this study. The MDA-MB-231 and MCF-7 cells were processed

as 2.3 described, and then harvested and re-seed in six-well plates, followed by culturing for two weeks at 37°C. Finally, the colonies in each well were photographed under a microscope.

Cell apoptosis by flow cytometry

Flow cytometry was performed to assess the function of harmine in promoting cell apoptosis. After incubated with a gradient concentration of harmine (0, 50, 100, and 150 μM) for 24 h, cells were collected and washed using cold PBS three times, followed by re-seeding in 0.2 mL buffer at a final density of 1×10^6 cells/mL with the DMEM containing Annexin V-FITC and PI (BD Biosciences, USA) for staining. After being incubated in a dark room for 15 min, cells in different stages were identified using an Accuri C6 flow cytometer (BD Biosciences) and analyzed using FloaJo 7.6.2 software. Finally, the apoptotic rate was expressed by GraphPad Prism 7.0 software.

Small interfering RNA transfection

siRNA-PTEN and siRNA-control were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Cells were plated into six-well plates at a density of 1×10^5 cells/well in DMEM and followingly treated with si-NC or si-PTEN with the assistance of Lipofectamine 2000 reagent (Invitrogen Corporation, Carlsbad, California, USA) according to the manufacturer's instructions. After transfection for 24 h, the medium was removed and the cells were washed and then treated with 240 μM PCB for 72 h. Then, the cells were treated with or without 100 μM harmine, and the PI3K signaling activity was subsequently detected.

Tumorigenesis assay

A total of 12 female Balb/C nude mice at 4-week-old were obtained from the Laboratory Animal Centre, Jinzhou Medical University. Then six mice were injected with 5×10^6 MDA-MB-231 and the other six mice were injected with MCF-7 cells subcutaneously. Followingly, mice were injected with harmine (50 mg/kg) or DMSO in the same volume every 3 days, respectively. After 3 weeks, the mice were sacrificed and the sizes of the tumors were measured. The Ki-67 expression was detected by immunohistochemical analysis.

Immunohistochemical analysis

Immunohistochemical analysis was employed to detect the expression of Ki-67, PI3K, and PTEN in subcutaneous tumors. Sections were first deparaffinized and were heated in citrate buffer (pH 6.0) for antigen retrieval, and subsequently blocked using 3.0% hydrogen peroxide and 10% goat serum. After incubated with anti-Ki67 (1:200; Proteintech), primary antibody at 4°C overnight, sections were incubated with the biotinylated secondary antibodies, then the sections were counterstained with hematoxylin, dehydrated, mounted on glass slides, and photographed using a microscope (Olympus) at 200× magnification.

Statistical analysis

Because the sample size was small and subjected to *t*-test, then the Student's *t*-test was used for the significance analysis between the two groups. However, the *t*-test would cause multiple comparisons during comparing the average values between multiple groups. In order to avoid the occurrence of false-positive results, the data comparison between the three groups and the four groups was analyzed using one-way analysis of variance followed by Tukey's post-hoc test at the 95% confidence interval. The results were expressed using GraphPad Prism 7.0 software (San Diego, USA). A significant difference was considered as $P < 0.05$. In this research, each experiment was independently performed three times.

Results

Harmine suppresses the proliferation activity of breast cancer cells

The structure of harmine is provided in Fig. 1a. As shown in Fig. 1b, the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) results revealed that the proliferation capacity of breast cancer cells (MCF-7, MDA-MB-231, and SKBR3) was suppressed by harmine, and the inhibitory effect was time and dose-dependent. However, the inhibition in normal breast cell MCF-10A was relatively minor compared with breast cancer cells. In addition, we performed a colony formation assay to further confirm the inhibition of harmine on the proliferation of breast cancer cells. Data in Fig. 1c suggested that the numbers of MCF-7 and MDA-MB-231 colonies decreased with the increased dose of harmine, which was consistent with the results in MTT assay. These results demonstrated that harmine could suppress the proliferation of breast cancer cells.

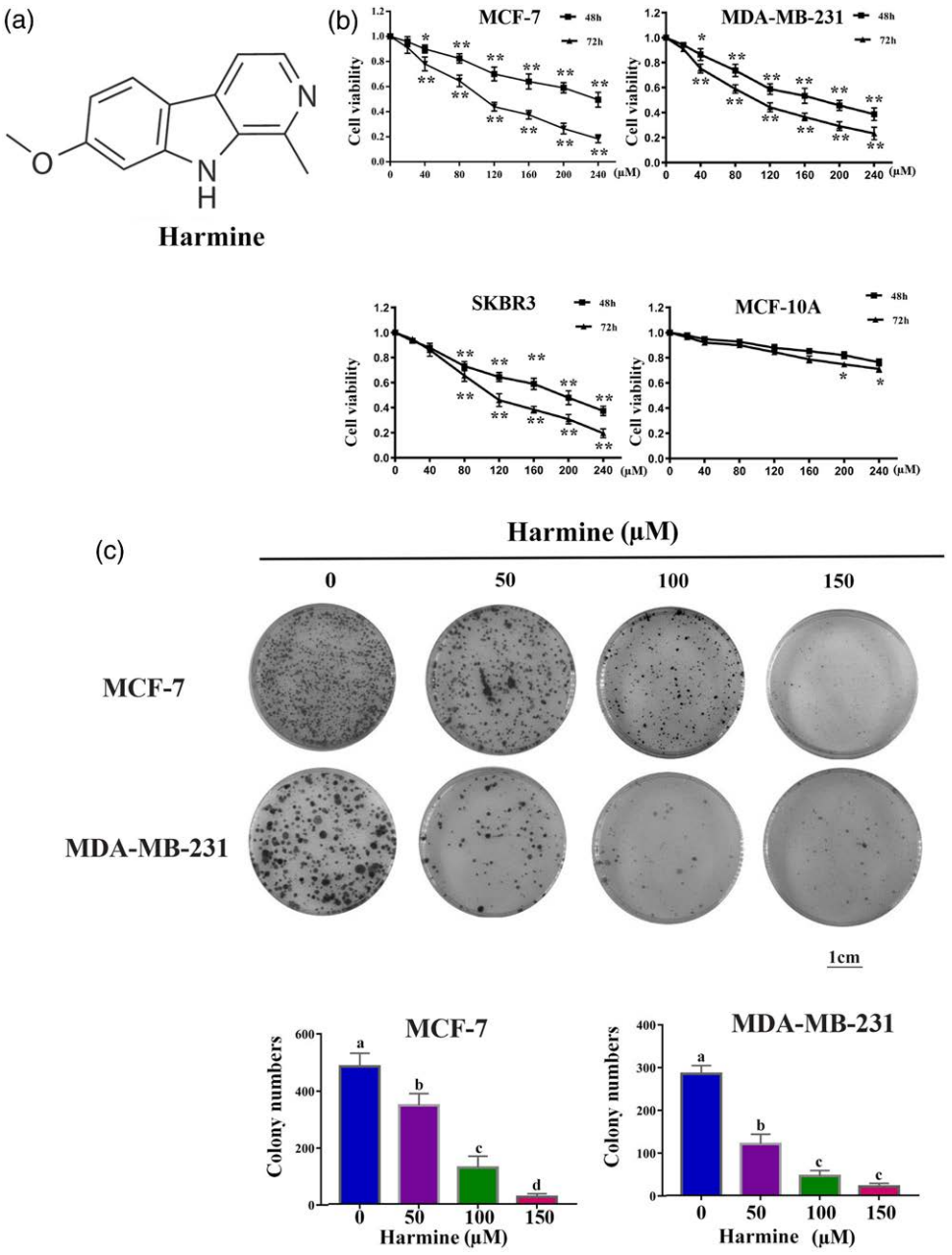
Harmine promotes apoptosis in breast cancer cells

To further explore the anti-cancer role of harmine in breast cancer, a flow cytometry assay was applied in this research. The results are shown in Fig. 2. After being treated with a gradient dose (0, 50, 100, or 150 μM) of harmine, MCF-7 and MDA-MB-231 cells were stained with Annexin V/PI. As the flow cytometry results shown, the percentage of apoptotic rates in MCF-7 and MDA-MB-231 were 8.3% and 8.6% (0 μM), 22.2% and 12% (50 μM), 30.5% and 21.8% (100 μM), and 43.7% and 43.1% (150 μM). The results of the flow cytometry assay suggested that harmine could induce apoptosis in breast cancer cells.

Harmine inhibits the migration, invasion, and epithelial–mesenchymal transition in breast cancer cells

To determine the migration and invasion capacities, transwell and wound healing assays were applied. As shown

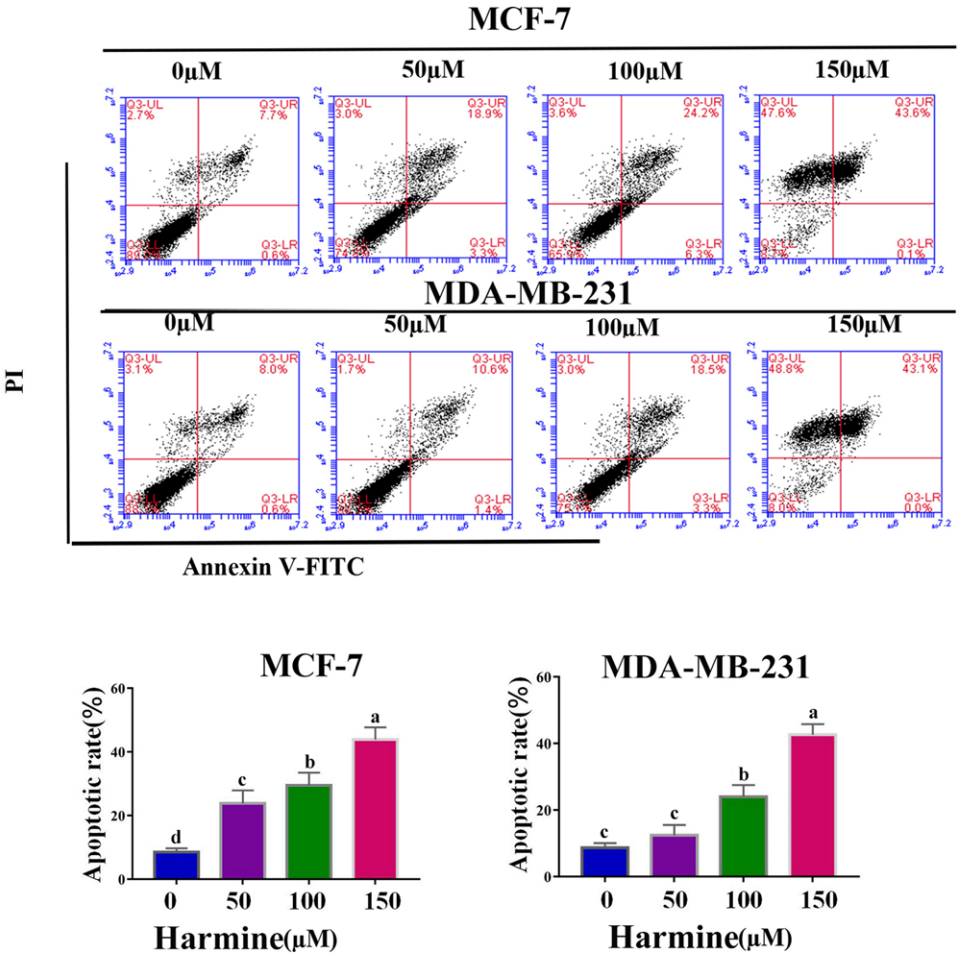
Fig. 1



Harmine suppresses the proliferation activity of breast cancer cells. (A) The structure of harmine. (B) MTT results showed that harmine suppressed the proliferation capacity of breast cancer cells (MCF-7, MDA-MB-231, and SKBR3) in time- and dose-dependent manners. (C) Colony formation assay indicated that harmine suppressed the proliferation capacity of MCF-7, MDA-MB-231, and SKBR3. * indicates $P<0.05$; ** indicates $P<0.01$. MTT, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide.

in Fig. 3a, the migration distance at 24 h of both cell lines decreased with the increasing harmine concentration. The invasion capacity was evaluated with transwell assay using the chambers coated with Matrigel solution, and the number of invaded cells was decreased by harmine treatment in a dose-dependent manner. Thence, the transwell and wound healing assays demonstrated that harmine could suppress the migration and invasion in MCF-7 and MDA-MB-231 cells dose-dependently. Since it was well known that the EMT process is critical for tumor invasion and metastasis, we further detected the alteration of EMT biomarkers (E-cadherin, N-cadherin, and vimentin,) after treatment with western blotting assay. As shown in Fig. 4, with the increase of harmine dose, E-cadherin expression increased, while N-cadherin and Vimentin decreased in MCF-7 and MDA-MB-231 cells. These data indicated that harmine could suppress the EMT process in breast cancer cells.

Fig. 2



Harmine promotes apoptosis in breast cancer cells. Flow cytometry assay data showed that harmine induces apoptosis in MCF-7 and MDA-MB-231 cells. The percentage of apoptotic rates in MCF-7 were 8.3% (0 μM), 22.2% (0 μM), 30.5% (0 μM) and 43.7% (0 μM); in MDA-MB-231 were 8.6% (0 μM), 12% (50 μM), 21.8% (100 μM) and 43.1% (150 μM).

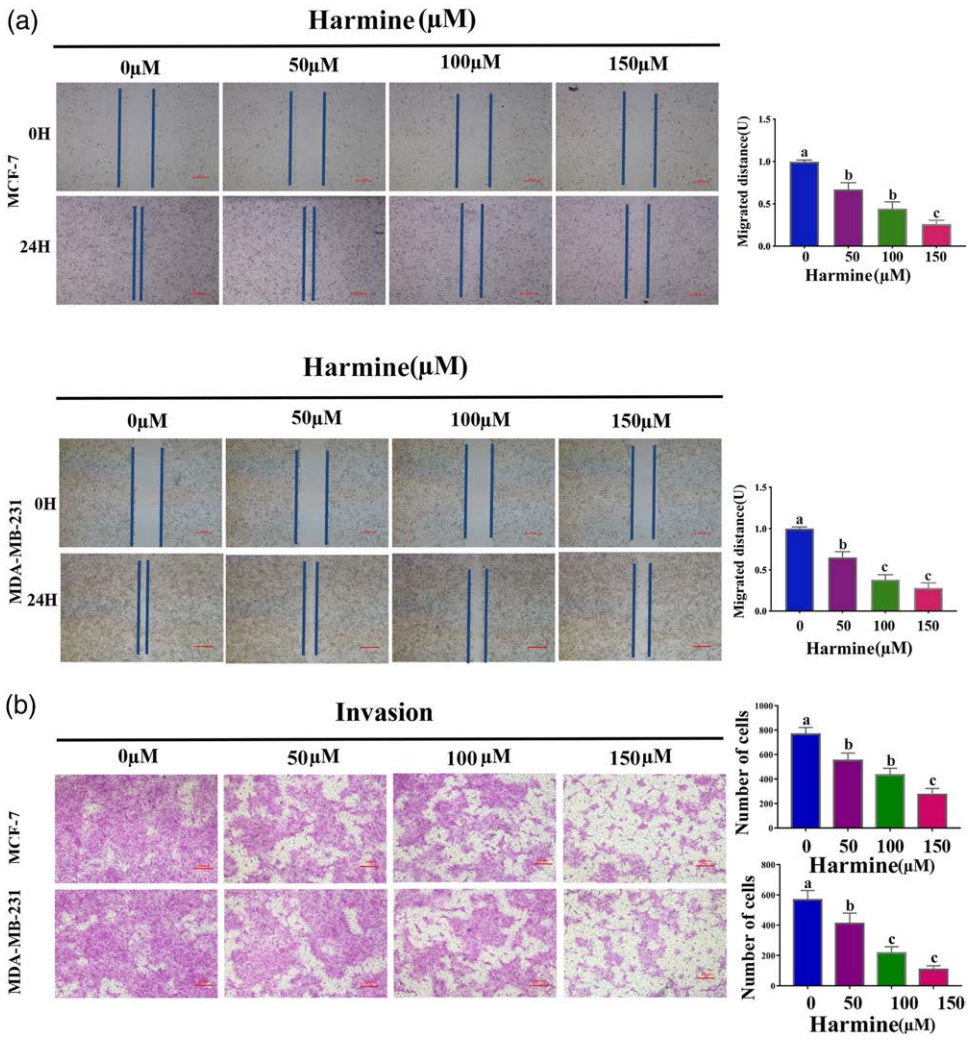
Harmine suppresses the activity of PI3K signaling pathway in breast cancer cells

PI3K, a key signaling pathway correlated with cell proliferation, and especially, it is well-known dysregulation of PI3K activity is closely associated with the carcinogenesis of multiple malignant diseases. Previous studies found that harmine could inhibit PI3K signaling in lung cancer. Thus, we were curious about if harmine could suppress the PI3K activity in breast cancer. The expression of PTEN, PI3K, Akt, phosphorylated Akt, mTOR, and phosphorylated mTOR were detected via western blotting assay, as shown in Fig. 5, after harmine treatment, PTEN was increased while PI3K was decreased. The phosphor-AKT (p-AKT Ser473 and p-AKT Thr308) were decreased but the total Akt level showed no significant alteration. Similarly, phosphor-mTOR (mTOR S2448 and mTOR S2481) were also decreased without a significant alteration of total mTOR level. Furthermore, siRNA targeting PTEN was introduced for verifying the impact on PI3K signaling of harmine. Compared to si-NC, si-PTEN transfection partly rescued the phosphorylation of Akt and mTOR, yet did not change the total Akt and mTOR levels (Fig. 6). These results suggest that harmine suppresses the malignant phenotypes at least by inhibiting the PI3K signaling.

Harmine suppresses the tumorigenesis of breast cancer cells *in vivo.*

To further detect the anti-tumor effect of harmine *in vivo*, BALB/C nude mice were implanted with MCF-7 or MDA-MB-231 cells. As shown in Fig. 7a, the tumor sizes in the harmine groups were significantly smaller than control groups. Followingly, the tumors were subjected to immunohistochemical staining to detect the expression, which represents cellular proliferation. As shown in Fig. 7b, harmine markedly decreased Ki-67 expression. These results indicate that injection of harmine effectively suppresses the tumorigenicity of breast cancer cells.

Fig. 3



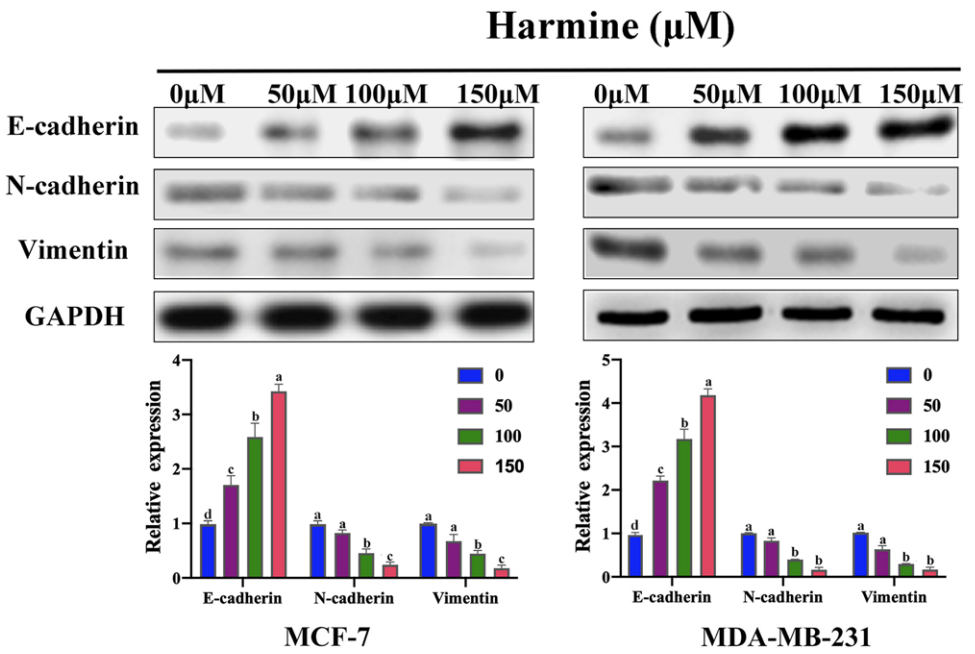
Harmine inhibits the migration, and invasion of breast cancer cells. (a) Wound healing assay results indicated that harmine suppressed the migration in MCF-7 and MDA-MB-231 cells in a dose-dependent manner. (b) transwell demonstrated that harmine suppresses the invasion of MCF-7 and MDA-MB-231 cells in a dose-dependent manner. Magnification 200x.

Discussion

Although a part of patients suffered from breast cancer and received better recovery after surgery, a large amount of breast cancer patients still face the risk of cancer relapse and metastasis [20]. Therefore, there is still an urgent need of finding suitable drugs against breast cancer. At present, multiple monomer compounds isolated from herbal medicines have been proven to suppress various biological events of tumor cells such as proliferation, invasion, and angiogenesis. For instance, baicalein, a flavone extracted from roots of *Scutellaria baicalensis*, has been reported to inhibit the metastasis of multiple cancers, such as hepatocellular carcinoma [21] and breast cancer [22]. Ma *et al*. proved baicalein inhibits the invasion of MDA-MB-231 cells via downregulating SATB1 and Wnt/β-catenin pathways [23]. Liu *et al*. proved that acetyl-keto-beta-boswellic acid, derived from *Boswellia serrata*, could inhibit the formation of small tubular structures via suppressing proliferation and migration of vascular endothelial cells, and therefore inhibit prostate cancer progression [24]. Harmine, the main effective component of *P. harmala L*, has also been shown to possess a cancer-suppressive function in several malignant tumor cells including breast cancer [8,25,26]. However, its influence on cell invasion of breast cancer remains not discovered. In this study, we further verified the ability of hermine in inhibiting cell proliferation, invasion, and EMT in breast cancer cell lines MCF-7 and MDA-MB-231, and further explored its impact on the PI3K signaling.

Under normal conditions, cell proliferation and apoptosis are subject to balanced regulation by relative genes [27].

Fig. 4



Harmine inhibits the EMT in breast cancer cells. Western blot assay showed that E-cadherin expression increased, while N-cadherin and vimentin decreased in MCF-7 and MDA-MB-231 cells in a dose-dependent manner, which indicated that harmine suppressed EMT process in MCF-7 and MDA-MB-231 cells. EMT, epithelial–mesenchymal transition.

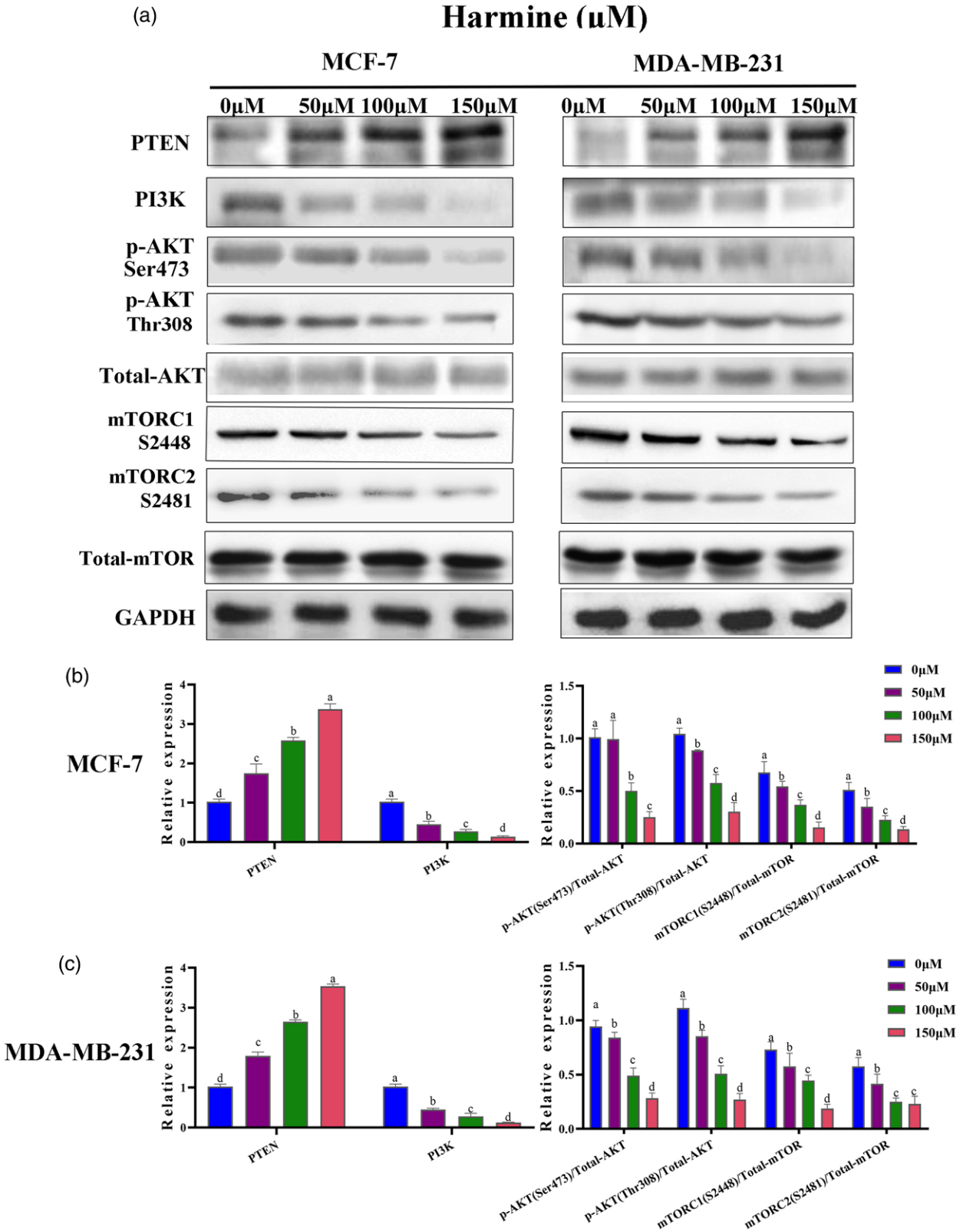
Uncontrolled proliferation and unstable programmed cell apoptosis are crucial features of malignant tumor cells. In addition, parts of tumor cells could break the extracellular matrix, detach from the primary focus, and spread to the surrounding tissues or distant organs [28]. In our study, harmine dose-dependently suppressed the proliferation, induced apoptosis, and inhibited migration and invasion in MCF-7 and MDA-MB-231 cells, indicating it may exert multiple tumor-suppressive effects against breast cancer. Therefore, we further explored the alterations in several important proteins which are crucial for tumorigenesis and metastasis.

EMT indicates a biological process in which the epithelial cells gain the properties of mesenchymal cells when cells undergo embryonic development or pathological processes [14,29]. During the EMT process, lots of alterations happen in cellular phenotypes and gene expressions. Epithelial cells usually possess epical-base polarity and tightly attached to each other, while mesenchymal cells are lack this attachment and could migrate freely [15]. Therefore, EMT process gains cancer cells the ability to break the cellular matrix to invade and metastasize [14]. EMT is a dynamic process, there are several biomarkers that could monitor the EMT level. E-cadherin and N-cadherin are the most common EMT markers, the E/N-cadherin switching is a key property of EMT [30], and vimentin is another typical mesenchymal biomarker [31]. In our research, after harmine treatment, the epithelial biomarker E-cadherin dose-dependently increased while mesenchymal biomarker N-cadherin and Vimentin decreased. Therefore, it could be indicated that harmine could reverse the EMT of breast cancer cells.

PI3K/AKT signaling pathway is indispensable for the regulation of multiple cellular phenotypes such as proliferation [32], survival [33], and autophagy [34]. Mountains of evidence support PI3K/Akt pathway is abnormally overactivated in multiple cancer types including breast cancer [35]. Moreover, small molecular drugs targeting PI3K/AKT pathway have been put into clinical trials and provided good efficacies [30]. For example, a phase III clinical trial suggested buparlisib, a pan-PI3K inhibitor, combined with fulvestrant effectively prolonged the progression-free survival for 1.9 months in comparison with a placebo [36]. Harmine has been proven to inhibit PI3K/Akt pathway in gastric cancer cells [10]. We considered whether it could exert a similar function in breast cancer. Therefore, we detected several biomarkers of this pathway, the results indicated that harmine significantly increased the expression of the typical cancer-suppressive gene PTEN, and suppressed the phosphorylation of Akt, moreover, interfering PTEN using siRNA transfection partly rescued the phosphorylation of Akt and mTOR, which further confirmed harmine suppresses the PI3K signaling activity at least partly by upregulation of PTEN.

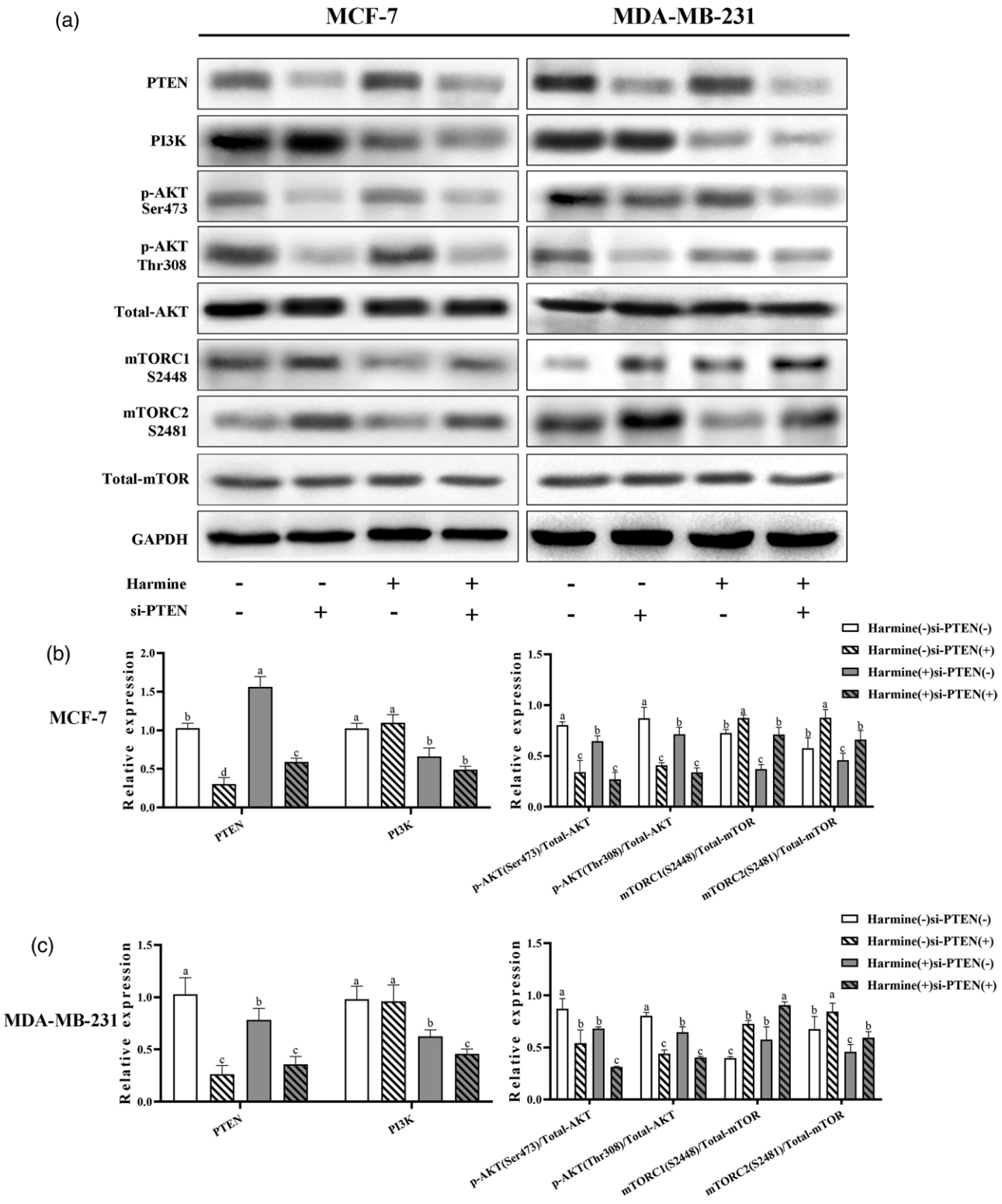
There are some limits in this study, our work has only evidenced that harmine exerts tumor suppressive function, and its safety and efficacy will further be demonstrated. Moreover, whether harmine impacts the activity of other

Fig. 5



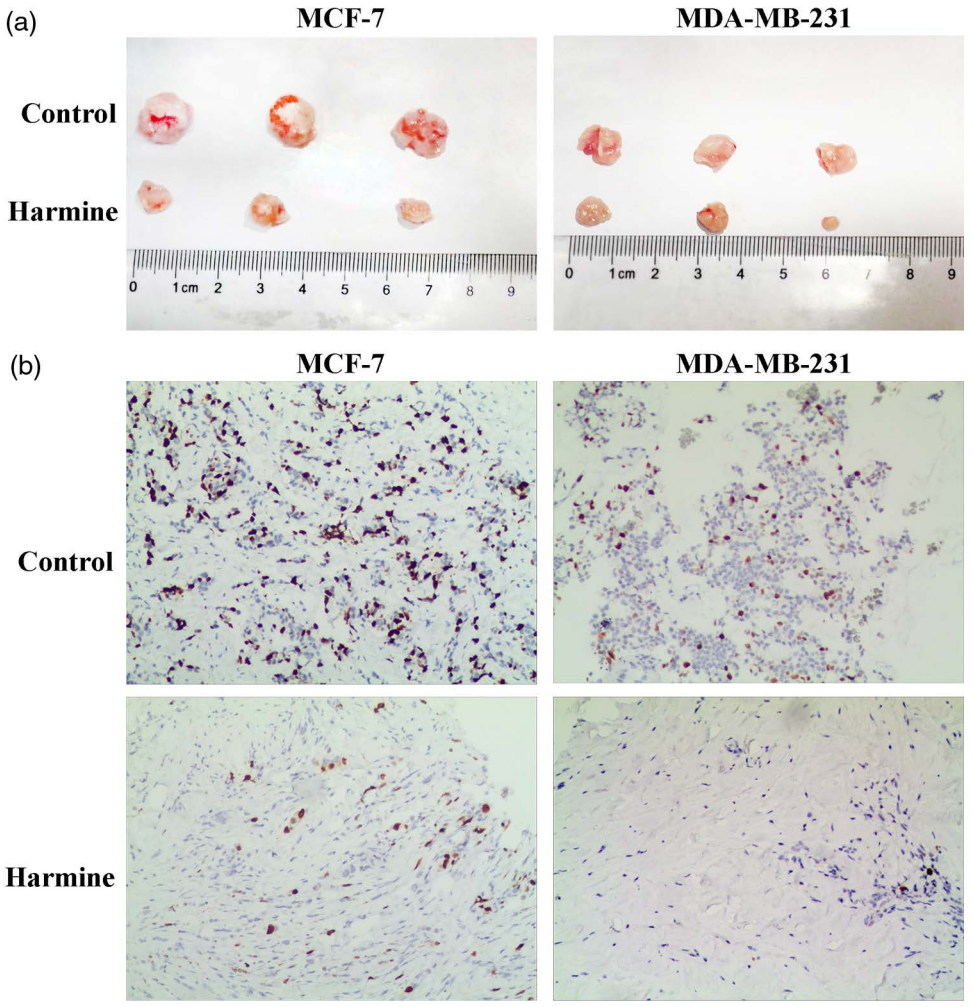
Harmine suppresses the activity of PI3K signaling pathway in breast cancer cells. Western blotting assay showed that PTEN expression increased, while PI3K, p-Akt, and p-mTOR were decreased in a dose-dependent manner. Furthermore, total Akt and total mTOR levels showed no significant alteration. Akt, protein kinase B; PTEN, phosphatase and tension homolog deleted from chromosome 10; PI3K, phosphatidylinositol 3 kinase.

Fig. 6



Harmine suppresses the PI3K signaling by upregulating PTEN. Compared to si-NC, si-PTEN transfection partly rescued the phosphorylation of Akt (p-Akt) and mTOR which were decreased by harmine treatment, yet did not change the total Akt and mTOR levels. Akt, protein kinase B; PTEN, Phosphatase and tension homolog deleted from chromosome 10; PI3K, phosphatidylinositol 3 kinase.

Fig. 7



Harmine suppresses the tumorigenesis of breast cancer cells *in vivo*. (a) The tumor sizes in harmine groups were significantly smaller than in control groups. (b) harmine markedly decreased Ki-67 expression.

signaling pathways is another question we are going to answer in future works.

In conclusion, our results confirmed the effect of Hermine in suppressing breast cancer cell proliferation, invasion, migration, and promoting apoptosis. Furthermore, we demonstrated that hermine could inhibit EMT process and PI3K/AKT signaling. All the data supports that Hermine might be a potential monomer compound against breast cancer. The underlying mechanism of its anticancer effect and its safety and efficacy need to be further in-vivo investigated.

Acknowledgements

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

In the majority of the experiments, the data analysis and article writing were performed by P.Y.; P.Y. assisted in part of the cell experiments (Transwell and MTT); X.K. participated in article writing and language modification; J.Y. designed the study and edited the article.

Conflicts of interest

There are no conflicts of interest.

References

1 Harbeck N, Gnant M. Breast cancer. *Lancet* 2017; **389**:1134–1150.
2 Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin* 2015; **65**:87–108.
3 Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, *et al.* Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; **136**:E359–E386.
4 Malvezzi M, Carioli G, Bertuccio P, Rosso T, Boffetta P, Levi F, *et al.* European cancer mortality predictions for the year 2016 with focus on leukaemias. *Ann Oncol* 2016; **27**:725–731.
5 Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, *et al.*; Early Breast Cancer Trialists' Collaborative Group. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet* 2011; **378**:771–784.

6 Patel K, Gadewar M, Tripathi R, Prasad SK, Patel DK. A review on medicinal importance, pharmacological activity and bioanalytical aspects of beta-carboline alkaloid "Harmine". *Asian Pac J Trop Biomed* 2012; **2**:660–664.
7 Zhang L, Li D, Yu S. Pharmacological effects of harmine and its derivatives: a review. *Arch Pharm Res* 2020; **43**:1259–1275.
8 Ding Y, He J, Huang J, Yu T, Shi X, Zhang T, *et al*. Harmine induces anticancer activity in breast cancer cells via targeting TAZ. *Int J Oncol* 2019; **54**:1995–2004.
9 Li YF, Gao Y, Liang BW, Cao XQ, Sun ZJ, Yu JH, *et al*. Patient-derived organoids of non-small cells lung cancer and their application for drug screening. *Neoplasma* 2020; **67**:430–437.
10 Li C, Wang Y, Wang C, Yi X, Li M, He X. Anticancer activities of harmine by inducing a pro-death autophagy and apoptosis in human gastric cancer cells. *Phytomedicine* 2017; **28**:10–18.
11 Prasad Kushwaha J, Baidya D, Patil S. Harmine-loaded galactosylated pluronic F68-gelucire 44/14 mixed micelles for liver targeting. *Drug Dev Ind Pharm* 2019; **45**:1361–1368.
12 Hamsa TP, Kuttan G. Harmine inhibits tumour specific neo-vessel formation by regulating VEGF, MMP, TIMP and pro-inflammatory mediators both in vivo and in vitro. *Eur J Pharmacol* 2010; **649**:64–73.
13 Owusu-Akyaw A, Krishnamoorthy K, Goldsmith LT, Morelli SS. The role of mesenchymal-epithelial transition in endometrial function. *Hum Reprod Update* 2019; **25**:114–133.
14 Ribatti D, Tamma R, Annese T. Epithelial-mesenchymal transition in cancer: a historical overview. *Transl Oncol* 2020; **13**:100773.
15 Kim H, Lee S, Shin E, Seong KM, Jin YW, Youn H, Youn B. The emerging roles of exosomes as EMT regulators in cancer. *Cells* 2020; **9**:861.
16 Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. *J Clin Invest* 2009; **119**:1420–1428.
17 Jayachandran A, Dhungel B, Steel JC. Epithelial-to-mesenchymal plasticity of cancer stem cells: therapeutic targets in hepatocellular carcinoma. *J Hematol Oncol* 2016; **9**:74.
18 Li Y, Guo D, Zhao Y, Ren M, Lu G, Wang Y, *et al*. Long non-coding RNA SNHG5 promotes human hepatocellular carcinoma progression by regulating miR-26a-5p/GSK3beta signal pathway. *Cell Death Dis* 2018; **9**:888.
19 Hamilton G, Rath B. Mesenchymal-epithelial transition and circulating tumor cells in small cell lung cancer. *Adv Exp Med Biol* 2017; **994**:229–245.
20 Wang Z, Han X. Clinical significance of breast-conserving surgery for early breast cancer and its impact on patient life quality of life. *J BUON* 2019; **24**:1898–1904.
21 Bie B, Sun J, Guo Y, Li J, Jiang W, Yang J, *et al*. Baicalein: a review of its anti-cancer effects and mechanisms in hepatocellular carcinoma. *Biomed Pharmacother* 2017; **93**:1285–1291.
22 Zhao X, Qu J, Liu X, Wang J, Ma X, Zhao X, *et al*. Baicalein suppress EMT of breast cancer by mediating tumor-associated macrophages polarization. *Am J Cancer Res* 2018; **8**:1528–1540.
23 Ma X, Yan W, Dai Z, Gao X, Ma Y, Xu Q, *et al*. Baicalein suppresses metastasis of breast cancer cells by inhibiting EMT via downregulation of SATB1 and Wnt/beta-catenin pathway. *Drug Des Devel Ther* 2016; **10**:1419–1441.
24 Lu M, Xia L, Hua H, Jing Y. Acetyl-keto-beta-boswellic acid induces apoptosis through a death receptor 5-mediated pathway in prostate cancer cells. *Cancer Res* 2008; **68**:1180–1186.
25 Hai-Rong C, Xiang H, Xiao-Rong Z. Harmine suppresses bladder tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis. *Biosci Rep* 2019; **39**:BSR20190155.
26 Uhl KL, Schultz CR, Geerts D, Bachmann AS. Harmine, a dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) inhibitor induces caspase-mediated apoptosis in neuroblastoma. *Cancer Cell Int* 2018; **18**:82.
27 Zhong X, Liu D, Jiang Z, Li C, Chen L, Xia Y, *et al*. Chrysin induced cell apoptosis and inhibited invasion through regulation of TET1 expression in gastric cancer cells. *Onco Targets Ther* 2020; **13**:3277–3287.
28 Machado Brandão-Costa R, Helal-Neto E, Maia Vieira A, Barcellos-de-Souza P, Morgado-Diaz J, Barja-Fidalgo C. Extracellular matrix derived from high metastatic human breast cancer triggers epithelial-mesenchymal transition in epithelial breast cancer cells through alphavbeta3 integrin. *Int J Mol Sci* 2020; **21**:2995.
29 Li H, Chen M, Yang Z, Wang Q, Wang J, Jin D, *et al*. Phillygenin, a MELK inhibitor, inhibits cell survival and epithelial-mesenchymal transition in pancreatic cancer cells. *Onco Targets Ther* 2020; **13**:2833–2842.
30 Lee JH, Chinnathambi A, Alharbi SA, Shair OHM, Sethi G, Ahn KS. Farnesol abrogates epithelial to mesenchymal transition process through regulating Akt/mTOR pathway. *Pharmacol Res* 2019; **150**:104504.
31 Cutone A, Colella B, Pagliaro A, Rosa L, Lepanto MS, Bonaccorsi di Patti MC, *et al*. Native and iron-saturated bovine lactoferrin differently hinder migration in a model of human glioblastoma by reverting epithelial-to-mesenchymal transition-like process and inhibiting interleukin-6/STAT3 axis. *Cell Signal* 2020; **65**:109461.
32 Wang B, Li J. Piceatannol Suppresses the proliferation and induced apoptosis of osteosarcoma cells through PI3K/AKT/mTOR pathway. *Cancer Manag Res* 2020; **12**:2631–2640.
33 Lin JY, Kuo W-W, Baskaran R, Kuo C-H, Chen Y-A, Chen WS, *et al*. Swimming exercise stimulates IGF1/PI3K/Akt and AMPK/SIRT1/PGC1 alpha survival signaling to suppress apoptosis and inflammation in aging hippocampus. *Aging (Albany NY)* 2020; **12**:6852–6864.
34 Fu X, Ming Y, Li C, Niu Y, Lin Q, Liu L, *et al*. Siniperca chuatsi rhabdovirus (SCRV) induces autophagy via PI3K/Akt-mTOR pathway in CPB cells. *Fish Shellfish Immunol* 2020; **102**:381–388.
35 Shi D, Zhao P, Cui L, Li H, Sun L, Niu J, *et al*. Inhibition of PI3K/AKT molecular pathway mediated by membrane estrogen receptor GPER accounts for cryptotanshinone induced antiproliferative effect on breast cancer SKBR-3 cells. *BMC Pharmacol Toxicol* 2020; **21**:32.
36 Baselga J, Im S-A, Iwata H, Cortés J, De Laurentiis M, Jiang Z, *et al*. Buparlisib plus fulvestrant versus placebo plus fulvestrant in postmenopausal, hormone receptor-positive, HER2-negative, advanced breast cancer (BELLE-2): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Oncol* 2017; **18**:904–916.