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Original Article

Harmine suppresses breast cancer cell migration and invasion by regulating TAZ-mediated epithelial-mesenchymal transition

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Abstract: Breast cancer is a highly lethal disease due to cancer metastasis. Harmine (HM), a β-carboline alkaloid, is present in various medicinal plants. Our previous study demonstrated that HM suppresses cell proliferation and migration by regulating TAZ in breast cancer cells and accelerates apoptosis. Epithelial-mesenchymal transition (EMT) plays an important role in the development of breast cancer by inducing the characteristics of cancer stem cells, cancer metastasis and recurrence. Overexpression of TAZ was shown to mediate EMT in breast cancer cells. We aimed to investigate whether HM inhibits EMT and metastasis of breast cancer cells by targeting TAZ. In this study, the cells treated with HM or with downregulated expression of TAZ showed an increase in epithelial markers and decrease in mesenchymal markers in breast cancer cells. Consistently, the breast cancer cells treated with HM or with downregulated expression of TAZ showed suppressed migration and proliferation. Moreover, TAZ overexpression reversed EMT and metastasis induced by HM in breast cancer cells. Thus, HM suppresses EMT and metastasis and invasion by targeting TAZ in breast cancer cells. HM can be used as an anticancer drug for breast cancer treatment and chemoprevention.

Keywords: Harmine, TAZ, epithelial-mesenchymal transition, metastasis, breast cancer cell

Introduction

Breast cancer is the most commonly diagnosed malignant tumor in women, with high morbidity and mortality in female patients. Most mortality from breast cancer is due to metastasis and strong invasion, which seriously threaten human health [1]. Most deaths from breast cancer are due to its high metastatic rate and strong invasiveness [2]. Therefore, elucidation of the specific mechanism of cancer metastasis is urgently needed.

Cancer metastasis is a complex mechanism involving cascades of continuous processes. Epithelial-mesenchymal transition (EMT) is recognized as an early event. Adhesive epithelial cells become motile and moving mesenchymal cells during the EMT process, and these cells show an altered phenotype, such as loss of apical basal polarity, cell-to-cell junctions and actin cytoskeleton reorganization. Instead, these cells develop mesenchymal-like properties, such as anteroposterior polarity and increased mobility and invasion. Moreover, the expression levels of epithelial markers such as E-cadherin are downregulated, and the expression levels of mesenchymal markers such as N-cadherin, Fibronectin and Vimentin are upregulated [3, 4]. These motile cancer cells have the ability to escape from the primary tumor position into surrounding tissues, resulting in tumor metastasis and relapse. Accumulating evidence suggests that EMT is mediated by various signaling pathways, such as the TGF-β, Hippo-YAP/TAZ, Wnt/β-catenin, hypoxia-inducible factor 1/2, and Notch pathways [5, 6]. The induction of EMT plays an essential role in the poor prognosis of several cancers, including breast cancer [7], gastric cancer [8] and hepatocellular carci-

noma [9]. The activation of EMT leads to therapeutic resistance, cancer stem cell production and metastasis to distant tissue. Therefore, EMT is important for metastasis of breast cancer cells.

Harmine (HM) is isolated from seeds of *Peganum harmala* and *Banisteriopsis caapiis* and is a natural beta-carboline alkaloid [10]. Studies have suggested that HM has remarkable antitumor activities that suppress proliferation, metastasis and invasion in breast cancer and induce apoptosis and autophagy [11, 12]. However, whether harmine exhibits antitumor effects by regulating EMT is unclear.

The transcriptional coactivator TAZ with a PDZ binding motif is known as a cancer gene expressed in various cancer cells, such as colorectal tumors [13], glioblastoma [14] and breast cancer [15]. Overexpression of TAZ has been reported to cause cancer cell proliferation, migration and invasion, and TAZ has been reported to contribute to EMT and metastasis of breast cancer [16, 17]. Our previous study confirmed that harmine promoted the proliferation and migration and facilitated the apoptosis of breast cancer cells by targeting TAZ [11], but the exact molecular mechanism by which harmine regulates TAZ is still unknown. Since TAZ is a major factor in EMT and metastasis, it is important to determine whether HM can affect the progression of breast cancer by inhibiting TAZ-mediated EMT.

In this study, we examined the effect of harmine on EMT and metastasis of breast cancer cells. We first showed that harmine inhibited EMT and migration by inhibiting TAZ activity in breast cancer cells. Our findings showed that harmine is a potential drug candidate for breast cancer.

Materials and methods

Tumor tissue samples

Animal experiments were approved at Tongji Medical College, Huazhong University of Science and Technology and complied with a guide for the care and use of experimental animals. The animal tumor tissue samples were obtained from a previous study of a mouse xenograft mouse model [11]. When nude BALB/c mice were inoculated with MCF-7 cells and the tumor size became 100 mm^3, they were treated with HM (40 mg/kg/day) or normal saline for 2 weeks, the mice were sacrificed, and the tumor tissue was collected. Clinical samples were obtained from The Central Hospital of Wuhan, and the experimental protocols were approved by the local ethics committee.

Immunohistochemistry staining

Immunohistochemistry was carried out on 5 μm thick paraffin sections. The sections were deparaffinized and incubated with 3% H_2O_2 for 30 min to eliminate endogenous peroxidase activity. Then, 3% BSA was added to prevent nonspecific reactions. The primary antibodies were incubated overnight at 4°C: anti-TAZ (#70148, Cell Signaling Technology, US), anti-E-cadherin (#610181, BD Biosciences, USA), anti-N-cadherin (#13116, Cell Signaling Technology, USA), anti-Vimentin (#5741, Cell Signaling Technology, USA) and anti-fibronectin (#ab2413, Abcam, UK). Subsequently, the sections were washed with PBS three times and cultured with biotinylated goat anti-rabbit secondary antibody (Beyotime Institute of Biotechnology, China) for 1 h at 37°C.

Diaminobenzidine tetrachloride (Beyotime Institute of Biotechnology, China) was used for dyeing. The dyed sections were photographed with an optical microscope (Olympus Corporation).

Cell culture and HM treatment

MDA-MB-231 and MCF-7 cells were purchased from the Shanghai Institute of Biochemistry and Cell Biology (Shanghai, China). The cells were maintained in DMEM (Gibco, USA) containing 10% FBS (Gibco, USA) at 37°C with 5% CO_2 in an incubator. HM was dissolved in DMSO at 200 mM and diluted in culture medium to the final experimental concentration.

Cell transfection

Cells were transfected with the pCMV6-TAZ expression vector or the pCMV6-control vector (Origene Technologies, USA) using Lipofectamine 3000 (Thermo Fisher Scientific, USA). TAZ siRNA (#S100111237) and negative control siRNA (#S100111230) were purchased from Qiagen (USA). Cells were transfected with siRNA transfection reagent in accordance with the manufacturer's instructions.

EdU assay

MDA-MB-231 and MCF7 cells were seeded in 96-well plates for 24 h, and the cells were treated with various concentrations of HM (0, 50, 100, 150 μM) after transfection with TAZ vector or siRNA for 24 h. After 24 h, the cells were cultured with 25 mmol/L EdU (RiboBio, China) for 2 h. All cells were immobilized with 4% paraformaldehyde for 30 min. The cell nuclei were stained using 5 μg/ml Hoechst. The dyed cells were captured by fluorescence microscopy (Olympus Corporation).

Colony formation assay

MDA-MB-231 and MCF-7 cells were trypsinized and seeded at 1×10^3 cells/well in 6-well plates in triplicate. After culture for 1 to 2 weeks, the cells were washed three times with PBS, fixed with 4% paraformaldehyde for 15 minutes and stained for 10 minutes. The number of colonies containing more than 50 cells was counted. Three independent experiments were carried out for this assay.

Transwell invasion assay

MDA-MB-231 and MCF-7 cells were subjected to transwell assays after transfection. The basolateral chamber was balanced with 600 μL of DMEM containing 10% FBS, while the apical chamber contained 200 μL of MDA-MB-231 cell or MCF-7 cell suspension in FBS-free DMEM. After culture for 24 h at 37°C with 5% CO_2, the transwell chambers were fixed for 15 min with 4% paraformaldehyde and then stained with 0.1% crystal violet for 10 min in accordance with the manufacturer's protocols for the transwell system (Corning, USA). The transmembrane cells were observed with an inverted fluorescence microscope.

Western blotting

Cell lysis buffer (#P0013B, Beyotime Institute of Biotechnology, China) was used to extract breast cancer cells. A BCA Protein Assay kit (#CW0014S, CWBio, China) was used to determine the protein concentration. The protein was transferred to a nitrocellulose membrane after separation by 10% SDS-PAGE. Nonspecific binding was blocked with 5% nonfat skim milk for 1 h at room temperature and subsequently incubated with primary antibodies at 4°C overnight. The primary antibodies included anti-TAZ (#70148, Cell Signaling Technology, US), anti-E-cadherin (#610181, BD Biosciences, USA), anti-N-cadherin (#13116, Cell Signaling Technology, USA), anti-Vimentin (#5741, Cell Signaling Technology, USA), anti-fibronectin (#ab2413, Abcam, UK) and anti-GAPDH (#5174, Cell Signaling Technology, USA). The protein was incubated with goat anti-rabbit IgG (#sc-2040, Santa Cruz Biotechnology, USA) or goat anti-mouse IgG antibody (#sc-2005, Santa Cruz Biotechnology, USA) conjugated with horseradish peroxidase for 1 h at room temperature after rinsing with TBST three times (10 min each). Image analysis was performed using Image-Pro Plus software (Version 7.0, Silver Springs, MD, USA) according to the target band relative to the GAPDH band.

RNA extraction and reverse transcription-quantitative PCR (RT-qPCR) analysis

Total RNA extracted from MDA-MB-231 cells and MCF-7 cells using TRIzol Reagent (#155-96018, Thermo Fisher Scientific, USA) was reverse transcribed into cDNA with a ReverTra Ace qPCR RT Kit (#FSQ-101, Toyobo Life Science, Japan). β-actin was used as the internal control, and the relative expression levels of target genes were calculated by relative quantification ($2^{-\Delta\Delta Ct}$ method). The primer sequences synthesized by Shanghai Sangon Biotechnology (Shanghai, China) for RT-qPCR are listed in the Supplementary Table 1.

Immunofluorescence staining

Breast cancer cells were seeded on chamber slides overnight. The cells were treated with harmine (0, 50, 100, 150 μM) for 24 h. Then, the cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100 in PBS and incubated with 3% bovine serum albumin for 30 min. The primary antibodies were incubated overnight at 4°C and washed three times with PBS. The cells were incubated with anti-mouse IgG-Alexa Fluor 488 (#A11001, Life Technologies, US) for 1 h at room temperature and washed with PBS 3 times. Then, 10 μg/ml Hoechst 33342 (#14533, Life Technologies, US) was used for nuclear staining. Images were obtained by fluorescence microscopy (Olympus Corporation).

Statistical analysis

All statistical data were analyzed using SPSS 22.0 software (IBM Corp., Armonk, NY, USA),

and the measured data are shown as the mean ± SD. Comparisons between the two groups were performed by Student's t test, and the difference between more than two groups was assessed using one-way ANOVA with Dunnett's post-hoc test. A value of $P<0.05$ and $P<0.01$ was considered statistically significant.

Result

EMT is induced in breast cancer patient tissues, and HM inhibits the expression of EMT-related molecules and activity of TAZ

TAZ, E-cadherin, N-cadherin, vimentin and fibronectin were monitored by IHC in breast cancer patient tissues and mouse mammary xenograft tumor tissues. We found that the expression of the epithelial marker E-cadherin was decreased, the expression of the mesenchymal markers N-cadherin, vimentin, and fibronectin was increased, and TAZ levels were upregulated in breast cancer patient tissues (**Figure 1A**). These results suggested that breast cancer might induce EMT activation and TAZ expression. Moreover, we examined whether HM inhibits EMT and TAZ activation in breast cancer. Paraffin-embedded tissues of mouse breast xenograft tumors treated with HM were obtained from previous experiments. In short, nude BALB/c mice were injected with MCF-7 cells and treated with HM (40 mg/kg/day). Four weeks later, tumor tissues were collected from all mice. Compared with that of the control group, the expression of E-cadherin was significantly elevated, and the expression of N-cadherin, vimentin, fibronectin and TAZ was reduced in the tumor tissues treated with HM (**Figure 1B**). We also determined TAZ activity after MDA-MB-231 and MCF-7 cells were treated with HM. MDA-MB-231 and MCF-7 cells were treated with HM (0, 50, 100, 150 μM) for 24 h, the TAZ phosphorylation levels were detected by western blotting, and TAZ nuclear localization was observed by immunofluorescence. HM increased the level of TAZ phosphorylation and inhibited the nuclear localization of TAZ (**Figure 1C** and **1D**). These results indicated that HM could suppress EMT and TAZ activation.

HM inhibits EMT, proliferation and invasion in breast cancer cells

More specifically, to study the regulation of EMT by HM, we treated MDA-MB-231 and MCF-7 cells with HM at final concentrations of 0, 50, 100, and 150 μM. The levels of E-cadherin, N-cadherin, vimentin and fibronectin were determined by RT-*q*PCR and western blotting. HM upregulated the expression of the epithelial marker E-cadherin and downregulated the expression of the mesenchymal markers N-cadherin, vimentin, and fibronectin in a dose-dependent manner (**Figure 2A** and **2B**), and cell morphology after HM treatment was observed. The EMT-like cell morphology was significantly inhibited after treatment with HM (**Figure 2C**). EMT plays an important role in breast cancer metastasis, and we investigated whether HM mediates the suppression of migration and invasion in breast cancer cells. For this purpose, MDA-MB-231 and MCF-7 cells were treated with different concentrations of HM (0, 50, 100, 150 μM), cell proliferation was evaluated through EdU assays, and colony formation, cell migration and invasion were determined by transwell invasion assays. We found that HM inhibited the growth and invasion of MDA-MB-231 and MCF-7 cells in a dose-dependent manner (**Figure 3A-C**). These data showed that HM suppressed EMT, proliferation and invasion of breast cancer cells in a dose-dependent manner.

TAZ overexpression accelerates mesenchymal transformation and promotes proliferation, migration and invasion of breast cancer cells

To investigate the regulation of EMT by TAZ, we transfected MDA-MB-231 and MCF-7 cells with either a TAZ-expressing plasmid (pCMV6-TAZ) or a control plasmid (pCMV6-control). The transfection efficiency of TAZ was determined by RT-qPCR analysis after 24 h of transfection and western blotting analysis after 48 h of transfection. The expression of TAZ was increased in the TAZ overexpression group (**Figure 4A** and **4C**). Previous studies have shown that the molecular regulatory mechanism of EMT is closely related to the growth, invasion and metastasis of malignant tumors [18, 19]; therefore, we examined TAZ and the expression of EMT-related molecules in breast cancer cells. We found that TAZ overexpression reduced the mRNA and protein levels of an epithelial marker (E-cadherin) but increased the mRNA and protein levels of mesenchymal markers (N-cadherin, fibronectin and vimentin) (**Figure 4B** and **4C**), inhibited the epithelial properties and resulted in a spindle-like cell

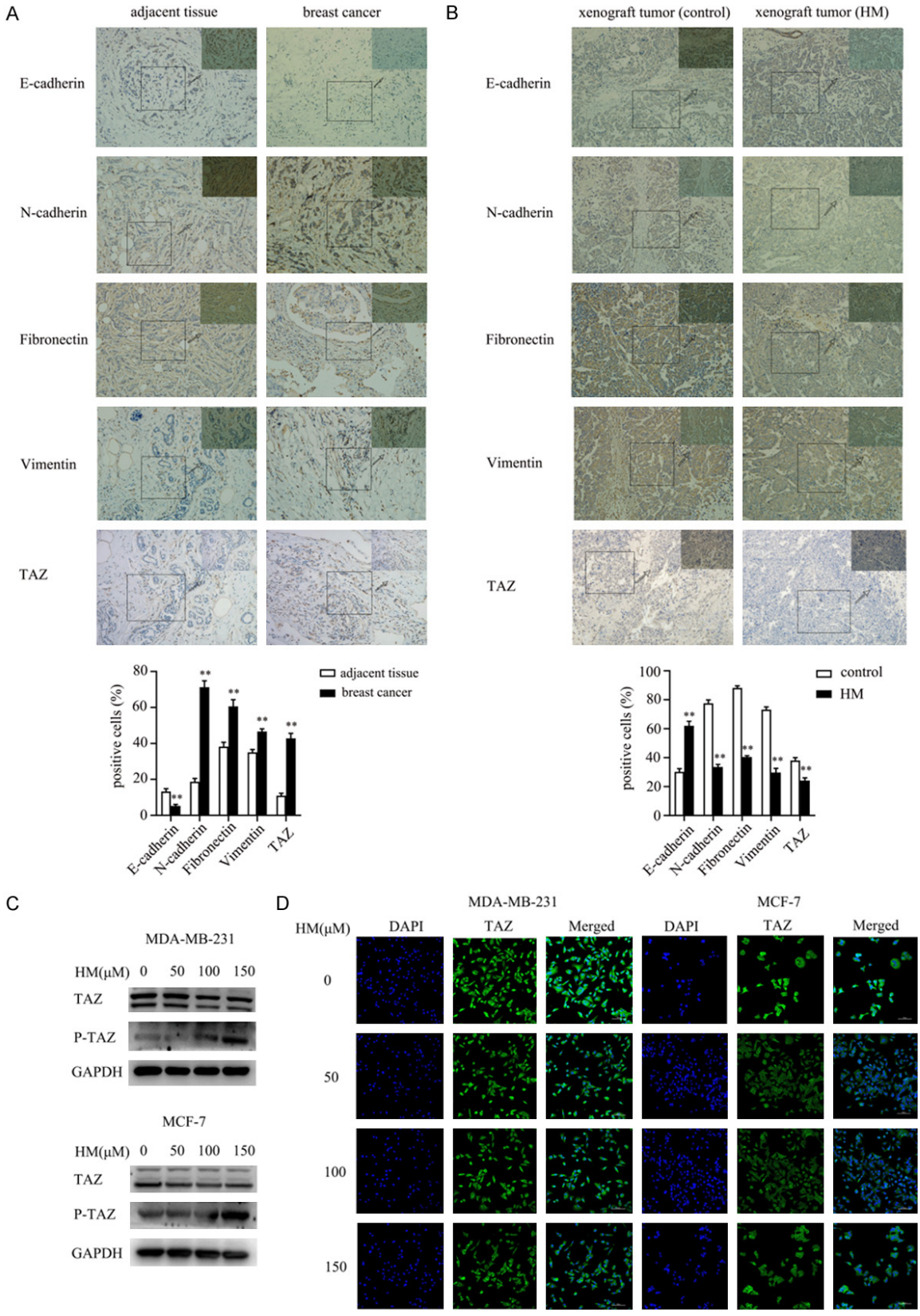


Figure 1. EMT is induced in breast cancer patients, and HM inhibits the expression of EMT-related molecules in mouse breast xenograft tumor tissues. A and B. EMT markers (E-cadherin, N-cadherin, vimentin and fibronectin)

were determined by IHC in breast cancer patient tissues and mouse breast xenograft tumor tissues. Magnification, ×200, ×400. C, D. MDA-MB-231 and MCF-7 cells were treated with HM (0, 50, 100, 150 μM) for 24 h. The TAZ phosphorylation level was detected via western blotting, and TAZ nuclear localization was observed by immunofluorescence. Magnification, ×200.

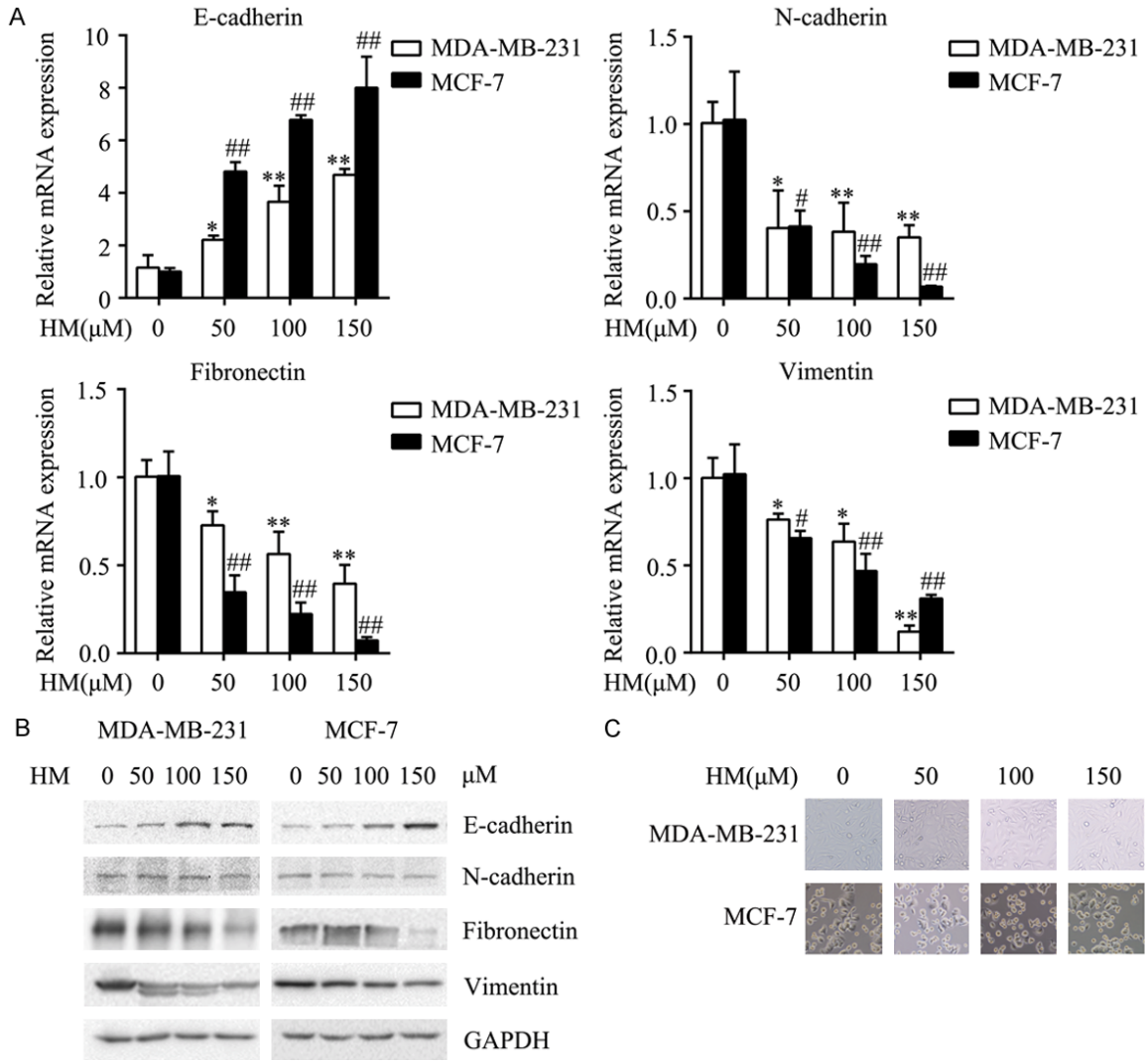


Figure 2. HM reduces the expression of EMT-related molecules. A, B. MDA-MB-231 and MCF-7 cells were treated with HM (0, 50, 100, 150 μM), and the relative mRNA and protein levels of EMT markers (E-cadherin, N-cadherin, vimentin and fibronectin) were determined by RT-*q*PCR and western blotting. C. Morphology of MDA-MB-231 and MCF-7 cells treated with HM (0, 50, 100, 150 μM) for 24 h. Data are presented as the mean ± SD of three independent experiments. **P*<0.05, ***P*<0.01, #*P*<0.05, ##*P*<0.01.

morphology in which the spindle fibroblast-like form was obtained (**Figure 4D**). In addition, the EdU assay and colony formation assay revealed that overexpression of TAZ promoted cell proliferation (**Figure 4E** and **4G**), and the transwell invasion assay indicated that overexpression of TAZ promoted migration and infiltration in MDA-MB-231 and MCF-7 cells (**Figure 4F**). These results suggested that TAZ overexpression can stimulate EMT, proliferation, migration and invasion of breast cancer cells.

TAZ knockdown reduces mesenchymal transformation and inhibits proliferation, migration and invasion of breast cancer cells

To further investigate the regulation of EMT by TAZ, we transfected MDA-MB-231 and MCF-7

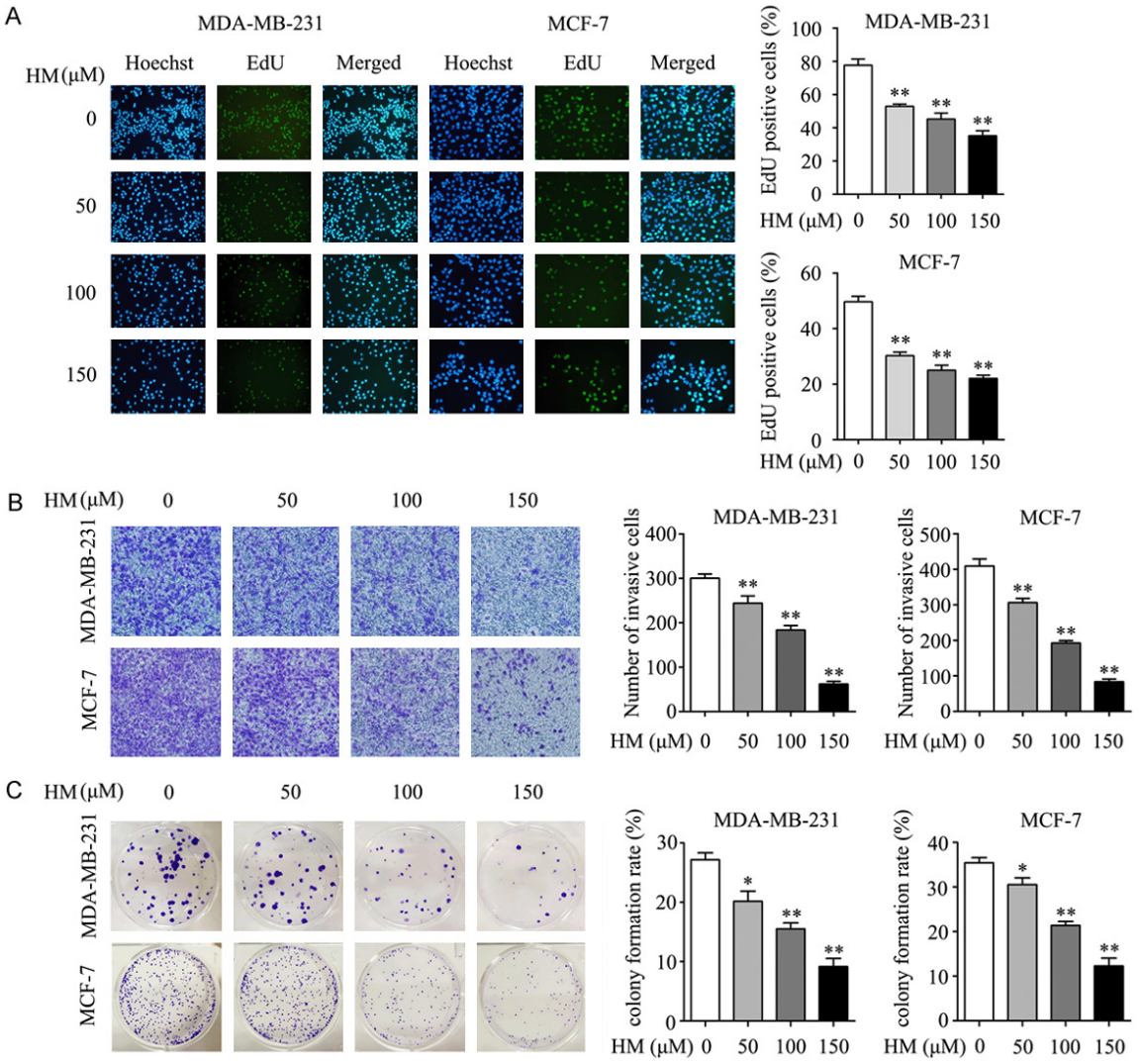


Figure 3. HM inhibits the proliferation and invasion of breast cancer cells. MDA-MB-231 and MCF-7 cells were treated with HM (0, 50, 100, 150 μM). A, C. Cell proliferation was determined by EdU assays (magnification, ×100) and colony formation assays, and the histograms show the EdU-positive cell rate and colony formation rate. B. Cell migration and invasion were analyzed by transwell invasion assays (magnification, ×100), and the histogram shows the numbers of invaded cells. Data are presented as the mean ± SD from three independent experiments. **P*<0.05 and ***P*<0.01.

cells with short interfering siRNA targeting TAZ (siTAZ) or nontargeting siRNA (siCON). The silencing efficiency of TAZ was determined by RT-qPCR analysis after 24 h of transfection and western blotting analysis after 48 h of transfection. The expression of TAZ was downregulated in the TAZ silencing group (**Figure 5A** and **5C**). TAZ knockdown caused upregulated expression of the epithelial marker E-cadherin and downregulated expression of the mesenchymal markers N-cadherin, fibronectin and vimentin at the mRNA and protein levels (**Figure 5B** and **5C**) and inhibited the EMT-like cell morphology (**Figure 5D**). In addition, TAZ knockout suppressed the growth of MDA-MB-231 and MCF-7 cells, as determined by EdU and colonization assays (**Figure 5E** and **5G**), and inhibited migration and invasion, as determined by transwell assays (**Figure 5F**). These results suggested that TAZ inhibition can reduce EMT, proliferation, migration and invasion of breast cancer cells.

TAZ mediates the EMT, migration and invasion in breast cancer cells regulated by harmine

Our previous results suggested that HM suppresses EMT, migration and invasion in breast

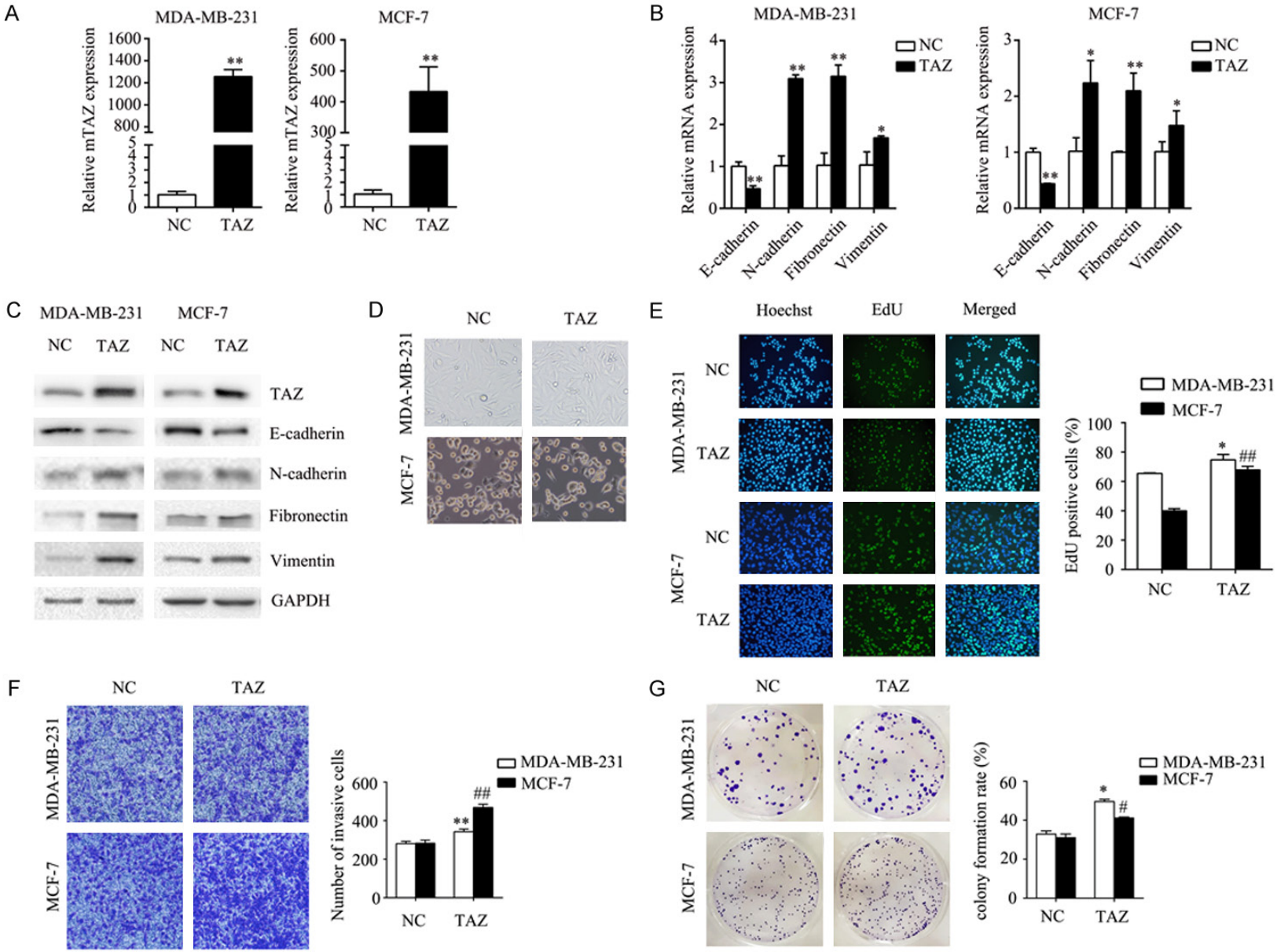

A
MDA-MB-231
MCF-7
Relative mTAZ expression
NC
TAZ
B
MDA-MB-231
MCF-7
Relative mRNA expression
NC
TAZ
E-cadherin
N-cadherin
Fibronectin
Vimentin
C
MDA-MB-231
MCF-7
NC
TAZ
TAZ
E-cadherin
N-cadherin
Fibronectin
Vimentin
GAPDH
D
NC
TAZ
MDA-MB-231
MCF-7
E
Hoechst
EdU
Merged
MDA-MB-231
MCF-7
EdU positive cells (%)
F
Number of invasive cells
G
colony formation rate (%)

Figure 4. TAZ overexpression is associated with increased proliferation, migration and invasion in breast cancer cells and the emergence of a mesenchymal phenotype. A, C. Relative mRNA levels and protein expression of TAZ in the negative control group and TAZ-overexpressing group of MDA-MB-231 and MCF-7 cells were detected by RT-*q*PCR analysis and western blotting assays. B, C. Relative mRNA levels and protein expression of the epithelial marker E-cadherin and mesenchymal markers (N-cadherin, fibronectin and vimentin) in TAZ-overexpressing MDA-MB-231 and MCF-7 cells were detected by RT-*q*PCR analysis and western blotting assays. D. Morphology of MDA-MB-231 and MCF-7 cells after TAZ transfection for 24 h. E, G. TAZ overexpression increased MDA-MB-231 and MCF-7 cell proliferation, as determined by EdU and colony formation assays. F. TAZ overexpression promoted MDA-MB-231 and MCF-7 cell migration and invasion, as determined by transwell invasion assays. Data are presented as the mean ± SD of three independent experiments. $^{*}P<0.05$, $^{**}P<0.01$, $^{\#}P<0.05$, $^{\#\#}P<0.01$.

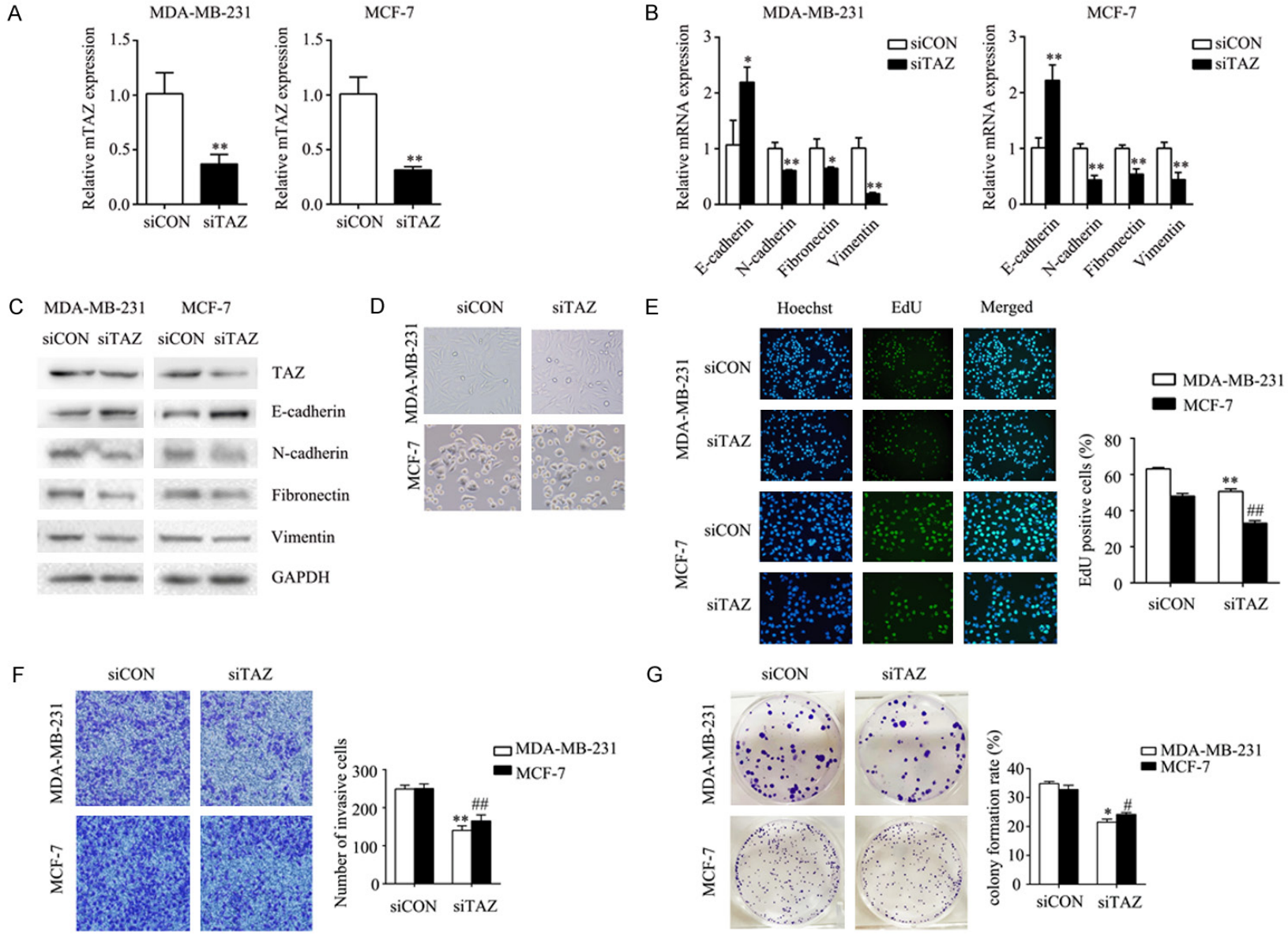
cancer cells. To determine whether TAZ meditates the process of HM-regulated EMT, we transfected MDA-MB-231 and MCF-7 cells with either control plasmid (pCMV6-control) or TAZ-expressing plasmid (pCMV6-TAZ). After 24 h of transfection, the cells were treated with HM (0, 50 μM) for 24 h, and then, the relative mRNA expression levels of E-cadherin, N-cadherin, fibronectin and vimentin were measured by RT-*q*PCR assays. As shown in **Figure 6A**, TAZ transfection inhibited HM-induced upregulation of E-cadherin expression and downregulation of N-cadherin, fibronectin, and vimentin expression. Moreover, western blotting was performed to assess the protein levels. We found that TAZ transfection inhibited the change in the protein level of EMT markers induced by HM (**Figure 6B**). Cell morphology was also observed, and the TAZ transfection group showed reversal of the HM-induced EMT-like cell morphology (**Figure 6C**). To investigate how TAZ mediates HM-induced migration and invasion, we examined migration and invasion in breast cancer cells. The results of the EdU assay and colony formation assay revealed that TAZ overexpression blocked HM-induced inhibition of cell proliferation compared with that of the negative control group (**Figure 7A** and **7C**). In addition, TAZ overexpression blocked HM-induced cell migration and invasion in a transwell invasion assay (**Figure 7B**).

Discussion

Breast cancer is derived from mammary epithelial tissue and is one of the most common cancers among women. Investigation of the molecular mechanisms of tumor development and metastasis is important. Previous studies have shown that harmine, originally isolated from *P. harmala* seeds, can suppress tumor cell proliferation and tumor progression in various cancers, including breast cancer [11, 20-22]. In this study, we identified the regulation of EMT-related molecules by HM in breast cancer cells and found that TAZ mediates HM-regulated EMT. In this study, we clarified a novel mechanism of TAZ-mediated EMT in breast cancer cells.

In previous studies, HM, a natural β-carboline alkaloid, had a significant inhibitory effect on various cancers, such as pancreatic cancer [22], thyroid cancer [23] and colorectal cancer [24]. Our previous studies also indicated that HM induces anticancer activity in breast cancer [11]. All this evidence indicated that HM functions as a tumor-suppressive molecule in tumor progression and metastasis. Epithelial-mesenchymal transition (EMT) is a process in which epithelial cells lose apical-basal polarity and cell-cell adhesion, transition to an invasive mesenchymal phenotype and play a critical role in cancer metastasis, progression and drug resistance [25]. Nevertheless, the relationship between HM and EMT in breast cancer cells has not been examined. In the current study, we investigated the changed levels of EMT markers and cell morphology after treatment with different doses of HM in vitro and in vivo. In vitro, MDA-MB-231 and MCF-7 cells were used to demonstrate that HM significantly increased the mRNA and protein levels of an epithelial marker (E-cadherin) but decreased the mRNA and protein levels of mesenchymal markers (N-cadherin, fibronectin and vimentin) in a dose-dependent manner and inhibited EMT-like cell morphology. In vivo, HM was found to inhibit EMT in MCF-7 cell-derived xenograft tumors. These results suggested that HM might affect EMT to suppress breast cancer cell growth and metastasis.

Recently, the molecular mechanisms of EMT and several signaling transduction pathways associated with EMT have been widely identified. The key signaling pathways involved in EMT include TGF-β signaling [26, 27], Sonic Hedgehog (SHH) signaling [28], Wnt signaling [29], etc. The Hippo/TAZ signaling pathway is


A
MDA-MB-231
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Relative mTAZ expression
siCON
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B
MDA-MB-231
MCF-7
Relative mRNA expression
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siTAZ
E-cadherin
N-cadherin
Fibronectin
Vimentin
C
MDA-MB-231
MCF-7
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N-cadherin
Fibronectin
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GAPDH
D
siCON
siTAZ
MDA-MB-231
MCF-7
E
Hoechst
EdU
Merged
MDA-MB-231
MCF-7
EdU positive cells (%)
F
siCON
siTAZ
MDA-MB-231
MCF-7
Number of invasive cells
G
siCON
siTAZ
MDA-MB-231
MCF-7
colony formation rate (%)

Figure 5. TAZ silencing prevents mesenchymal transformation and inhibits breast cancer cell proliferation and invasion. A, C. Relative mRNA levels and protein expression of TAZ in the TAZ knockdown group and the control group of MDA-MB-231 and MCF-7 cells were detected by RT-*q*PCR analysis and western blotting assay. B, C. Relative mRNA levels and protein expression of the epithelial marker E-cadherin and mesenchymal markers (N-cadherin, fibronectin and vimentin) in TAZ knockdown MDA-MB-231 and MCF-7 cells were detected by RT-*q*PCR analysis and western blotting assays. D. Morphology of MDA-MB-231 and MCF-7 cells transfected with TAZ siRNA for 24 h. E, G. TAZ knockdown suppressed MDA-MB-231 and MCF-7 cell proliferation, as determined by EdU assay and colony formation assay. F. TAZ knockdown inhibited MDA-MB-231 and MCF-7 cell migration and invasion, as determined by transwell invasion assays. Data are presented as the mean ± SD of three independent experiments. **P*<0.05, ***P*<0.01, #*P*<0.05, ##*P*<0.01.

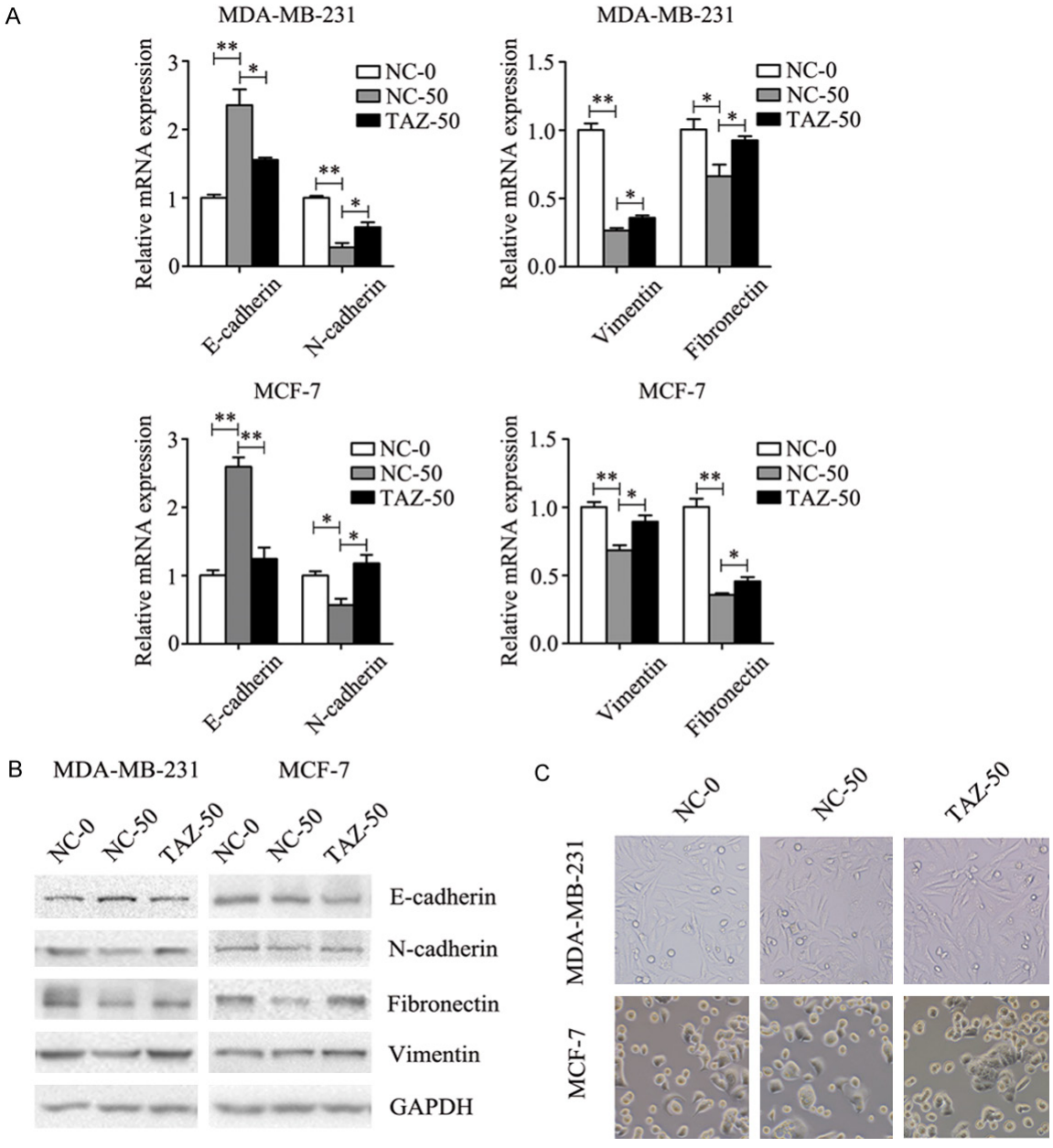

Figure 6. TAZ overexpression mediates the epithelial-mesenchymal transition induced by harmine. A. MDA-MB-231 and MCF-7 cells were transfected with pCMV6-control and pCMV6-TAZ and then treated with HM (0, 50 μM) for 24 h after 24 h of transfection. The relative mRNA levels of the epithelial marker E-cadherin and mesenchymal markers (N-cadherin, fibronectin and vimentin) were detected by RT-*q*PCR analysis. B. MDA-MB-231 and MCF-7 cells were transfected with pCMV6-control and pCMV6-TAZ and then treated with HM (0, 50 μM) for 48 h after 24 h of

transfection. The relative protein levels of the epithelial marker E-cadherin and mesenchymal markers (N-cadherin, fibronectin and vimentin) were detected by western blotting analysis. C. Morphology of MDA-MB-231 and MCF-7 cells after transfection with pCMV6-control and pCMV6-TAZ and treatment with HM (0, 50 μM) for 48 h after 24 h of transfection. Data are presented as the mean ± SD of three independent experiments. *P<0.05, **P<0.01.

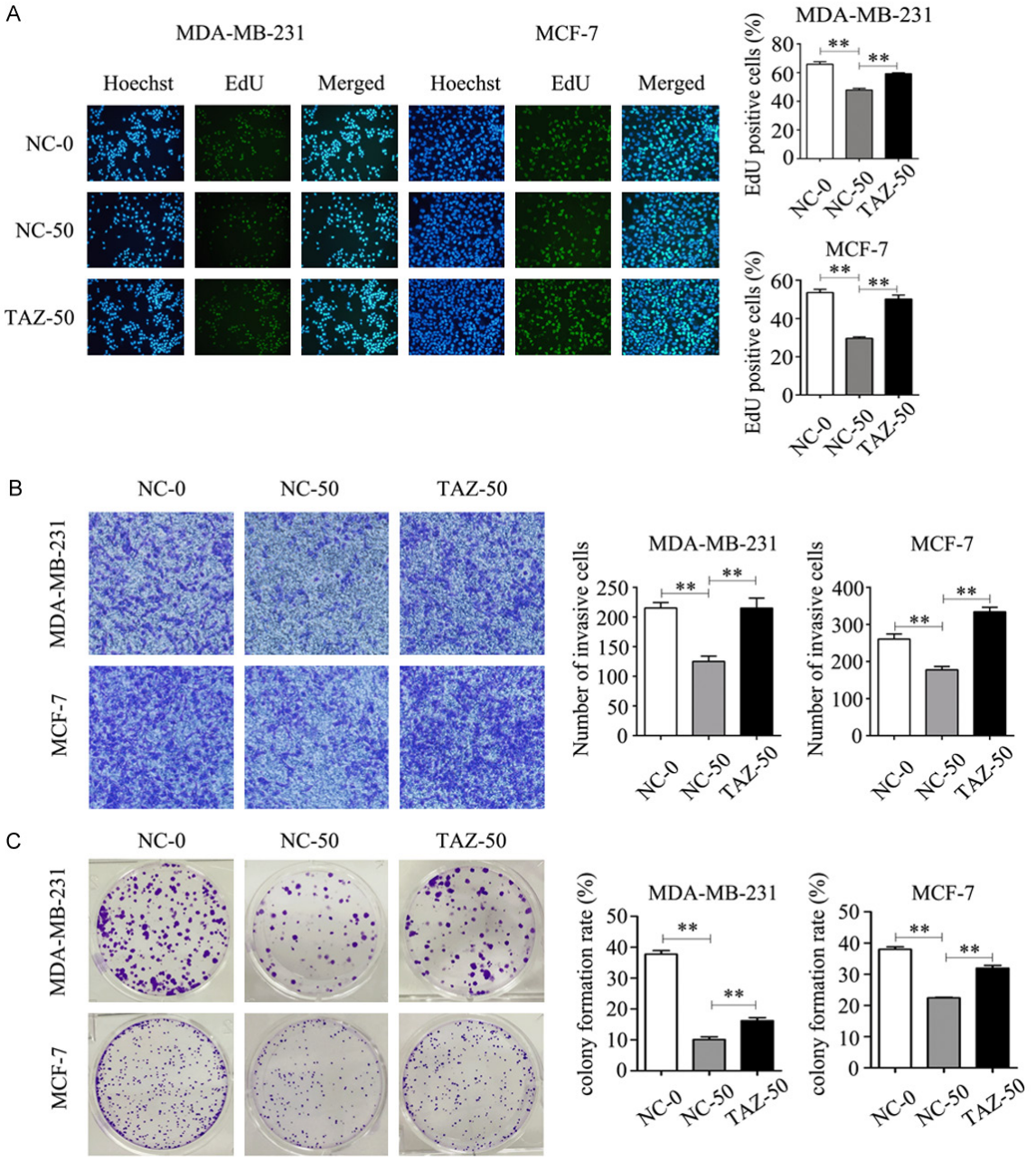

Figure 7. TAZ overexpression blocked the migration and invasion of breast cancer cells regulated by harmine. A, C. The proliferative activities of MDA-MB-231 and MCF-7 cells transfected with pCMV6-control or pCMV6-TAZ with or without HM (50 μM) were determined by EdU and colony formation assays. B. Migration and invasion of MDA-MB-231 and MCF-7 cells transfected with pCMV6-control or pCMV6-TAZ with or without HM (50 μM) were evaluated by transwell invasion assays. Data are presented as the mean ± SD of three independent experiments. *P<0.05, **P<0.01.

an important signaling pathway that regulates intracellular environmental stability, stem cell self-renewal and tissue regeneration, while YAP/TAZ is the main downstream effector mol-

ecule of the Hippo pathway in mammals [30, 31]. Previous studies have reported that the Hippo signaling pathway is associated with tumor cell migration and metastasis as well as the process of EMT. Li Yuan et al. demonstrated that JSD reverses EMT and inhibits the invasion and metastasis of colorectal cancer cells through the Hippo signaling pathway [32]. Other studies have demonstrated that inhibition of YAP can suppress the occurrence of EMT and metastasis of pancreatic cancer cells and hepatocellular carcinoma [33, 34].

In the present study, MDA-MB-231 and MCF-7 cells were constructed with TAZ overexpression and knockdown cell models to investigate the regulation of EMT markers and cell morphology by TAZ. TAZ downregulated the expression of the epithelial marker E-cadherin, upregulated the expression of mesenchymal markers (N-cadherin, fibronectin and vimentin) and induced EMT-like cell morphology in breast cancer cells. Furthermore, cell proliferation, migration and invasion were determined. All these results indicated that TAZ may accelerate mesenchymal transformation and promote proliferation, migration and invasion in breast cancer cells.

The enhanced intercellular mesenchymal state and cell motility associated with the EMT program provide the basic conditions for the migration and invasion of tumor cells [35]. Thus, a variety of studies utilizing human cancer cell lines demonstrated that tumor cells decompose basic membrane and cell-cell junctions to support migration and invasion as single cells during the progression of EMT [36, 37].

Earlier studies revealed that TAZ and miR-135b participate in positive feedback loops to regulate EMT and metastasis of osteosarcoma [38]. In this study, we examined whether TAZ mediates HM-induced EMT in breast cancer cells. Experiments were performed using MDA-MB-231 and MCF-7 cells to show that HM increases the level of TAZ phosphorylation, inhibits the nuclear localization of TAZ and may reverse EMT and inhibit migration and invasion by inhibiting the expression of TAZ.

In conclusion, the present study validated that harmine could reverse the progression of EMT and inhibit the proliferation and invasion of breast cancer cell lines. Moreover, TAZ was found to reverse the EMT and metastasis induced by HM in breast cancer cells. In this study, we revealed a novel anticancer molecular mechanism of HM in breast cancer cells, which will provide a new basis for the treatment of breast cancer.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. RT-qPCR primers (listed 5' to 3')

E-cadherin F'	TGGGTAGATGGGCTCACAGG	E-cadherin R'	CACTTCGTCTCGGTGGAAGC
N-cadherin F'	TGGTCTGGTGGTTGACTGGT	N-cadherin R'	CACCAAGTGGTCTGGATGGC
Fibronectin F'	AACAGACCGCTGCCATGAAG	Fibronectin R'	GGCTTGCAGGTCCATTCTCC
Vimentin F'	CATGCAAGTAGCTGGGCCTC	Vimentin R'	ATGCGAACTGCAAGGTCTGG
TAZ F'	GTGCTGCTGTCCCTCATTCC	TAZ R'	CCATTCCTCCTCACCAACGC
β-actin F'	TAGTTGCGTTACACCCTTTC	β-actin R'	CTGTCACCTTCACCGTTC