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Original Article

HDAC11 is related to breast cancer prognosis and inhibits invasion and proliferation of breast cancer cells

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Abstract: Objective: Histone deacetylases (HDACs) not only regulate histone acetylation but also participate in many pathophysiologic processes, especially the development of cancer, including breast cancer. However, whether Histone deacetylase 11 can influence breast cancer is still unknown. This study investigated the relationship between HDAC11 expression in breast cancers and clinicopathologic parameters, and used small interference RNA (siRNA) to determine the biological behavioural changes after knockdown of HDAC11. Methods: Immunohistochemical (IHC) staining was employed to detect the expression of HDAC11 in a tissue microarray (TMA) of 145 patients with invasive ductal breast carcinoma. Transwell and wound healing assays were employed to analyze cell invasion and migration. The proliferation ability of cells was determined by Cell Counting Kit (CCK8). Results: The results show that the expression of HDAC11 was positively correlated with the overall survival (OS) of breast cancer patients. Specific HDAC11 knockdown enhanced MDA-MB-231 cell proliferation, migration, and invasion. Conclusion: In conclusion, this study found that HDAC11 expression is positively correlated with the overall survival rate of patients. HDAC11 can inhibit the invasion and proliferation of breast cancer cells to a certain extent and can be used as a good prognosis marker.

Keywords: Breast neoplasms, histone deacetylases, invasion, metastasis, prognosis

Introduction

The latest global cancer data for 2020 indicate that breast cancer has become the world's largest cancer, and the number of cancer deaths in China exceeds 3 million annually [1]. Cancer initiation and progression is a genetic disease, related to epigenetic abnormalities and genetic alterations [2]. Epigenetic alterations can be regulated by histone deacetylases (HDACs) through chromatin modification. The HDAC-induced abnormal epigenetic modification plays a crucial role in tumorigenesis and development [3]. The expression of numerous oncogenes and tumour suppressor genes are subject to abnormal transcription due to the abnormal modification of HDACs, while these abnormally transcribed genes will participate in malignant biologic behaviours such as tumour metastasis, angiogenesis, proliferation, invasion, differentiation and migration [4, 5]. HDACs regulate the steps related to the proliferation, invasion and migration of cancers [6, 7]. In addition, there is a relationship between HDAC expression and clinical prognosis in patients with breast cancer and other invasive cancers [8, 9]. Through studying the expression of various HDAC isoforms, Park et al pointed out that HDAC1, 6 and 8 participate in the invasion of breast cancer [10]. Some scholars have investigated whether HDACs (HDAC1, HDAC7, and HDAC9) play a role in breast carcinoma [11-13]. Therefore, HDACs could become therapeutic targets for treating breast cancer [14]. As an epigenetic therapy used to treat various malignant tumours, the histone deacetylase inhibitor (HDACi) can block HDAC activity and show certain anticancer effects, such as cutaneous

T-cell lymphoma, and diffuse B-cell lymphoma [15, 16]. At present, some HDACi have entered the clinical trial phase [17]. HDAC11 was discovered in 2002 [18]. HDAC11 has Class I and Class II HDAC features and is specifically divided into a new independent group, Class IV HDAC. This class of HDAC has only one member. Although most functions of HDAC11 are related to immune function [19-21], the role of HDAC11 in childhood neuroblastoma development has also been disclosed recently [22]. According to the study results, as HDAC11 has a significant influence on carcinogenesis, it is necessary to investigate the role of HDAC11 in tumorigenesis. In addition, the present study also investigated whether HDAC11 is related to breast cancer.

Materials and methods

Patient and tissue samples

A tissue microarray was collected from paraffin specimens from the affiliated hospital of Southwest Medical University. Inclusion criteria: Female breast cancer patients over 28 years of age with pathologically confirmed invasive ductal carcinoma and complete pathological data, without other serious underlying diseases and without other systemic malignancies. Exclusion criteria: Patients with pathological diagnosis of non-invasive ductal carcinoma, incomplete pathological data or combined with malignant tumors of other systems. The study included 145 women aged 29 to 83 years who had histologically diagnosed invasive ductal breast cancer. Participants' average age was 55 years, and the median age was 54 years. A typical representative tumor area was selected from 145 paraffin specimens. Cylindrical core tissue specimens (0.6 mm in diameter) were obtained from the obvious areas of each paraffin block and then precisely arrayed into the new acceptor paraffin block (20×35 mm) using a precision instrument [23]. All patients with paraffin specimens underwent surgery between 2003 and 2006, including axillary lymph node dissection and modified radical mastectomy. The follow-up period was 7 to 150 months. Patients without a clear histopathologic diagnosis on the tissue chip or patients with insufficient cancer cells in their samples were excluded. The clinico-pathologic parameters and the relevant dates for following up the patients in the long term were collected from the hospital.

Para-carcinoma tissues from 21 invasive ductal carcinoma cases were obtained from the Department of Breast Surgery, The First People's Hospital of Yibin, Yibin, Sichuan, P. R. China between March 2019 and May 2019. Participants were all women, ranging in age from 30 to 68, with an average age of 48 and a median age of 47. The para-carcinoma tissues were used as normal tissue controls. The present study was approved by the Ethics Committee of the First People's Hospital of Yibin City affiliated to Southwestern Medical University.

Immunohistochemical staining

The expression of HDAC11 was evaluated using immunohistochemical staining (IHC). ALL samples were dewaxed in xylene twice for 15 minutes each time and rehydrated in various alcohol solutions with a decreasing concentration gradient. Antigen was retrieved in 10 mmol/L EDTA buffer (pH 8.0) in a microwave oven (800 W) for 16 minutes, 3% H_2O_2 was used as a blocking agent at room temperature for 1 minute to prevent non-specific staining. The specimens were incubated with anti-human HDAC11 rabbit monoclonal primary antibody (cat. no. PB0674; 1:300; Boster Biological Technology, Ltd.) at 4°C overnight. The specimens were then washed for 5 min with PBS. Subsequently, the specimens were incubated with the secondary antibody (Goat anti rabbit: Biotin, ab207995, 1:4000) at 37°C for 1 h. Chromogen detection was performed using 3,3'-diaminobenzidine. Specimens were counterstained with haematoxylin for 5 minutes, to allow for clear visualization of the nuclei and cytoplasm of the cells. The resulting specimens were observed using a confocal microscope with objective magnification of 40 X, evaluated in three randomly selected high-power fields.

Scoring the staining results

IHC staining related to HDACs was interpreted in accordance with the intensity (3, strong; 2, moderate; 1, mild; and 0, negative) and the positive cell proportion (4, ≥66%; 3, ≥33% and <66%; 2, ≥10% and <33%; 1, <10%; and 0, negative). The intensity and the proportion were multiplied to calculate the HDAC IHC scores. The examined cases were classified into two groups based on IHC scores: high expression (8-12) or low expression (0-6) [24]. Due to tableting and staining, the final tissue

microarray had 139 points available for evaluation. Moreover, there were 21 non-tumor breast tissue sample controls. The stained tissue microarray was evaluated by 2 pathologists who had no prior clinicopathologic information regarding the samples. Inconsistencies were resolved using a confocal microscope (Leica TCS SP8 CARS) with a magnification of 40 X to allow for a simultaneous second examination of the specimens by the two pathologists.

Cell culture

Breast cancer (BC) cell lines (MDA-MB-175, MDA-MB-231, SK-BR-3, BT-474 and MCF-7) were provided by Dr. Juan-Juan Qiu (Department of Breast Surgery, West China Hospital, Sichuan University). SK-BR-3 and MDA-MB-231 cell lines were cultured in DMEM (Gibco, Thermo Fisher Scientific, Inc.) with 1% penicillin-streptomycin solution (Gibco, cat. No. 15140-122), and 10% FBS (HyClone, cat. No. SH30071.03) at 37°C and 5% CO_2. MCF-7 cells and BT-474 cells were cultured in RPMI 1640 (Gibco, Thermo Fisher Scientific, Inc.) with 10% FBS (HyClone, cat. No. SH30071.03) and 1% penicillin-streptomycin solution (Gibco, cat. No. 15140-122) at 37°C and 5% CO_2. Leibovitz's L-15 (Gibco, Thermo Fisher Scientific, Inc.) with 10% FBS (HyClone, cat. No. SH30071.03) and 1% penicillin-streptomycin solution (Gibco, cat. No. 15140-122) was used to culture MDA-MB-175 cells at 37°C and 5% CO_2.

Small interfering RNA (siRNA) transfection

The siRNAs (#1, stB0001617A; #2, stB0001617B; #3, stB0001617C; and non-targeted control, siN05815122147) were purchased from Guangzhou RiboBio Co., Ltd. Untreated as blank group. The siRNAs used were as follows: #1, stB0001617A: #2, stB0001617B: #3, stB0001617C. MDA-MB-231 cells were counted with a cell counting chamber and subsequently, cells were seeded into 6-well plates at a density of 1.5×10^6 cells/well. Cells were incubated at 37°C overnight and transfection was performed at 50% confluence. According to the manufacturer's protocol, cells were transfected with 50 nm siRNA using riboFECTTMCP transfection reagent (Guangzhou RiboBio Co., Ltd.). Subsequent experimentation was performed 48-72 h post-transfection.

Western blotting

MDA-MB-231 cells 72 h post-transfection, five untreated breast cancer cell lines and the tissue specimens were analysed by western blotting. For the extraction of tissue protein, the cancer and para-carcinoma tissues were sufficiently ground using a grinding machine prior to protein extraction. Total protein was extracted using radioimmunoprecipitation assay lysis buffer (Biosharp) mixed with phosphatase inhibitor mixture (100X; 100:1), followed by sufficient cleavage reaction on ice for 1 h. Subsequently, an equivalent amount of SDS-PAGE loading buffer (Beijing Zoman Biotechnology Co., Ltd.) was added and the resulting mixture was boiled for 15 min. Total protein was quantified using a bicinchoninic acid assay. The proteins were separated via 12% SDS-PAGE gel separation and were subsequently transferred to PVDF membranes. The membranes were blocked with 5% milk at 4°C. Subsequently, the membranes were incubated with primary antibodies targeted against HDAC11 (cat. no. PB0674; 1:300; Boster Biological Technology), β-actin (cat. no. ab8227; 1:5,000; Abcam) at 4°C overnight. The membranes were washed with TBST. Following the primary antibody incubation, the membranes were incubated with a horseradish peroxidase-conjugated anti-mouse anti-rabbit secondary antibody with 1:1000 (Goat anti rabbit: Biotin, ab207995, and Goat anti mouse: Biotin, ab6788). Protein bands were visualised using an enhanced chemiluminescent detection substrate (Beijing 4A Biotech Co., Ltd.). Blots were performed in duplicate and β-actin was used as the loading control.

Reverse transcription-quantitative PCR (RT-qPCR)

Basal HDAC11 protein expression was assessed in the following five breast cancer cell lines: MCF-7, BT-474, SK-BR-3, MDA-MB-175 and MDA-MB-231. The MDA-MB-231 cells with the highest HDAC11 expression levels were selected for further experimentation. According to the manufacturer's protocol, total RNA was extracted from the cells at 48 and 120 h post-transfection using the RNA isolation kit (Foregene Co., Ltd.). The purity and concentration of the RNA were measured on a ScanDrop100 ultra-micro nucleic acid analyser

(Analytik Jena AG). RNA (2 μg) was reverse-transcribed to cDNA using a PCR instrument. Subsequently, qPCR was performed on a CFX96 Touch Real-Time PCR instrument (Bio-Rad Laboratories, Inc.). The following primer pairs were used: HDAC11 forward, 5'-TCAGGGAGGAGATGGACTGAA-3' and reverse, 5'-GCCCCAGGCCAAACAGATTA-3'; and β-actin forward, 5'-TCAAGATCATTGCTCCTGAG-3' and reverse, 5'-ACATCTGCTGGAAGGTGGACA-3'. The following thermocycling conditions were used: Predenaturation at 96°C for 40 sec; initial denaturation at 96°C for 5 sec; and 40 cycles of annealing and elongation at 60°C for 30 sec. HDAC11 mRNA levels were quantified using the $2^{-\Delta\Delta Cq}$ method and normalized to the internal reference gene β-actin [25]. qPCR was performed in duplicate with three independent repeated experiments.

Transwell assay

Transwell inserts consisting of upper and lower chambers separated by a membrane (pore size, 8 μm; Corning, Inc.) were used to assess cell invasion. The upper chamber of the Transwell inserts were pre-coated with Matrigel (cat. no. 3356234; Beijing You Nikang Technology Co., Ltd.) and incubated at 37°C overnight. Subsequently, a total of 1.2×10^6 MDA-MB-231 cells were plated in the upper chamber with DMEM. DMEM supplemented with 10% FBS was plated in the lower chambers. Following incubation at 37°C for 24 h, cells on the upper membrane surface were removed with a cotton swab. Cells invading to the lower chamber were fixed with 10% formaldehyde for 30 min at room temperature, and were subsequently stained with 1% crystal violet for 40 min at room temperature. Stained cells were counted in five randomly selected fields of view using an OBSERVER D1/AX10 camera HRC inverted fluorescence microscope (Zeiss; magnification, ×100). ImageJ software (version 2.9; National Institutes of Health) was used to quantify the number of invading cells in the lower chamber. The assay was performed in duplicate with three independent repeated experiments.

Wound healing assay

MDA-MB-231 cells 72 h post-transfection were incubated at 37°C to 90% confluency. A pipette tip was used to make scratches in the cell layer. Images of the wound were captured at the same position using an OBSERVER D1/AX10 cam HRC inverted fluorescence microscope with a magnification of 100 times at 0, 24 and 48 h. ImageG software (version 2.9) was used to quantify wound healing and subsequently, a histogram was plotted.

Cell proliferation assay

The Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Inc.) was used to assess cell proliferation of transfected MDA-MB-231 cells at 24 h post-transfection, according to the manufacturer's protocol. Briefly, MDA-MB-231 cells were seeded into a 96-well plate at a density of 2×10^3 cells/well. Subsequently, 10 μl CCK-8 solution was added to each well and the cells were incubated for 1 h at 37°C. The optical density values were measured at a wavelength of 450 nm using the MultiskanGO microplate reader (Thermo Fisher Scientific, Inc.). The assay was performed in duplicate with three independent repeated experiments.

Statistical analysis

Data are presented as the mean ± SD, unless otherwise stated. Statistical analyses were performed using SPSS software (version 23; IBM Corp.). Inter-group differences in the cell proliferation assay were assessed by repeated measures ANOVA followed by the LSD post hoc test. Inter-group differences in the transwell and wound healing assays were assessed by one-way ANOVA followed by the LSD post hoc test. Differences in HDAC11 mRNA expression levels were assessed by an unpaired Student's t-test. The χ^2 test was used to assess the relationship between the expression of HDAC11 and clinicopathologic parameters. Kaplan-Meier survival analysis was performed to evaluate the prognostic significance of HDAC11 and the log-rank test generated the survival curves. A Cox proportional-hazards model was established for univariate and multivariate survival analyses. Overall survival time (OS) was the period of time from diagnosis to disease-related death. $P<0.05$ was considered a significant difference.

Results

Expression of HDAC11 and the relationship between HDAC11 and clinicopathological data

HDAC11 expression was observed in both the cytoplasm and the nuclei (**Figure 1A**). The

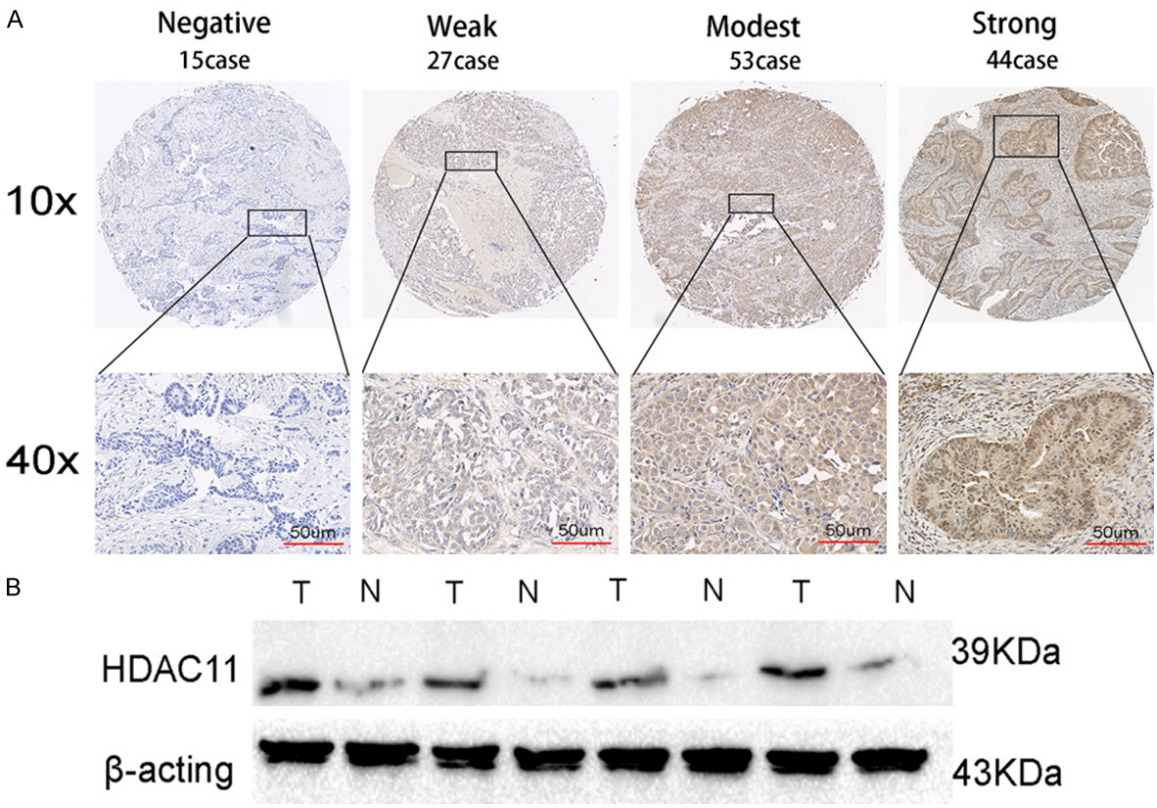

Figure 1. The expression results of HDAC11. A. IHC revealed the expression of HDAC11 protein in both the cytoplasm and nuclei of cancer cells. Negative, weak, moderate and strong staining are shown. Magnification: ×10, ×40. B. Breast cancer tissues had higher HDAC11 expression than adjacent normal tissues. β-actin was a loading control. T, tumour; N, normal tissue.

Table 1. Results of immunohistochemical staining scores

Immunohistochemical staining score	0	1	2	3	4	6	8	9	12
Number	15	7	9	5	7	4	23	39	30

results of immunohistochemical staining scores are shown in **Table 1**. A higher number of cases displayed high HDAC11 expression in the malignant breast cancer tissues (66.0%) compared to the normal mammary tissues (23.8%) (*P*<0.001; **Table 2**). HDAC11 expression was higher in breast cancer tissues compared to normal tissues (**Figure 1B**). Patients were classified into two groups based on the HDAC11 immunohistochemical scoring results, and out of the patients with invasive ductal breast cancer, 47 displayed low expression (34%) and 92 displayed high expression (66%) (**Table 2**). High HDAC11 expression was associated with estrogen receptor (ER)-negative (*P*=0.032) and human epidermal growth factor receptor 2 (HER2)-negative (*P*=0.027) status (**Table 2**). In addition, HDAC11 expression was associated with clinical stage (*P*=0.017) and pT stage (*P*=0.011). The results indicated that 76.5% of pT2 tumours and 75.0% of clinical phase II breast tumours displayed high HDAC11 expression (**Table 2**). However, no significant association was identified between HDAC11 expression and histologic grade (*P*=0.088), progesterone receptor status (*P*=0.183), nodal status (*P*=0.230), or age (*P*=0.814).

Association between HDAC11 expression and overall survival rate

Multivariate Cox proportional-hazards analysis suggested that low HDAC11 protein expression [hazard ratio (HR) =2.265; 95% confidence interval (CI), 1.197-4.289; *P*=0.015], ER-negative status (HR=2.162; 95% CI, 1.038-4.503;

Table 2. Association between HDAC11 expression and clinicopathologic data

Data	Total No. of case	HDAC11high No. (%)	HDAC11low No. (%)	*P*-value
Tissue				<0.001*
Adjacent-carcinoma tissues	21	5/21	16/21	
Cancer tissue	139	92/139	47/139	
Age (yr)				0.814
≤50	67	45/67	22/67	
>50	72	47/72	25/72	
pT stage				0.011*
pT1	43	21/43	22/43	
pT2	68	52/68	16/68	
pT3	27	18/27	9/27	
Clinical Stage				0.017*
I	35	20/35	15/35	
II	76	57/76	19/76	
III	24	11/24	13/24	
Nodal status				0.230
pN0	21	15/21 (71.4%)	6/21 (28.6%)	
pN1	51	30/51 (58.8%)	21/51 (41.2%)	
pN2	49	37/49 (75.5%)	12/49 (24.5%)	
pN3	18	10/18 (55.6%)	8/18 (44.4%)	
Histologic Grade				0.088
G1	33	18/33	15/33	
G2	67	43/67	24/67	
G3	38	30/38	8/38	
HER2 status				0.027*
Negative	68	51/68	17/68	
Positive	70	40/70	30/70	
ER				0.032*
Negative	65	49/65	16/65	
Positive	74	43/74	31/74	
PR				0.183
Negative	63	38/63	25/63	
Positive	76	54/76	22/76	

*$P<0.05$ as calculated by the χ^2 test. HDAC11, histone deacetylase 11; HER2, human epidermal growth factor receptor 2.

Table 3. Multivariate analysis of overall survival

Factor	Unfavorable factor	OS	
		HR (95% CI)	*P*-value
Pt Status	pT2	0.302 (0.104-0.879)	0.028
HER2	Positive	1.944 (1.029-3.672)	0.041
ER	Negative	2.162 (1.038-4.503)	0.039
HDAC11 Status	Low expression	2.265 (1.197-4.289)	0.015

HER2, human epidermal growth factor receptor 2; HDAC11, histone deacetylase 11; HR, hazard ratio; CI, confidence interval.

P=0.039) and HER2-positive status (HR=1.944; 95% CI, 1.029-3.672; *P*=0.041) were independent risk factors of OS in patients with invasive ductal breast cancer (**Table 3**). The univariate survival analysis, involving the log-rank test and the Kaplan-Meier method, defined OS as the time period between diagnosis and disease-related death. The results suggested that high HDAC11 expression significantly improved the OS of patients with invasive ductal breast cancer (*P*=0.023; **Figure 3B**). However, patients with ER-negative or HER2-positive invasive ductal breast cancer status displayed

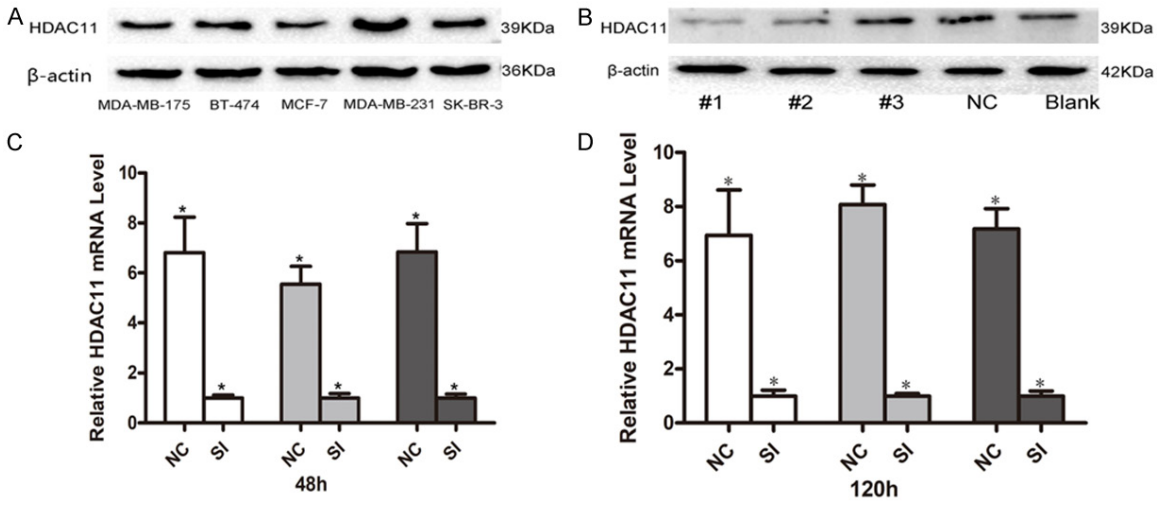

Figure 2. Basal expression of HDAC11 in breast cancer cell lines and efficiency detection after HDAC11 knockdown. A. Western blot analysis was carried out to evaluate the basal expression of HDAC11 protein in 5 breast cancer cell lines. β-actin was the loading control. B. MDA-MB-231 cells were transfected with sh-HDAC11 (si-HDAC11#1, si-HDAC11#2 and si-HDAC11#3) or non-targeted siRNA (siControl), blank control. The levels of HDAC11 were examined by Western blot 72 hours after transfection. β-actin was the loading control. C and D. siRNA knockdown kinematic characteristics selected #1 si-HDAC11 transfection to further test the transfection effect by quantitative real-time PCR. C and D. Different colors represent the pCR experiments performed by 3 different experimental groups. The data are the mean ± SD of three independent experiments. $P<0.05$. Group differences in all experiments were calculated through the independent sample t-test.

significantly reduced OS ($P=0.018$ and P= 0.026, respectively; **Figure 3A** and **3C**).

HDAC11 knockdown enhances the invasion, migration, and proliferation of breast cancer cells

The triple-negative MDA-MB-231 cells displayed the highest levels of HDAC11 expression out of the five breast cancer cell lines that were assessed (**Figure 2A**), and were therefore used to investigate whether HDAC11 knockdown had an effect on the invasion, migration, and proliferation of breast cancer cells. Western blot analysis suggested that the three HDAC11-siRNAs reduced the levels of HDAC11 protein expression at 72 h post-transfection compared with the siControl and blank cells (**Figure 2B**). Therefore, #1 HDAC11 siRNA was used to further analyse the HDAC11 knockdown by RT-qPCR (**Figure 2C** and **2D**). According to the Transwell invasion assays, MDA-MB-231 cell invasion was significantly increased by 49.1% in cells transfected with si-HDAC11 compared with cells transfected with non-targeting siRNA (**Figure 4A** and **4B**). A wound healing assay was performed to analyse cell motility. HDAC11 knockdown MDA-MB-231 cells displayed stronger migratory ability compared with MDA-MD-231 cells treated with non-targeted control (**Figure 4C** and **4D**). The CCK-8 assay suggested that HDAC11 knockdown MDA-MB-231 cells had a stronger proliferative ability compared to non-targeted control or blank MDA-MB-231 cells (**Figure 4E**).

Discussion

The influence of HDAC11 on the genesis and development of breast cancer is currently unknown. The present study investigated the expression of HDAC11 protein in breast cancer tumour tissues. Similarly, Seo *et al* [24] analyzed the relationship between the expression of multiple HDACs (HDAC1, HDAC2, HDAC3 and HDAC6) in an invasive ductal breast cancer tissue microarray and clinicopathologic parameters and prognosis, using immunohistochemical staining. The results of the previous study suggested that high HDAC1 expression was related to the upregulation of HER2 and similarly, that high HDAC6 expression was related to ER expression and HER2 upregulation. In the present study, HER2 and ER negative status was associated with high HDAC11 expression. Additionally, results suggested that HDAC11 expression was associated with clinical stage and pT. Using univariate analysis, Seo *et al* [24]

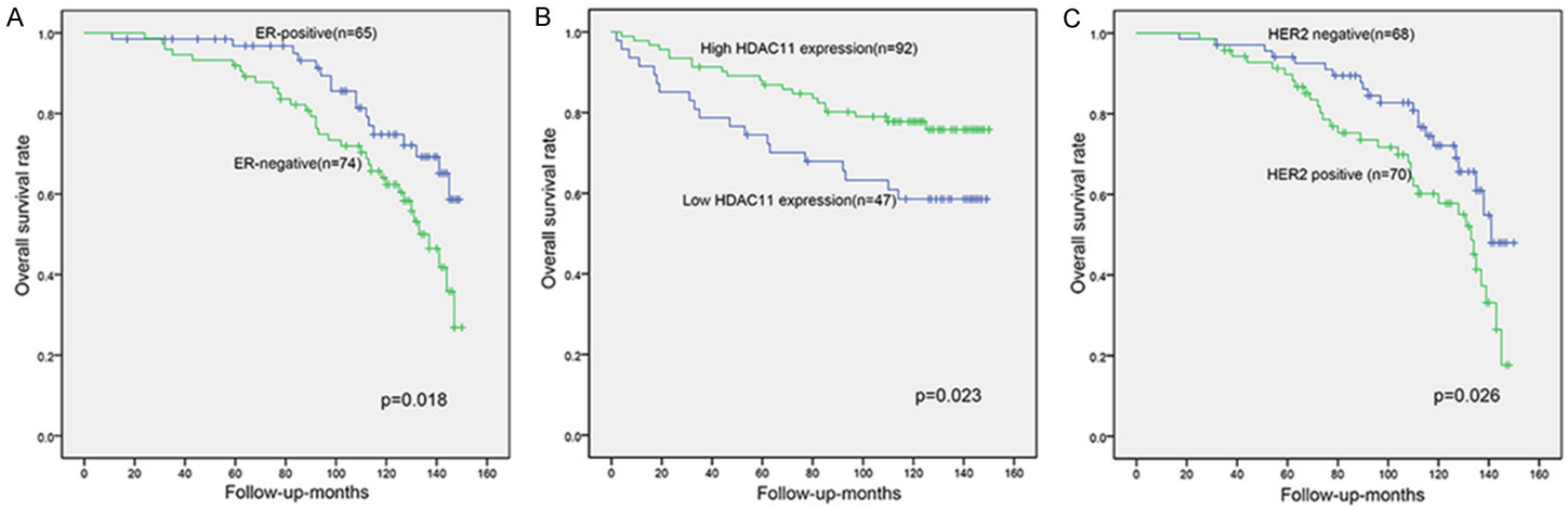

Figure 3. Univariate survival analysis. A. ER-positive expression in breast cancer patients was closely related to improved OS (*P*=0.018). B. High HDAC11 expression predicted better OS in patients (*P*=0.023). C. HER2-negative expression predicted prolonged OS in breast cancer patients (*P*=0.026).

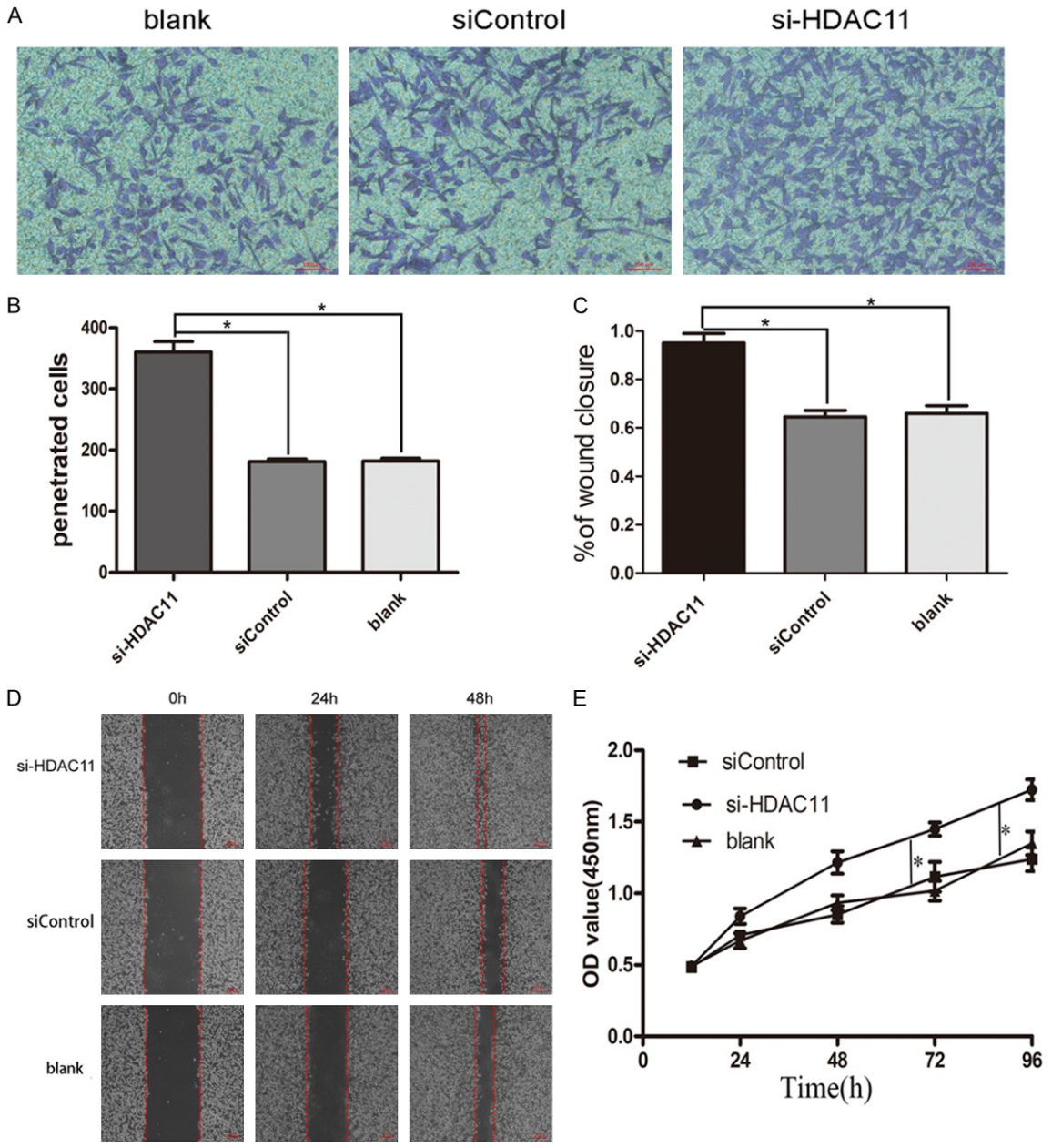

Figure 4. HDAC11 knockdown strengthens the invasion, migration and proliferation of BC cells. A and B. At 72 hours after transfection with si-HDAC11#1 and siControl. Untreated MDA-MB-231 cells were blank controls. The invasiveness of MDAMB-231 was assessed through Matrigel invasion chambers. Magnification, ×100. A. The average number of invading cells in five random microscopic fields. The results of the three experiments are presented. *P<0.05. C and D. A scratch wound-healing assay was used to evaluate MDA-MB-231 cell motility after transfection. Magnification, ×40. Columns, mean ± SD of three experiments. *P≤0.05. E. Knockdown HDAC11 increases proliferation of cells. Group differences in all experiments were calculated through LSD of repeated measure ANOVA and LSD of one-way ANOVA.

reported that high HDAC2 expression was related to improved OS in patients with ER negative tumor status. Moreover, multivariate analysis suggested that high HDAC2 expression was associated with improved disease-free survival. The univariate analysis conducted in the present study suggested that high HDAC2 expression was associated with improved OS. The highly expressed genes or proteins in cancer are generally regarded to exert carcinogenic effects. However, in the present study, high HDAC11 expression was associated with

improved prognosis, suggesting that HDAC11 may partly suppress the genesis and development of breast cancer. This result is not consistent with the traditional viewpoint. Furthermore, Chen *et al* [26] also reported that c-Jun dimer protein 2 (JDP2), a tumor suppressor gene, was highly expressed in liver cancer tissues, and that patients with liver cancer with high JDP2 expression displayed improved survival rates.

In vitro experiments suggested that HDAC11 appeared to have significant effects on tumour suppressor genes. HDAC11 knockdown enhanced the invasion, migration, and proliferative ability of MDA-MB-231 cells. Zhang *et al* reported that HDAC11 overexpression suppressed the invasion and metastasis of BLBC breast cancer cells, *in vivo* and *in vitro*. Furthermore, HDAC11 knockdown enhanced the invasion and metastasis of BLBC breast cancer cells [27]. HDAC11 blocks the activity of Twist to suppress the invasion of BLBC breast cancer cells by identifying and binding to the amino acid terminal of Twist to suppress transcription and mediate the expression of the Twist target gene, hyaluronan synthase (HAS2) [27]. HAS2 is the member of the hyaluronic acid synthase family in vertebrates. The catalysis product of HAS2 is hyaluronic acid, which is a macromolecular polysaccharide present in the extracellular matrix that plays a role in cell migration [28, 29]. Previous studies have reported that HAS2 participates in tumor epithelial-mesenchymal transition and invasion processes; however, the precise mechanism remains unclear [30-32]. Leslie *et al* [33] reported that HDAC11 suppressed the E2F7 and E2F8 cell cycle inhibitors, and that HDAC11 suppression enhanced the distant metastasis of breast cancer via the lymph nodes (LNs). This might partially explain the role of HDAC11 in promoting cancer cell survival within the LNs. Furthermore, Leslie *et al* [33] also reported that HDAC11 deletion resulted in weakened RRM2 suppression, and the enhanced RRM2 effect was related to the migration and metastatic phenotypes of numerous tumors [33].

In conclusion, the present study suggested that HDAC11 expression was associated with the OS of patients with invasive ductal breast cancer. In addition, *in vitro* experiments suggested that HDAC11 suppressed the migration and proliferation of breast cancer cells. However, the precise mechanism of HDAC11 in breast cancer requires further investigation. Previous studies have reported that multiple HDACs participate in breast cancer genesis and development. HDACi is a potential therapeutic strategy that could be used to restore acetylation and gene expression, and has the possible benefit of enhanced tolerance compared with cytotoxic chemotherapeutic agents [14]. Histone modification, including acetylation and deacetylation, is involved in the progression and prognosis of multiple tumors, including breast cancer. Therefore, histone modification may be a promising therapeutic target [34, 35]. However, our study suggested that caution should be taken when inhibiting HDAC11 activity, specifically for the treatment of breast cancer.

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All patients provided written informed consent.

Disclosure of conflict of interest

None.

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