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LncRNA DLEU1/microRNA-300/RAB22A axis regulates migration and invasion of breast cancer cells

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Abstract. – OBJECTIVE: To explore the possible role of deleted in lymphocytic leukemia 1 (DLEU1) in regulating the metastasis of breast cancer and its underlying mechanism.

PATIENTS AND METHODS: The expression levels of DLEU1 in 60 cases of breast cancer tissues and adjacent normal tissues were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). Survival analysis and receiver operating characteristic (ROC) curves were introduced to analyze the correlation between DLEU1 expression and the clinical features of enrolled breast cancer patients. After altering expressions of DLEU1, RAB22A or microRNA-300 by plasmid transfection, the abilities of breast cancer cells to migrate and invade were evaluated by transwell assay. Western blot was conducted to detect protein level changes of epithelial-mesenchymal transition (EMT)-related genes regulated by DLEU1, RAB22A or microRNA-300. Dual-luciferase reporter gene assay was performed to detect the binding relation between microRNA-300 with DLEU1 and RAB22A.

RESULTS: DLEU1 expression remains higher in breast cancer tissues than in normal adjacent tissues, and has a certain diagnostic value. The overall survival of breast cancer patients was negatively correlated with DLEU1 expression. Overexpression of DLEU1 or RAB22A, or microRNA-300 knockdown could enhance the migratory and invasive capacities, as well as increase EMT ofBT549 cells. On the contrary, knockdown of DLEU1 or RAB22A, or microRNA-300 overexpression in MCF-7 cells obtained the opposite trends of cellular behaviors. Dual-luciferase reporter gene assay confirmed that DLEU1 and RAB22A could bind to microRNA-300. Further verification showed that RAB22A expression was positively regulated by DLEU1, but negatively regulated by microRNA-300. Finally, we found that DLEU1 overexpression could reverse the inhibitory effects of microRNA-300 on proliferation, migration, and EMT of breast cancer cells.

CONCLUSIONS: DLEU1 is highly expressed in breast cancer, which promotes migration, invasion, and EMT of breast cancer cells by targeting microRNA-300 to mediate RAB22A.

Key Words:
Breast cancer, DLEU1, MicroRNA-300, EMT.

Introduction

Breast cancer is the most common female malignancy and the second cancer-related cause of death in women[1]. Despite advances have been made in improving early diagnosis and treatment, the mortality of breast cancer remains high in developed countries[2]. Researches on the pathogenesis of breast cancer are still necessary to enhance the therapeutic, diagnostic, and prognostic values of breast cancer. The onset and death of solid tumor are generally caused by infiltrated tumor cells. Tumor cell migration has become well-studied in exploring the potential mechanism of tumor metastasis[3]. Metastatic tumors cannot be surgically removed and are resistant to chemotherapy[4]. Seriously, about 90% of cancer deaths are caused by distant metastasis of tumors[5]; however, the molecular mechanisms of breast cancer migration are not well elucidated. Biomarkers for predicting the metastatic progression of breast cancer are lacking. Explorations of key factors and pathways in mediating migration and progression of breast cancer contribute to guide the early diagnosis and treatment, thereby reducing the mortality rate and improving the prognosis.

Only a small part of the mammalian genome is transcribed as protein-coding genes in the genome[6], and most are transcribed as non-coding RNAs[7]. Long non-coding RNA (lncRNA) is a class of RNAs with a length of more than 200 bp that could not encode proteins[8]. The biological functions of some lncRNAs in tumor progression and metastasis have been identified. LncRNA HOTAIR can promote breast cancer metastasis by inducing chromatin rearrangement[9]. MALAT1 exerts a key

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regulatory role in the metastasis of lung cancer cells[10]. NKILA can inhibit breast cancer metastasis by suppressing IκB phosphorylation[11].

The competitive endogenous RNA (ceRNA) hypothesis proposes that endogenous RNAs such as lncRNA, pseudogene, and mRNA may contain the binding sites of the corresponding miRNAs. CeRNAs competitively bind to these miRNAs and thus attenuate their inhibitory effects on targets[12]. Some certain lncRNAs could be used as ceRNAs, and participate in the cellular activities of tumor cells. For example, lncRNA-ATB upregulates ZEB1 and ZEB2 by competitive binding to the miR-200 family, thereby accelerating epithelial-mesenchymal transition (EMT), invasion, and migration[13].

LncRNA DLEU1 is closely related to tumor diseases. Scholars have shown that deleted in lymphocytic leukemia 1 (DLEU1) deficiency occurs at 13q14.3 in certain solid tumors and hematopoietic malignancies such as atypical or classical spindle cell lipoma, chronic lymphocytic leukemia, and small lymphocytic lymphoma. In addition, lncRNA DLEU1 is an oncogene in gastric and ovarian cancer[14,15]. However, the role and potential mechanism of lncRNA DLEU1 in breast cancer remain unclear. This study aims to investigate whether lncRNA DLEU1 can act as an oncogene in breast cancer and its specific molecular mechanism.

Patients and Methods

Tissue Samples

60 cases of breast cancer undergoing surgery from December 2016 to October 2018 in Cancer Hospital of China Medical University were enrolled. The invasive breast cancer tissues and adjacent non-tumor tissues were surgically resected and stored at –80°C. The collection of clinical specimens was approved by the Cancer Hospital of China Medical University Medical Ethics Committee**,** and all participating patients have signed informed consents.

Cell Lines

Normal breast cells (MCF-10A) and breast cancer cell lines (BT549, MCF-7, and HCC38) were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) containing 10% of fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% penicillin-streptomycin. All cells were maintained in the Cancer Hospital of China Medical University laboratory.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

Tissue or cells were lysed in TRIzol (Invitrogen, Carlsbad, CA, USA) on ice. The lysate was incubated with chloroform for extraction and isopropanol for precipitation. The precipitate was washed with 75% ethanol, air dried and diluted in 20 μL of diethyl pyrocarbonate (DEPC) water (Beyotime, Shanghai, China). 500 ng of RNA of each sample were reversely transcribed into complementary deoxyribose nucleic acid (cDNA). QRT-PCR was detected by SYBR® Green Master Mix (TaKaRa, Otsu, Shiga, Japan) according to the instructions with denaturation at 95°C for 15 min, followed by 40 cycles of 94°C for 15 s, 55°C for 30 s, and 72°C for 30 s. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) served as an internal reference. The primer sequences were as follows: GAPDH, F: 5′-CACCCACTCCTCCACCTTTG-3′, R: 5′-CCACCACCCTGTTGCTGTAG-3′; DLEU1, F: 5′- CTGCCTCCGTGGTAGCTATG-3′, R: 5′-GACCTCATAGGATCGCTGGC-3′; RAB22A, F: 5′-GTGTGTCTGCTCGGGGATAC-3′, R: 5′-GCCCCTATTGTTGGGTTGATGT-3′.

Transfection

Cells with good growth condition were inoculated into the six-well plate with 3×10^4 cells per well. Transfection was performed at 75-85% of the confluence. 1.5 mL of serum-free medium and 500 μL of the mixture of transfection plasmid and Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) were added into each well. The complete medium was replaced after 4-6 h. Transfection plasmids were constructed by GenePharma (Shanghai, China). DLEU1 siRNA, sense: 5′-GCAGUCUGUUCUGAACAUAdTdT-3′, antisense: 5′-UAUGUUCAGAACAGACUGCdTdT-3′; RAB22A siRNA, sense: 5′-GGAUACAGCUGGACAAGAA-3′, antisense: 5′-UUCUUGUCCAGCUGUAUCC-3′; MicroRNA-300 mimic, 5′-UAUACAAGGGCAGACUCUCUCU-3′; MicroRNA-300 inhibitor: 5′-UAUACAAGGGCAGACUCUCUCU-3′.

Migration and Invasion Assays

Fibronectin (FN) was diluted to a final concentration of 100 μg/mL. Matrigel was diluted with a

serum-free medium at a ratio of 1:9. The bottom of each chamber was coated with 50 μL of FN and placed in a clean bench for 2 h for air dry. The inner side of the chamber was coated with 100 μL of Matrigel and placed in an incubator overnight. 100 μL of suspension (3×10^4 cells/mL) was added to the upper layer of the transwell chamber. 600 μL of medium with 10% FBS was added to the bottom layer of the chamber. After incubating for 24 h, fixation with methanol, trypan blue staining and phosphate-buffered saline (PBS) wash for three times were performed. Penetrating cells were photographed under a microscope. Migration assay procedures were as same as those indicated above, except for Matrigel pre-coating.

Western Blot

The total protein was extracted using the cell lysate to determine the protein expression. The protein sample was quantified by bicinchoninic acid (BCA; Abcam, Cambridge, MA, USA), separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel electrophoresis, and blocked with 5% skim milk. The membranes were then incubated with the primary antibody and the corresponding secondary antibody. Band exposure was developed by chemiluminescence.

Dual-Luciferase Reporter Gene Assay

Based on the binding sites, we constructed DLEU1 WT, DLEU1 MUT, RAB22A WT, and RAB22A MUT. Cells were seeded in a 96-well plate, co-transfected with 50 pmol/L microRNA-300 mimics or negative control and 80 ng wild-type plasmid or mutant-type plasmid for 48 h. Cells were lysed and detected for the luciferase activity.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 software (IBM, Armonk, NY, USA) was utilized for statistical analysis. The quantitative data were represented as mean ± standard deviation ($\bar{x}\pm s$). The *t*-test was used to compare the differences between the two groups. The Kaplan-Meier plotter analysis was utilized to analyze the survival of breast cancer patients. The correlation between clinical features and DLEU1 expression was compared using the chi-square test. The receiver operating characteristic (ROC) curve was plotted by GraphPad Software (La Jolla, CA, USA). $p<0.05$ was considered statistically significant.

Results

DLEU1/microRNA-300/RAB22A Expression in Breast Cancer

DLEU1 expression in 60 breast cancer tissues and adjacent tissues was determined by qRT-PCR. The results showed that DLEU1 was highly expressed in breast cancer (Figure 1A). Correlation between DLEU1 expression and clinical characteristics of breast cancer patients was listed in Table I. Subsequently, enrolled breast cancer patients were divided into high level and low-level group based on the median expression of DLEU1. No significant differences in age and

Table I. Correlation between DLEU1 expression and clinical features in breast cancer patients (n = 60).

Clinicopathologic features	Number of cases	DLEU1 expression		*p*-value
		Low (n=30)	High (n=30)	
Age (years)				0.4362
≤50	33	18	15	
>50	27	12	15	
Gender				0.5982
Male	24	13	11	
Female	36	17	19	
Tumor size				0.0201*
≤2 cm	31	20	11	
>2 cm	29	10	19	
TNM stage				0.0195*
I-II	27	18	9	
III-IV	33	12	21	
Lymph node metastasis				0.0184*
Absent	25	17	8	
Present	35	13	22	

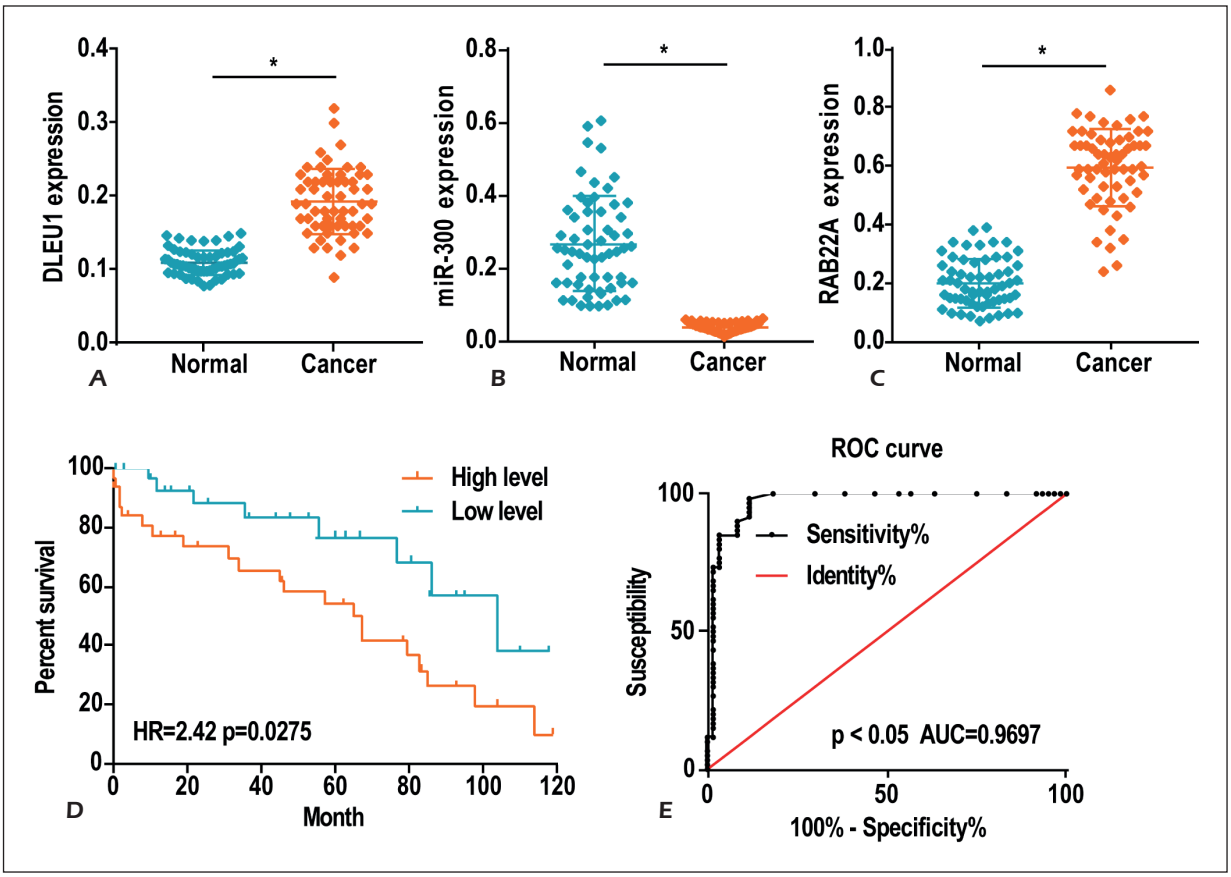

Figure 1. DLEU1/miR-300/RAB22A expression in breast cancer. QRT-PCR data showed that **A**, the expression of DLEU1 in 60 breast cancer tissues was significantly higher than that in the normal tissues. **B**, Expression of miR-300 in 60 breast cancer tissues was significantly lower than that in the normal tissues. **C**, Expression of RAB22A in 60 breast cancer tissues was significantly higher than that in the normal tissues. **D**, Overall survival of breast cancer patients with high expression of DLEU1 was significantly lower than those with low expression. **E**, ROC curve of DLEU1 expression and the diagnostic sensitivity of breast cancer.

sex were found between the two groups. Specifically, DLEU1 expression was positively correlated to the TNM stage, tumor size, and distant metastasis of breast cancer. Through bioinformatics prediction, microRNA-300 was believed to bind to DLEU1 and RAB22A could bind to microRNA-300. Therefore, we further determined expression levels of microRNA-300 and RAB22A in breast cancer tissues and adjacent tissues. The data revealed that microRNA-300 was lowly expressed, whereas RAB22A was highly expressed in breast cancer compared to adjacent tissues (Figures 1B and 1C). By analyzing the prognostic data of patients, it was found that breast cancer patients with high level of DLEU1 were expected to have a shorter overall survival as compared to low level group (Figure 1D). ROC curves further verified the diagnostic value of DLEU1 to distinguish breast cancer tissues from normal breast tissues (AUC=0.9697, Figure 1E). These results suggested that DLEU1, microRNA-300, and RAB22A may be involved in the development of breast cancer.

DLEU1 Overexpression Promoted Migratory and Invasive Potentials of Breast Cancer Cells

To investigate the effects of DLEU1 on cellular behaviors of breast cancer cells, we first detected the expression of DLEU1 in breast cancer cells (BT549, MCF-7, and HCC38) and normal breast cells (MCF-10A) by qRT-PCR. Identically, DLUE1 was highly expressed in breast cancer cells compared to normal breast cells (Figure 2A). Since DLEU1 was differentially expressed in BT549 and MCF-7, they were selected for subsequent cell experiments. BT549 cells were transfected with DLEU1 overexpression plasmid, while MCF-7 cells were transfected with DLEU1 siRNA. QRT-PCR verified their sufficient transfection efficacy

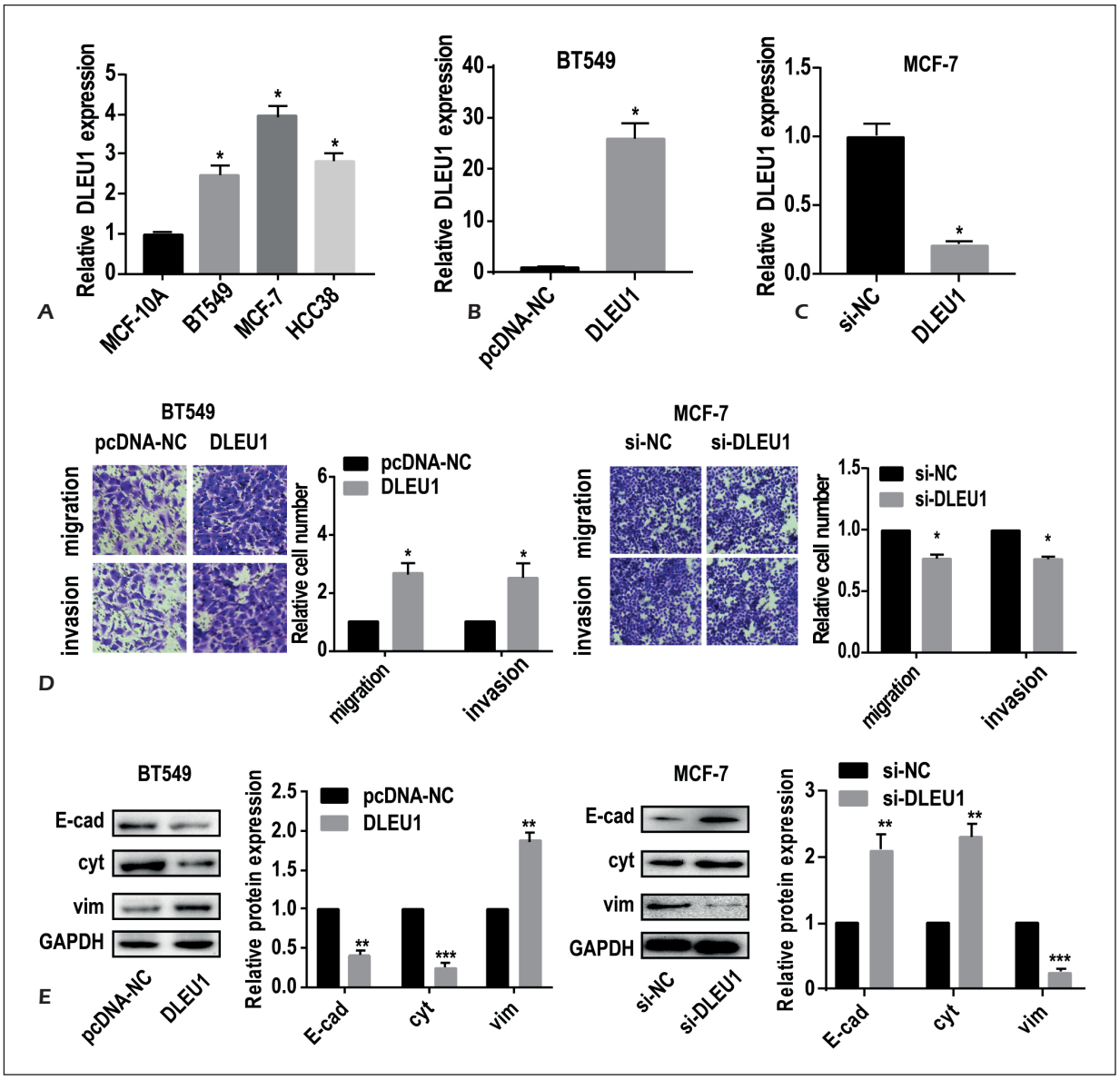

Figure 2. DLEU1 overexpression promoted migratory and invasive potentials of breast cancer cells. **A**, QRT-PCR detected the expression levels of DLEU1 in normal breast cells (MCF-10A), as well as breast cancer cell lines (BT549, MCF-7, and HCC38). **B**, In BT549 cells, the expression of DLEU1 significantly increased after overexpression of DLEU1. **C**, In MCF-7 cells, the expression of DLEU1 significantly decreased after knockdown of DLEU1. **D**, Transwell assay showed that after overexpression of DLEU1 in BT549 cells, the invasive and migratory abilities were enhanced. After knockdown of DLEU1 in MCF-7 cells, the invasive and migratory abilities decreased (magnification: 40×). **E**, Western blot analysis showed that after overexpression of DLEU1 in BT549 cells, protein expressions of E-cadherin and cytokeratin decreased, and the expression of vimentin increased. After knockdown of DLEU1 in MCF-7 cells, protein expressions of E-cadherin and cytokeratin increased, and the expression of vimentin decreased.

(Figures 2B and 2C). The transwell assay indicated that DLEU1 overexpression promoted the abilities of breast cancer cells to migrate and invade, whereas DLEU1 knockdown obtained the opposite results (Figure 2D). Protein expressions of EMT-related genes were determined by Western blot. As the results elucidated, DLEU1 overexpression in BT549 cells downregulated E-cadherin and cytokeratin, but upregulated vimentin. On the contrary, the DLUE1 knockdown in MCF-7 cells upregulated E-cadherin and cytokeratin, but downregulated vimentin (Figure 2E). These results indicated that high expression of DLEU1 promoted invasive and migratory potentials of breast cancer cells.

DLEU1 Upregulated RAB22A Expression

To test whether DLEU1 can participate in the development of breast cancer through targeting RAB22A, the expression level of RAB22A in breast cancer cells was first detected. QRT-PCR showed highly expressed RAB22A in breast cancer cells compared to controls (Figure 3A). Transfection of RAB22A overexpression plasmid in BT549 cells successfully upregulated RAB22A expression, and transfection of RAB22A siRNA markedly downregulated its expression in MCF-7 cells at both mRNA and protein levels (Figures 3B-3D). QRT-PCR and Western blot

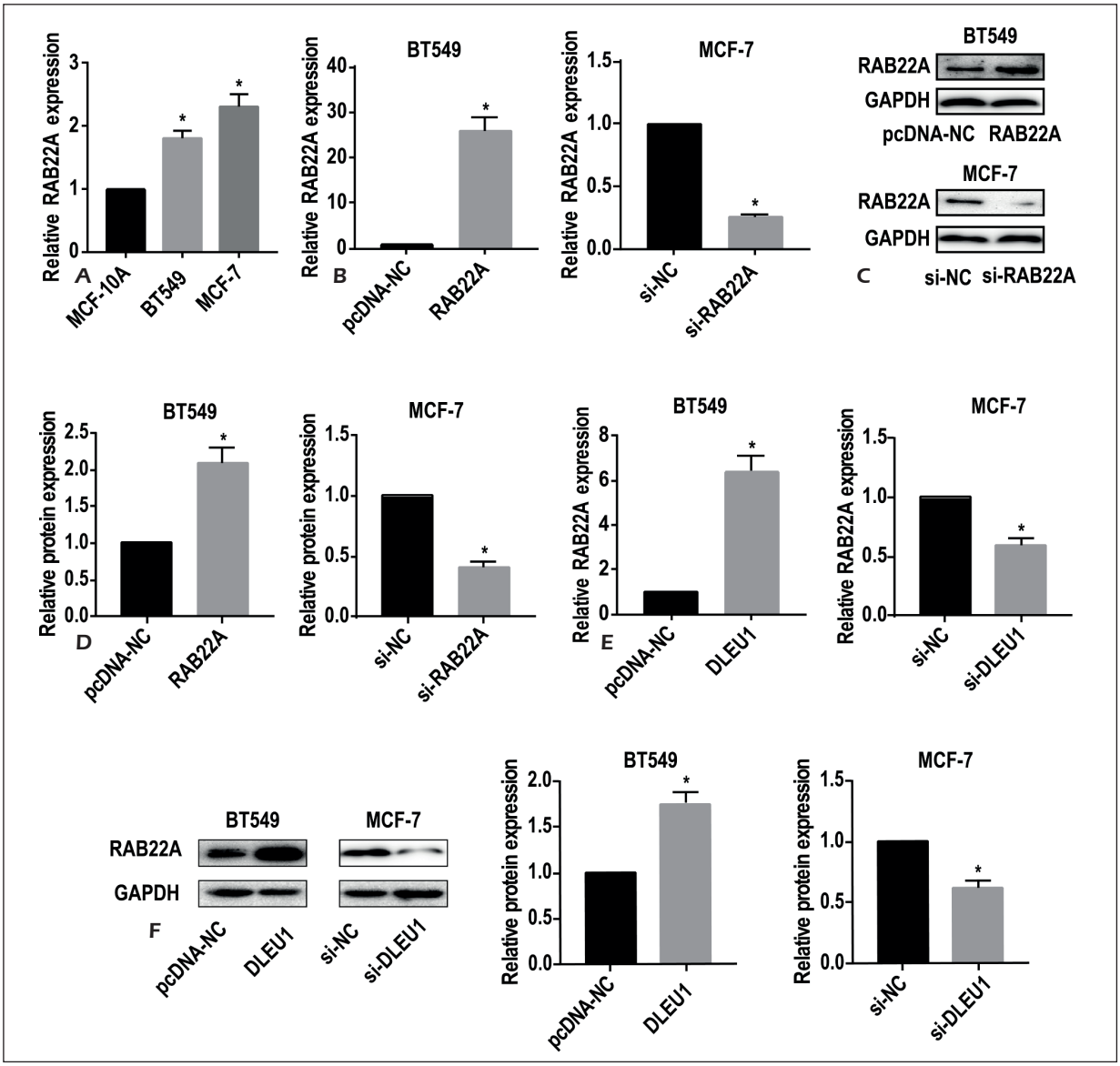

Figure 3. DLEU1 upregulated RAB22A expression. ***A***, QRT-PCR was used to detect the expression level of RAB22A in normal breast cells (MCF-10A) and breast cancer cell lines (BT549, MCF-7). ***B***, In BT549 cells, the mRNA expression of RAB22A significantly increased after overexpression of RAB22A. In MCF-7 cells, the mRNA expression of RAB22A significantly decreased after RAB22A knockdown. ***C-D***, Western blot analysis showed that the protein expression of RAB22A significantly increased in BT549 cells after overexpression of RAB22A. In MCF-7 cells, the protein expression of RAB22A was significantly decreased after knockdown of RAB22A. ***E***, QRT-PCR assay showed that the expression of RAB22A significantly increased in BT549 cells after overexpression of DLEU1. In MCF-7 cells, the expression of RAB22A significantly decreased after knockdown of DLEU1. ***F***, Western blot analysis showed that the expression of RAB22A significantly increased in BT549 cells after overexpression of DLEU1. In MCF-7 cells, the expression of RAB22A significantly decreased after knockdown of DLEU1.

both showed that DLUE1 could positively regulate RAB22A expression in breast cancer cells (Figures 3E and 3F).

RAB22A overexpression promoted migratory and invasive potentials of breast cancer cells

Transwell assays were carried out to evaluate the effect of RAB22A on migratory and invasive potentials of breast cancer cells. It was shown that RAB22A overexpression promoted migratory and invasive rates of breast cancer cells, and RAB22A knockdown yielded the opposite trends (Figure 4A). Its regulatory effect on EMT was assessed by Western blot. Protein levels of E-cadherin and cytokeratin decreased, and vimentin expression increased in BT549 cells overexpressing RAB22A. For comparison, upregulated E-cadherin and cytokeratin, as well as downregulated vimentin were seen in MCF-7 cells with RAB22A knockdown (Figure 4B).

DLEU1 and RAB22A Could Bind to MicroRNA-300

To further demonstrate that DLEU1 was involved in the development of breast cancer through the microRNA-300/RAB22A axis, we first examined the expression of microRNA-300 in breast cancer cells, which was markedly reduced (Figure 5A). Transfection efficacy of microRNA-300 mimics and inhibitor was verified (Figure 5B). By searching for the binding sequences of microRNA-300 to DLUE1 and RAB22A, we constructed wild-type and mutant-type plasmids of DLUE1 and RAB22A (Figure 5C). Luciferase activity decreased in DLEU1 WT and RAB22A WT group; however, we did not observe changes in luciferase activity of DLEU1 MUT and RAB22A MUT group (Figure 5D). QRT-PCR verified the negative correlation between mRNA expressions of microRNA-300 and RAB22A, which was the same at protein levels (Figures 5E and 5F). These results discovered that DLEU1 and RAB22A could bind to microRNA-300.

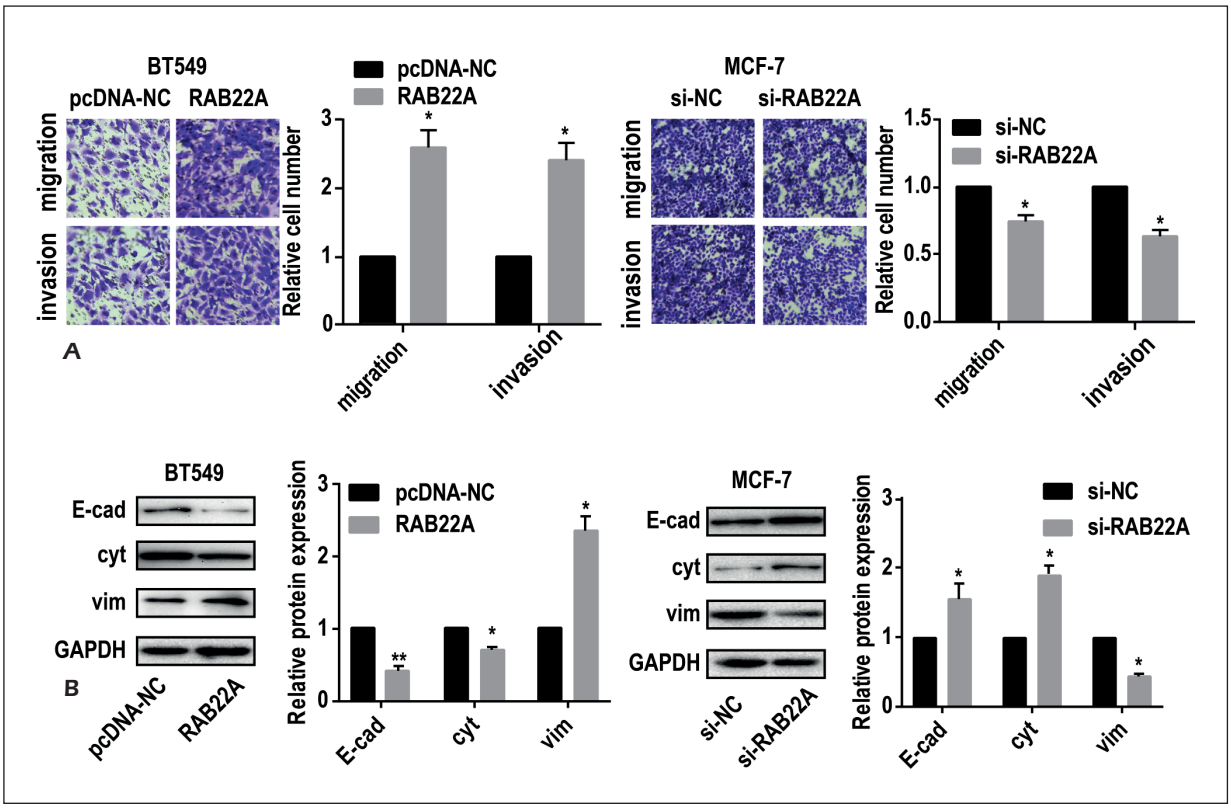

Figure 4. RAB22A overexpression promoted migratory and invasive potentials of breast cancer cells. **A**, Transwell assay showed that after overexpression of RAB22A in BT549 cells, the invasive and migratory abilities were enhanced. After RAB22A knockdown in MCF-7 cells, the invasive and migratory abilities decreased (magnification: 40×). **B**, Western blot analysis showed that after overexpression of RAB22A in BT549 cells, expressions of E-cadherin and cytokeratin decreased, and the expression of vimentin increased. After RAB22A knockdown in MCF-7 cells, expressions of E-cadherin and cytokeratin increased, and the expression of vimentin decreased.

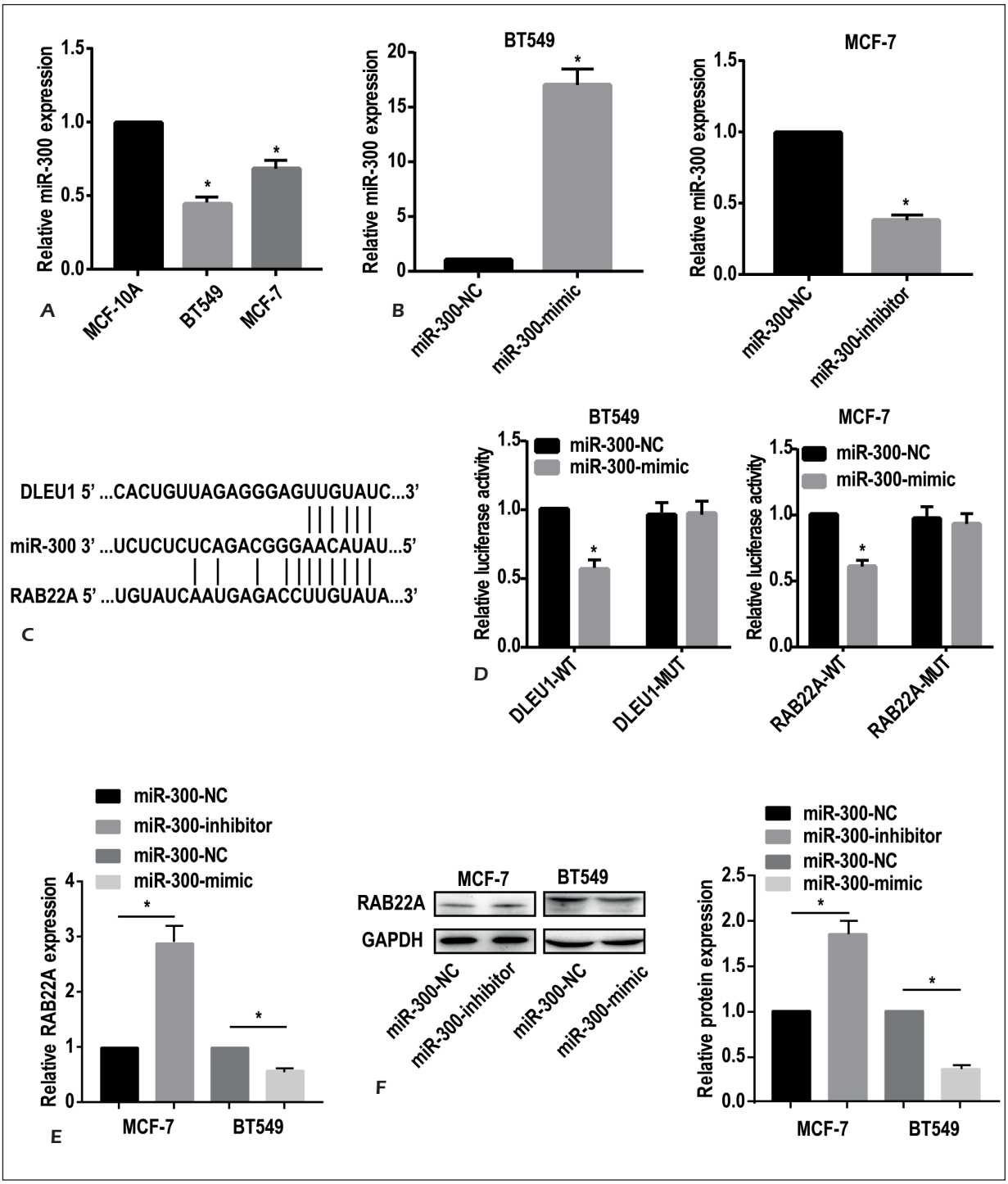

Figure 5. DLEU1 and RAB22A could bind to miR-300. **A**, QRT-PCR was used to detect the expression level of miR-300 in normal breast cells (MCF-10A) and breast cancer cell lines (BT549, MCF-7). **B**, QRT-PCR showed that in BT549 cells, the expression of miR-300 significantly decreased after overexpression of miR-300. In MCF-7 cells, knockdown of miR-300 significantly upregulated RAB22A expression. **C**, DLEU1 and RAB22A could bind to miR-300. **D**, Overexpression of miR-300 in BT549 cells allowed fluorescence quenching of wild-type DLEU1 and RAB22A. **E**, QRT-PCR assay showed that the expression of RAB22A significantly increased in MCF-7 cells after knockdown of miR-300. In BT549 cells, the expression of RAB22A significantly decreased after overexpression of DLEU1. **F**, Western blot analysis showed that the expression of RAB22A significantly increased in MCF-7 cells after knockdown of miR-300. In BT549 cells, the expression of RAB22A significantly decreased after overexpression of DLEU1.

DLEU1 Knockdown Reversed the Effects of MicroRNA-300 on Migratory and Invasive Potentials of Breast Cancer Cells

We performed rescue experiments to clarify the role of DLEU1/microRNA-300/RAB22A axis in breast cancer. Transfection of microRNA-300 mimic in BT549 cells inhibited mRNA and protein levels of RAB22A, which were reversed by DLEU1 overexpression (Figure 6A). Subsequently, the upregulated mRNA and protein levels of RAB22A in MCF-7 cells with microRNA-300 knockdown were reversed by transfection of DLEU1 siRNA (Figure 6B). Notably, co-overexpression of microRNA-300 and DLUE1 reversed the increased migratory and invasive potentials of BT549 cells overexpressing microRNA-300 (Figure 6C). Compared to those overexpressing microRNA-300, BT549 cells co-overexpressing microRNA-300 and DLUE1 presented lower levels of E-cadherin and cytokeratin, but a higher level of vimentin (Figure 6D). The enhanced migratory and invasive potentials of MCF-7 cells with microRNA-300 knockdown were reversed by the knockdown of both microRNA-300 and DLUE1 (Figure 6E). Western blot results revealed that decreases in E-cadherin and cytokeratin, as well as an increase in vimentin in MCF-7 cells with microRNA-300 knockdown were reversed by DLUE1 knockdown (Figure 6F).

Discussion

Breast cancer is the most common malignant tumor in women and its morbidity and mortality are increasing in recent years[1]. Metastatic breast cancer is one of the important causes of female death. It is urgent to elucidate the mechanisms related to the development and metastasis of breast cancer.

Volders et al[16] have found lncRNAs and miRNAs in human transcriptomes, which belong to non-coding RNAs. LncRNA has been found to be involved in a variety of biological processes such as chromatin modification, transcriptional regulation, mRNA stability, miRNA, and protein function regulation[17]. Abnormal expression of lncRNA may be present in tumors, showing a great involvement in the tumorigenesis[18]. Many lncRNAs have been found to be abnormally expressed in breast cancer and be used as therapeutic targets[19]. For example, lncRNA MEG3 is lowly expressed in breast cancer, which is closely related to the prognosis[20]. H19 is highly expressed in breast cancer, which is capable of promoting proliferation, invasion, and drug resistance of breast cancer cells[21]. HOTAIR is closely related to breast cancer metastasis, drug resistance, and poor prognosis of patients as an oncogene[22]. The important roles of lncRNA in tumors have gradually become a guidance direction in molecular biology and clinical researches. LncRNA DLEU1 is found to promote colorectal cancer progression by activating KPNA3[23]. DLEU1 also promotes the development of endometrial cancer by regulating SP1 expression through miR-490 sponging[24]. DLEU1 is associated with poor prognosis of gastric cancer and promotes cell proliferation by inhibiting KLF2[25]. In this study, DLEU1 was highly expressed in breast cancer tissues and cells. It presented a certain diagnostic value and was remarkably associated with the overall survival of breast cancer patients. *In vitro* experiments indicated that DLEU1 overexpression promoted proliferative and invasive capacities, as well as accelerated EMT in breast cancer cells.

MicroRNAs exert crucial roles in many physiological processes of tumor development such as cell differentiation, cell proliferation, apoptosis, cell cycle, etc.[26]. Functionally, they serve as oncogenes or tumor-suppressor genes in tumors based on their target genes[27]. MicroRNA-300 is 22 nucleotides in length. It is downregulated in various tumors and closely related to the occurrence and progression of tumors[28]. High expression of microRNA-300 in glioma tissues and glioma hepatocytes inhibits cell differentiation into astrocytes and neurons[29]. MicroRNA-300 promotes proliferative and metastatic rates in osteosarcoma and gastric cancer[30]. Notably, microRNA-300 is downregulated in head and neck squamous cell carcinoma, and breast cancer. Overexpression of microRNA-300 suppresses EMT and reverses the EMT phenotype of tumor cells[31]. In this study, we found that microRNA-300 knockdown inhibited the development of breast cancer.

CeRNA theory proposes that RNAs can competitively bind to oncogenic or carcinostatic miRNAs to abolish or alleviate the inhibitory effect of miRNA on the target genes[32,33]. Our work revealed that DLEU1 was involved in the development and metastasis of breast cancer by sponging microRNA-300 to regulate the RAB22A expression. Researches[34,35] suggested that RAB22A is associated with biological behaviors of tumor cells. RAB22A expression is higher in malignant tumors than paracancerous tissues, and higher expression of RAB22A indicates a worse prognosis, which is are consistent with our results. Hence, we believed that RAB22A was involved in the occurrence and progression of breast cancer.

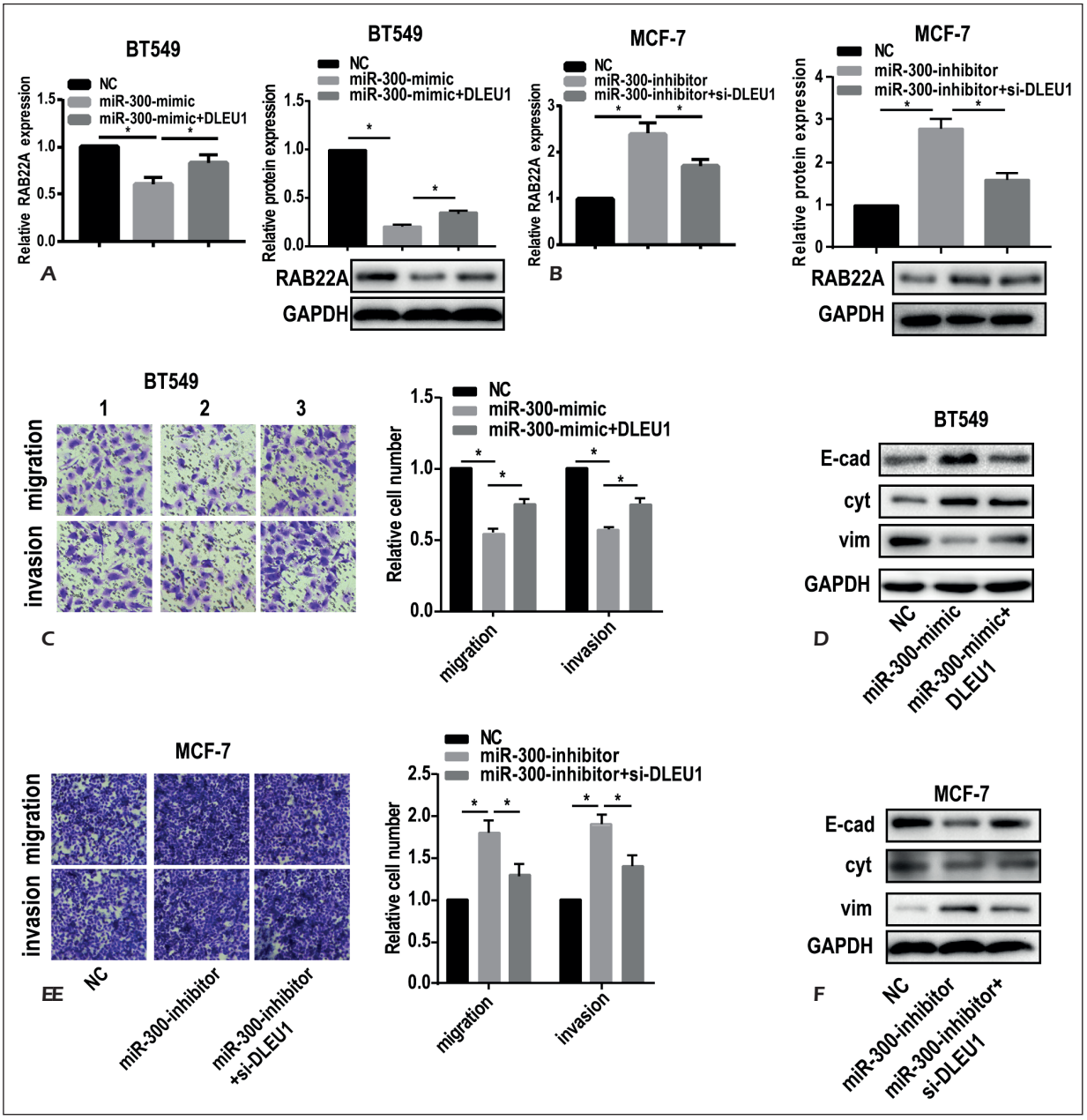

Figure 6. DLEU1 knockdown reversed the effects of miR-300 on migratory and invasive potentials of breast cancer cells. **A**, QRT-PCR and western blot analysis showed that co-overexpression of miR-300 and DLEU1 in BT549 cells reversed the decrease in RAB22A expression induced by overexpression of miR-300. **B**, QRT-PCR and western blot analysis showed that knockdown of miR-300 and DLEU1 in MCF-7 cells reversed the increase of RAB22A expression induced by miR-300 (magnification: 40×). **C**, Transwell assay showed that co-overexpression of miR-300 and DLEU1 in BT549 cells reversed the decreased migratory and invasive abilities induced by overexpression of miR-300. **D**, Western blot analysis showed that co-overexpression of miR-300 and DLEU1 in BT549 cells reversed the increased expressions of E-cadherin and cytokeratin, and the decreased vimentin expression induced by overexpression of miR-300. **E**, Transwell assay showed that knockdown of miR-300 and DLEU1 in MCF-7 cells reversed the enhanced migratory and invasive abilities induced by miR-300 knockdown (magnification: 40×). **F**, Western blot analysis showed knockdown of miR-300 and DLEU1 in MCF-7 cells reversed the decrease in expressions of E-cadherin and cytokeratin, and increased expression of vimentin induced by miR-300 knockdown alone.

EMT[36] is the transformation process of polar epithelioid cells into migratory mesenchymal-like cells under specific pathological or physiological conditions. EMT leads to the downregulation of epithelioid surface markers, loss of cell polarity, cytoskeleton reorganization, and enhancement of invasive and mi-

gratory capacities[36]. Besides, the interstitial molecular markers (e.g. N-cadherin and Vimentin) upregulate and the anti-apoptotic ability enhances during the process of EMT. EMT is believed to promote wound healing, tissue regeneration, inflammatory response, and tumor metastasis[37]. In this study, the overexpression of DLEU1, RAB22A or the knockdown of microRNA-300 could promote EMT. More importantly, DLEU1 knockdown reversed EMT in breast cancer; however, there are some shortcomings in this research. We only focused on migratory and invasive changes in breast cancer, whereas other cellular behaviors were not explored in our experiments. Meanwhile, *in vivo* verification is also required to demonstrate the biological function of DLEU1 in breast cancer.

Conclusions

DLEU1 expression is highly expressed in breast cancer, which promotes migration, invasion, and EMT of breast cancer cells by targeting miR-300 to mediate RAB22A.

Conflict of Interests

The authors declared that they have no conflict of interests.

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