miR-143 Suppresses Epithelial–Mesenchymal Transition and Inhibits Tumor Growth of Breast Cancer Through Down-Regulation of ERK5

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Epithelial–mesenchymal transition (EMT) plays a pivotal role in the development of cancer invasion and metastasis. Many studies have significantly enhanced the knowledge on EMT through the characterization of microRNAs (miRNAs) influencing the signaling pathways and downstream events that define EMT on a molecular level. In this study, we found that miR-143 suppressed EMT. Up-regulating miR-143 enhanced E-cadherin-mediated cell–cell adhesion ability, reduced mesenchymal markers, and decreased cell proliferation, migration, and invasion in vitro. In vivo, the xenograft mouse model also unveiled the suppressive effects of miR-143 on tumor growth. Additionally, we demonstrated that up-regulating extracellular signal regulated kinase 5 (ERK5) was associated with poor prognosis of breast cancer patients. Moreover, we observed an inverse correlation between miR-143 and ERK5 in breast cancer tissues. miR-143 directly targeted seed sequences in the 3′-untranslated regions of ERK5. Furthermore, we revealed that the downstream molecules of glycogen synthase kinase 3 beta (GSK-3β)/Snail signaling were involved in EMT and modulated by ERK5. In summary, our findings demonstrated that miR-143 down-regulated its target ERK5, leading to the suppression of EMT induced by GSK-3β/Snail signaling of breast cancer. © 2015 Wiley Periodicals, Inc.

Key words: miR-143; ERK5; GSK-3β; breast cancer; EMT

INTRODUCTION

microRNAs (miRNAs), a class of non-coding RNAs, have emerged as critical players in cancer pathogenesis and progression by modulating many pathological aspects related to tumor development, migration, proliferation, and metastasis [1,2]. The major function of miRNAs is to post-translationally silence gene expression by binding with the 3′-untranslated region (3′-UTR) in target mRNAs, which can occur through one or multiple mechanisms, such as direct cleavage, translational repression, or destabilization of mRNA [3,4].

Recently, miR-143 was found to decrease K-RAS expression by directly binding to K-RAS 3′-UTR in prostate cancer cell lines, and it functions as a tumor suppressor [5]. miR-143 can also inhibit the proliferation and invasion of hepatocellular carcinoma cells via the down-regulation of Toll-like receptor 2 expression [6]. miR-143 was also reduced in human clinical specimens of glioma, and miR-143 over-expression suppresses glioma cell migration, invasion, and slows tumor growth by inactivating N-RAS and inhibiting the phosphatidylinositol 3-kinase (PI3K)/AKT, and mitogen-activated protein kinase (MAPK)/extracellular signal regulated kinase (ERK) signaling [7]. More importantly, the data from in situ hybridization for miR-143 and automated detection of ERK5 expression provided a model to develop future strategies in stratifying patients for targeted therapy in prostate cancer [8].

ERK5 is a member of the MAP kinase family [9]. Three out of the four MAP kinase pathways (i.e., ERK, JNK, and p38) have been described to participate in EMT, which is mainly characterized by loss of cell–cell adhesion structures, impaired polarity, and absence of E-cadherin expression. Cells treated with MEK5, a binding partner for ERK5, can increase factors regulating EMT, including Twist, LEF-1, and matrix metallopeptidase 2 (MMP2), indicating that the MEK5/ERK5 pathway induces the mesenchymal-like

Conflicts of interest: The authors declare no conflicts of interest regarding the publication of this paper.

Limin Zhai and Chuanxiang Ma are the co-first authors and equally contributed to this study.

Grant sponsor: National Nature Scientific Foundation of China; Grant numbers: 30901779; 81471048; Grant sponsor: Natural Science Foundation of Shandong Province; Grant number: BS2011YY060

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Received 8 April 2015; Revised 5 September 2015; Accepted 17 November 2015

DOI 10.1002/mc.22445

Published online 30 November 2015 in Wiley Online Library (wileyonlinelibrary.com).
phenotype in breast cancer [10,11]. However, the function of ERK5 remains unknown in EMT.

In our study, we confirmed that miR-143 expression was experimentally declined in breast cancer specimens, and ERK5 was down-regulated directly by miR-143. To gain insight into the roles of miR-143 in breast cancer, we overexpressed miR-143. Our data suggested that miR-143 enhanced E-cadherin–mediated cell–cell adhesion, and suppressed cell migration through ERK5. Furthermore, the GSK-3β/Ncad signaling pathway was involved in the suppression of EMT by miR-143 in breast cancer.

MATERIALS AND METHODS

Patient Samples

Breast cancer specimens were obtained from 86 patients at the Weifang Medical University Affiliated Hospital after surgical resection. A total of 19 breast tissue samples from individuals confirmed to be free of malignancy were allocated into the negative control group. No patient in the current study received chemotherapy or radiation therapy before the surgery. This study was approved by the Institutional Review Board of Weifang Medical University Hospital, and informed consent was obtained from each patient. All the fresh samples were stored at −80°C.

Immunohistochemical Staining and Scoring System

The labeled streptavidin biotin method was used for immunohistochemistry. After deparaffinization and rehydration, the slides were soaked in sodium citrate buffer under 96°C for 5 min for antigen retrieval. After blocking by bovine serum albumin (BSA), primary antibodies were added to the slides for overnight storage at 4°C. The slides were then incubated with biotinylated secondary antibodies at room temperature for 30 min, followed by HRP-Streptavidin for 15 min. After 3,3′-diaminobenzidine (DAB) staining, the results were graded for intensity (0-negative, 1-weak, 2-moderate, and 3-strong) and percentage of positive cells (0, 1 [1–24%], 2 [25–49%], 3 [50–74%], and 4 [75–100%]), with discrepancies resolved by consensus. The grades were multiplied to determine a consensus. The grades were multiplied to determine a consensus.

Quantitative Real-Time PCR (qPCR)

Total RNA was extracted from cells using TRIzol (Invitrogen, CA). cDNA was reverse transcribed from total RNA samples using poly (A). qPCR analyses were performed to detect ERK5 mRNA using SYBR Premix Ex Taq (TaKaRa, Dalian, China), and GAPDH was used as an internal control. The PCR primers were as follows: ERK5 forward: 5′-GCCCTGTGTTCCAGATGTG-3′ and ERK5 reverse: 5′-CAGGCTGCAGAGTCAGATCA-3′; GAPDH forward: 5′-GGAGAGAAGTGAGGCTCG-3′ and GAPDH reverse: 5′-TGGAGAAGATGGATGGGGA-3′. An miRcute miRNA qPCR detection kit (TIANGEN, Beijing, China) was used to quantify the expression levels of mature miR-143 according to the protocol provided. We used endogenous control (RNU48) to normalize the expression levels of miRNA. Hsa-miR-143 primer sequences were 5′-GGAGAGAAGTGAGGCTCG-3′ and 5′-GTTAGACCTACCTTTTGATGG-3′. The expression levels were quantified using the comparative ΔCt method. miRNA quantification is shown as mean ± standard error from three independent experiments performed in triplicate.

Adhesion Assay

Six-well plates were precoated with 20 mg/mL fibronectin (Sigma–Aldrich). MCF-7 or T47D cells (0.5 × 10⁶ cells/well) transfected with either miR-143 mimic or mock were added to each well, and incubated for 4 h at 37°C and 5% CO₂. The unattached cells were washed. The attached cells were fixed with 70% ethanol, followed by staining with 0.1% crystal violet in 20% ethanol for 1–2 min. Crystal violet was dissolved in 10% acetic acid, and the absorbance value was measured at 597 nm. Wells coated with BSA served as a negative control, whereas cells that were incubated in complete culture medium for 8 h served as a positive control. The cell matrix adhesion index was calculated as the OD value (test-negative control)/OD value (positive control-negative control). Each test group was assayed in triplicate and repeated at least three times.

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MTT Assay of Breast Cancer Cell Proliferation

To detect the rate of cell proliferation, \(1 \times 10^4/\text{mL}\) MCF-7 or T47D cells were plated into 96-well culture plates. After 24 h of quiescence, the cells were cultured for 72 h with or without 10% FBS. At the end of the treatment, 10 \(\mu\)L of MTT (5 mg/mL) was added. The cells were incubated for 4 h at 37°C, and 100 \(\mu\)L of DMSO was added. After shocking for 10 min, the OD_{540} value was detected using a microplate reader.

Cell Migration and Invasion Assays

Cells were grown to confluence in a six-well plate and then wounded using a 200 \(\mu\)L sterile pipette tip. The wounded area was photographed at 0 and 24 h. The distance traveled by cells at the migrating edge after 24 h was calculated by measuring three separate points within each field of view from at least three separate experiments. The Boyden chamber technique was performed. In brief, 8 \(\mu\)m pore polycarbonate filters were pre-coated with 50 \(\mu\)L of 1 mg/mL Matrigel (BD Science, MD). Single cell suspensions (1 \(\times\) 10^6 cells/well) were prepared and placed on the top chambers for 24 h. Non-invasive cells on the upper surface of filters were removed by wiping with a cotton swab. Invasive cells that penetrated the filters were stained with 0.1% crystal violet. Each invasion assay was repeated three times.

Immunofluorescence

Cells were grown on glass cover slips in a six-well plate and then fixed with 4% paraformaldehyde in PBS (pH 7.4) for 15 min. The cells were permeabilized with 0.2% Triton X-100 plus 1% normal goat serum in PBS for 5 min on ice. Cover slips were incubated with respective primary antibodies at 1:100 dilution overnight at 4°C. After three washes with 1% normal goat serum in PBS, incubation with fluorescein-conjugated secondary antibodies (Invitrogen) at 1:100 dilution was performed. Cells were mounted with medium containing 4,6-diamidino-2-phenylindole (DAPI) and analyzed using fluorescence microscopy.

Western Blot

Total protein extracts were obtained as described previously. About 30 \(\mu\)g of total protein was separated on 8% denaturing SDS-polyacrylamide gel, and the proteins were blotted onto nitrocellulose membranes. The membranes were incubated with primary antibodies against \(\beta\)-actin (Rabbit polyclonal, 1:4000, Maxim, China); N-cadherin (BD Transduction); ERK5, GSK-3\(\beta\) (Cell Signaling); E-cadherin, ZO-1, vimentin, pGSK-3\(\beta\) (Santa Cruz, CA); Snail, Zeb1, Zeb2, and Twist (1:500, BD Biosciences, USA) in 5% non-fat dry milk in TBST overnight. After washing, blots were probed with the respective secondary antibodies for 1 h. Western blots were developed using an ECL Plus Chemiluminescence Detection Kit (Beyotime, China). All immunoblotting experiments were repeated at least three times.

Luciferase Activity Assay

The 3′-UTR of ERK5 was amplified by PCR from human genomic DNA using primers forward 5′-GAGCAGGAAGAAGGGG-3′ and reverse 5′-GAGGACGACTGGTAGGTTGACG-3′, and cloned into a pGL3 vector (Promega, WI) to generate the wild-type (WT) construct. For mutant plasmid (MUT), overlap extension PCR was used. MCF-7 cells were co-transfected with 0.5 \(\mu\)g of pGL3 basic vectors and different concentrations of miR-143 precursor as indicated. The MCF-7 cells were cultured in 24-well plates and then transfected with 100 ng of WT or MUT ERK5 constructs by Lipofectamine 2000 (Invitrogen). At 24 h after transfection, luciferase activity was measured. Renilla luciferase activity was normalized for \(\beta\)-galactosidase activity to correct for differences in transfection efficiency. The measurements were carried out in triplicate.

In Vivo Tumorigenesis Assay

MCF-7 cells (0.5 \(\times\) 10^7 cells/100 L) expressing miR-143 mimic or mock were injected into the fat pad of 5-week-old nude mice. Mice were killed 35 d after fat pad injection. Tumor sizes were measured using a vernier caliper every 2 d when the tumors were apparent, and the tumor curve was recorded accordingly. Horizontal (h) and vertical (v) diameters were determined, and tumor volume was calculated according to the following formula: \(V = 4/3\pi(1/2\sqrt{h \times v})^3\) [13].

RESULTS

Inhibitory Effect on EMT in Breast Cancer Cells by Up-Regulating miR-143 In Vitro

To investigate the role of miR-143 on adhesion in breast cancer cells, MCF-7, post-EMT MCF-7, T47D, and post-EMT T47D cells were transfected with miR-143 mimic and mock, and allowed to develop for 1–2 d. Two different morpholinos were used to ensure specificity, and we verified the overexpression and knockdown of miR-143 using Northern blots (data not shown). As shown in Figure 1a, the adhesion ability of cells with miR-143 overexpression was enhanced. On the contrary, the adhesion ability of cells declined when miR-143 was inhibited using siRNA compared with using scramble siRNA (Figure 1b). We then investigated whether the molecular alterations in EMT occur in miR-143 mimic cells by examining adherent junction proteins, including E-cadherin protein. Immunostaining of cells with enhanced miR-143 expression showed that E-cadherin protein was significantly up-regulated in
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the cell membrane, compared with the levels detected in control cells (Figure 1c). However, vimentin expression in miR-143 mimic cells was declined compared with that in miR-143 mock cells (Figure 1c). Western blot was also applied to detect the expression of EMT markers, expression of epithelial marker, E-cadherin, and ZO-1 were increased significantly, by contrast, the expression of mesenchymal markers, vimentin, and N-cadherin, appeared to be down-regulated (Figure 1d, e). The majority of EMT-associated transcription factors were down-regulated by miR-143 overexpression in MCF-7 and T47D cells, with Snail being the most severely affected (Figure 1f, g). Taken together, these findings demonstrated that miR-143 could inhibit EMT in breast cancer cells.

miR-143 Attenuates Tumor Growth

Cell growth proliferation was assessed by MTT assay. The results showed that miR-143 significantly inhibited cell proliferation in in MCF-7 and T47D cells (Figure 2a). To further explore the relationship between miR-143 and tumorigenesis in vivo, a xenograft model of MCF-7 cells in nude mice was adopted. In brief, MCF-7 cells that were overexpressed or not for miR-143 were injected into the fat pad of nude mice (Figure 2b). Tumor volume was calculated every 2 d, and the growth curves of tumors were plotted accordingly (Figure 2c). Finally, all mice were killed to harvest the xenograft. The tumor volume and weight from the miR-143 overexpression group were reduced significantly compared with those from the control group.

Figure 1. Ectopic miR-143 inhibits EMT in breast cancer cells in vitro (a and b) Ectopic miR-143 facilitated cellular adhesion in MCF-7 cells. (c) Fluorescence microscopic staining of E-cadherin (green) and vimentin (red) was indicated in MCF-7 cells. Nuclear DNA was stained with DAPI. Scale bar represents 10 μm. (d and e) Expression levels of epithelial markers, E-cadherin and ZO-1, as well as mesenchymal markers, N-cadherin and vimentin, were examined by immunoblotting in miR-143 overexpression MCF-7 and T47D cells. (f and g) Expression levels of EMT-associated gene proteins were detected by Western blot. Shown are the mean values of the relative expression in miR-143 overexpression versus vector control in MCF-7 and T47D cells respectively. *P < 0.05, **P < 0.01.

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(Figure 2d), moreover, the control group exhibited evidently faster growth rates.

Relationship Between miR-143 and ERK5 in Breast Cancer Cells and Tissues

To investigate the direct effects of miR-143 on ERK5 expression in breast cancer cell lines, miR-143 overexpression experiments were carried out. Transfection of miR-143 mimic into breast cancer MCF-7 and T47D cells resulted in an increase in miR-143 levels (Figure 3a). Up-regulated miR-143 expression in MCF-7 cells generated a decrease in ERK5 protein expression in a time-dependent manner compared with that in the mock vector cells (Figure 3b and c), similarly, ectopic expression of miR-143 could also reduce the expression of ERK5 protein 48h later in T47D cells (Figure 3d). Subsequently, we verified the expression levels of miR-143 and ERK5 in breast tumor tissues from 86 patients. As shown in Figure 3e and f, breast tumor tissues exhibited lower miR-143 and higher ERK5 than normal breast tissues. We also found an inverse correlation between miR-143 and ERK5 mRNA expression (Figure 3g \( r^2 = 0.68, \ P < 0.05; \) Pearson’s correlation).

Positive Expression of ERK5 is Associated With Poor Prognosis for Breast Cancer

Immunohistochemical staining for ERK5 in breast cancer tissues and the corresponding normal tissues is shown in Figure 4a. ERK5 was prominently expressed in breast cancer tissues, primarily in the cytoplasm of tumor cells. Comparisons of the ERK5 protein levels between the cancer and normal breast tissues are shown in Figure 4b. The results demonstrated that ERK5 levels significantly increased in breast cancer tissues compared with those in normal tissues (\( P < 0.05 \)). Kaplan–Meier analysis via the log-rank test was performed to calculate the effect of ERK5 expression on breast cancer survival in different subgroups (TNM) of breast cancer patients stratified in relation to histologic staging. High ERK5 expression was markedly associated with reduced overall survival in the T1+T2, N2+N3, and M0 breast cancer subgroups (Figure 4c1, c4, and c5, \( P < 0.05 \)). High ERK5 expression showed significantly shorter median survival time of patients than that of patients with low ERK5 expression level, indicating that ERK5 was a valuable prognostic marker for breast cancer patients.
miR-143 Down-Regulates ERK5 by Targeting its 3′-UTR

We performed a comprehensive survey for target genes according to consensus miR-143 binding sites using two independent informatics software (TargetScan, miRanda). The findings indicated a sequence located at bases 120–127 of the ERK5 3′-UTR (NM_002749), which was highly complementary with the seed sequence of miR-143 (Figure 5a). To experimentally validate this target, luciferase assay was performed. In the presence of miR-143, the relative luciferase activity of breast cancer cell lines with the WT construct was significantly reduced, whereas no significant suppressive effect by miR-143 was found in cells transfected with the MUT construct (Figure 5b). Moreover, knockdown of miR-143 with miR-143 siRNA increased luciferase activity in MCF-7 cells (Figure 5c). These results suggested a direct and specific interaction of miR-143 on ERK5 3′-UTR in breast cancer cells. Taken together, our data suggested that ERK5 was a target gene of miR-143.

miR-143 Suppresses Migration and Invasion Through ERK5

The aforementioned results displayed that miR-143 contributed to the suppressive effect of EMT of breast cancer. Moreover, levels of ERK5 were down-regulated in breast cancer cells with miR-143 overexpression. Therefore, to explore whether the effect of miR-143 on migration and invasion in breast cancer cells is mediated by ERK5, we employed an expression construct that encoded the ERK5 open reading frame (ERK5-ORF) lacking the 3′-UTR, which yielded an mRNA that was resistant to miRNA-mediated suppression. Western blot showed no significant difference in ERK5-ORF between the miR-143 mock and miR-143 mimic cells in migration and invasion when ERK5-ORF was overexpressed (Figure 6a). Furthermore, we established four groups in miR-143 mimic and mock cells containing the ERK5-ORF construct and ERK5-ORF mock for cell migration and invasion assays. Results showed that the ectopic expression of ERK5-ORF was capable of partially promoting the invasion ability in the miR-143 mock cells, whereas the invasion ability of the miR-143 overexpression cells without ERK5-ORF was reduced. The migration ability of these four groups also showed similar results. The quantification results of invasion and migration are shown in Figure 6b. Taken together, these findings demonstrated that miR-143 suppressed migration and invasion by down-regulating ERK5 in breast cancer cells.

GSK-3β/Snail Signaling Pathway is Involved in EMT in Breast Cancer Cells

The MEK5/ERK5 pathway has been confirmed to induce progression to the EMT phenotype in breast cancer. The increase in GSK-3β phosphorylation by p38 MAPK-stabilized Snail protein was also observed...
Figure 4. Positive expression of ERK5 is associated with poor prognosis for breast cancer (a) ERK5 immunostaining was located at the cytoplasm of breast cancer specimens and normal breast tissues. (b) Higher ERK5 expression was displayed in breast cancer tissues. (c) Kaplan–Meier curves with univariate analyses (log-rank) for patients with low ERK5 (blue line) versus high ERK5 (red line). (c1 and 2) T1 + T2 stage and T3 + T4 stage groups; (c3 and 4) N0 + N1 stage and N2 + N3 stage groups; (c5 and 6) M0 stage and M1 stage groups.
During EMT in different cancer types [14]. In the present study, to identify whether GSK-3β is involved in EMT and modulated by ERK5, two groups were established in MCF-7 cells with or without the down-regulation of ERK5 using a specific siRNA. Interestingly, the expression of pGSK-3β obviously declined in a dose-dependent manner in cells with siERK5 (Figure 7a). Given that stabilization of Snail was mediated by regulating GSK-3β activity [15], we treated MCF-7 cells with GSK-3β inhibitor AR-A014418, and determined Snail expression by Western blot. We found that the level of Snail decreased in cells with AR-A014418 but not in the control cells (Figure 7b). The data displayed that ERK5 participated in the EMT suppressed through the GSK-3β/Snail signaling pathway in breast cancer cells.

DISCUSSION

Metastasis, a complex process allowing primary tumor cells to colonize distant sites, accounts for 90% of the mortality in breast cancer patients [3]. A prerequisite for cancer metastasis to occur is that at least a subpopulation of malignant cells should acquire the ability to migrate and invade focally [16]. To achieve this ability, breast cancer cells have to partially or completely undergo a transformation from epithelial to mesenchymal, which is a critical step during cancer metastasis [17,18]. Therefore, a better understanding of the molecular mechanisms underlying EMT is required to facilitate the development of effective therapeutic strategies for breast cancer patients.

miRNAs could negatively regulate EMT-related genes at the post-transcriptional level, and play critical roles in cancer metastasis [19,20]. Previous studies reported that miR-143 is associated with the regulation of EMT, and miR-143 is significantly dysregulated in many human tumor types, including prostate cancer, hepatocellular carcinoma, non-small cell lung cancer, and gastric cancer [6,21]. However, the role of miR-143 in breast cancer is barely explored. In the present study, we performed functional assays after transfecting miR-143 mimic into breast cancer cells. The expression levels of E-cadherin and ZO-1 increased, whereas the levels of mesenchymal markers (vimentin and N-cadherin) decreased in MCF-7 and T47D cells. In vivo, the results displayed that miR-143 could reduce the tumor volume and weight of orthotopically implanted breast tumors. Ectopic miR-143 lowered the expression of EMT-associated transcription factors, with Snail being the most affected. In brief, these findings displayed that miR-143 functioned as an EMT suppressor.

In many cancer types, ERK5 participates in tumor cell proliferation and survival, and regulates cell focal adhesion and tumor metastasis [22], whereas knock-down of ERK5 levels by siRNA reduces the hepatocyte
growth factor-induced migration of MDA-MB-231 breast cancer cells [23]. To reveal the influence of ERK5 on the prognosis of breast cancer patients, we recruited breast cancer specimens and Kaplan–Meier analyses were performed. There was prominent survival difference between ERK5 low and ERK5 high groups in T1+T2, and M0 breast cancer subgroups. These results suggested that positive ERK5 expression was a predictor of poor prognosis marker for early breast cancer. Moreover, down-regulation of ERK5 with specific siRNA remarkably declined cell migration and invasion ability.

**Figure 6.** miR-143 suppresses migration and invasion through ERK5 (a) Ectopic miR-143 did not influence the expression of ERK5-ORF. (b) Ectopic ERK5-ORF could partially reverse the inhibition of miR-143 on migration and invasion in MCF-7 cells. *P < 0.05.
qRT-PCR confirmed that miR-143 was negatively correlated with ERK5 in breast cancer cells and tissues in vitro and vivo. To identify whether ERK5 is a target gene of miR-143, bioinformatic methods were used. As expected, in the ERK5 3'-UTR, one potential binding site of miR-143 with high complementarity was found, which was located at bases 120–127 of the ERK5 3'-UTR (NM_002749). Moreover, luciferase activity assay revealed that ectopic expression of miR-143 inhibited the expression of the reporter vector containing the WT sequence of ERK5 3'-UTR. Furthermore, co-transfection of miR-143 mimic and ERK5-ORF led to the enhancement of migration and invasion of breast cancer cells with high expression of ERK5. Together, these findings testified that miR-143 participated in the MAPK-signaling pathway by directly regulating ERK5 expression, and through down-regulation of ERK5, miR-143 suppressed migration and invasion in breast cancer.

Glycogen synthase kinase 3β (GSK-3β) is a multifunctional serine/threonine protein kinase that has important roles in metabolism [24]. GSK-3β has been implicated to play roles in cancers that are resistant to chemo-, radio-, and targeted therapy [25]. GSK-3β activated by protocadherin 9 was found to participate in the inhibition of EMT in hepatocellular carcinoma [26]. In turn, the phosphorylation of serine-9 inactivates GSK-3β, thereby leading to the stabilization of Snail [15]. A previous study reported that the AKT signaling pathway negatively regulates GSK-3β [27]. AKT activation (pAkt 473) mediates GSK-3β inactivation by phosphorylating pSer9 of GSK-3β, however ERK signaling functions when AKT signaling is absent [28]. Coincidentally, p38 MAPK also inactivates GSK-3β by direct phosphorylation at its COOH terminus, which also leads to the accumulation of β-catenin [14]. Therefore, similar to AKT and p38 MAPK signaling, the ERK pathway was also considered to participate in the regulation of GSK-3β inactivation. In the present study, to detect whether ERK5 is implicated in the suppression of EMT by the GSK-3β/Snail pathway, we employed siERK5 and GSK-3β inhibitor (AR-A014418) for detecting the expression levels of GSK-3β and Snail in breast cancer cells. The results revealed that GSK-3β/Snail cascades were down-regulated in the cells treated with siERK5 and GSK-3β inhibitor. However, the specific regulatory mechanism by which ERK5 modulated GSK-3β should be further ascertained.

In summary, the present study established an association between miR-143 and ERK5 expression, and highlighted the importance of this regulation for the E-cadherin–mediated adhesion and migration of breast cancer. We also demonstrated that the GSK-3β/Snail signaling pathway was implicated in the suppression of EMT by the crosstalk of miR-143/ERK5 in breast cancer. These data provide novel evidence for making the miR-143/ERK5 pathway a novel target for breast cancer therapy.
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