miR-27a promotes human breast cancer cell migration by inducing EMT in a FBXW7-dependent manner

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Abstract. Increasingly, evidence has revealed that aberrant microRNA (miRNA) expression is involved in breast cancer carcinogenesis and further progression, including metastasis. miRNA (miR)-27a was previously identified to be abnormally expressed and to serve pro-oncogenic functions in multiple human cancer types, including breast cancer. However, its functions and underlying mechanisms in breast cancer remain poorly understood. In the present study, it was demonstrated that miR-27a was significantly upregulated in breast cancer tissues and cell lines compared with their normal counterparts. Overexpression of miR-27a resulted in enhanced cell migration by inducing epithelial-to-mesenchymal transition, while its knockdown effectively reversed these cellular events. The present study additionally confirmed for the first time, to the best of our knowledge, that F-box and WD repeat domain containing 7 (FBXW7) is a downstream target gene of miR-27a in human breast cancer cells. FBXW7 is underexpressed in breast cancer tissues and cell lines, and is an independent positive factor for the overall survival rate of patients with breast cancer. Notably, the ectopic expression of FBXW7 may effectively suppress the epithelial-to-mesenchymal transition and migratory activity of breast cancer cells, in addition to reversing the cell migration mediated by miR-27a. Altogether, the results of the present study indicated the important function of miR-27a in regulating the metastasis of breast cancer in a FBXW7-dependent manner, and provide evidence for the potential application of miR-27a in breast cancer therapy.

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Introduction

Human breast cancer is the second leading cause of cancer-associated mortality in women globally (1). Metastasis, as one of the unique characteristics of late-stage cancer, accounts for the majority of morbidity and mortality in breast cancer (2). Therefore, the investigation of more effective therapeutic approaches and agents to treat metastatic breast cancer, or to overcome the metastasis of breast cancer cells, is urgently required, by identifying the underlying molecular mechanism accounting for its metastatic capacity. Notably, epithelial-to-mesenchymal transition (EMT) is a crucial step in cell migration, which confers upon cancer cells increased invasive properties (3). Targeting the EMT process is a potential approach for suppressing breast cancer cell metastasis.

MicroRNAs (miRNAs) are a group of small non-coding RNAs, which are known as regulatory RNA molecules, post-transcriptionally modulating the expression of downstream genes through base pairing with the 3'-untranslated region (3'-UTR) of the corresponding mRNAs, and resulting in either mRNA degradation or translation inhibition (4,5). Growing evidence indicates that miRNAs serve critical roles in the proliferation, migration and invasion of tumor cells (6). miRNA (miR)-27a is located on chromosome 19 and has been demonstrated to be abnormally expressed in numerous types of cancer, including breast cancer, gastric cancer, cervical cancer and esophageal cancer (7-10). The widespread overexpression of miR-27a in cancer has resulted in the assumption that miR-27a is an oncogenic microRNA. Recently, miR-27a was reported to promote the proliferation, migration and invasion of colorectal cancer by targeting family with sequence similarity 172 member A (11). Furthermore, the suppression of miR-27a sensitizes colorectal cancer stem cells to TNF superfamily member 10 (TRAIL) by promoting the formation of the apoptotic protease activating factor-1-caspase-9 complex (12). However, it appears that not all cancer types are able to benefit from miR-27a. For example, miR-27a significantly increases the TRAIL sensitivity of acute myeloid leukemia by targeting PLAG1 zinc finger (13) and is also able to inhibit metastasis by suppressing EMT in human hepatocellular carcinoma (14). Therefore, the biological function of miR-27a is variable in different types of cancer.

F-box and WD repeat domain-containing 7 (FBXW7), a member of the F-box family that constitutes one of the four subunits of the ubiquitin protein ligase complex termed SKP1-cullin-F-box, has been identified to be involved in the ubiquitin-dependent proteolysis of oncoprotein substrates, including c-Myc proto-oncogene protein, neurogenic locus notch homolog protein, serine-/threonine-protein kinase MTOR, transcription factor AP-1 and myeloid cell leukemia-1, and contributes to carcinogenesis (15-18). FBXW7 regulates diverse cellular processes, including cell proliferation and differentiation (19). Additionally, FBXW7 serves critical roles in regulating the metastasis of cancer cells by targeting the EMT process. In renal cancer cells, the upregulation of FBXW7 is able to effectively suppress metastasis by inhibiting EMT (20). FBXW7 is also able to regulate EMT, in part through the Ras homolog gene family member A signaling pathway in gastric cancer (21). Recently, FBXW7 was identified to suppress EMT in non-small-cell lung cancer by promoting the degradation of snail family transcriptional repressor 1 (Snail) (22). Notably, it was reported that miR-223 was able to enhance the doxorubicin resistance of non-small cell lung cancer by promoting EMT by targeting FBXW7 (23). Generally, FBXW7 is considered to be a tumor suppressor in a variety of human cancer types, although not all (24).

In the present study, the functions and underlying mechanisms of miR-27a in human breast cancer migration were investigated. It was demonstrated that the expression of miR-27a was upregulated in human breast cancer tissues and cell lines. miR-27a was able to significantly inhibit the expression of FBXW7 to promote EMT and human breast cancer cell migration. The regulatory association between miR-27a and FBXW7 was also confirmed. The present study suggested that the miR-27a/FBXW7 axis may be a clinically beneficial target for identifying patients with high-risk cancer with distant metastasis.

Materials and methods

Clinical specimens and cell culture. Twenty primary breast cancer biopsy specimens and corresponding paracancerous tissues were collected from patients at the Taizhou Hospital of Zhejiang Province (Taizhou, China) who underwent surgery between December 2016 and July 2017, and were immediately stored in liquid nitrogen until use. All patients provided written informed consent for the use of these clinical materials in research, and the Institutional Ethics Committee of the Taizhou Hospital of Zhejiang Province ethically approved the project. The human breast cancer cell lines MDA-MB-231 and SKBR3, in addition to the normal human mammary cell line MCF-12A, were purchased from the American Type Culture Collection (Manassas, VA, USA) and cultured in Dulbecco's modified Eagle's medium (DMEM; Hyclone; GE Healthcare Life Sciences, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone; GE Healthcare Life Sciences), 100 U/ml penicillin (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) and 100 g/ml streptomycin (Sigma-Aldrich; Merck KGaA). All cell lines were cultured in a humidified incubator in an atmosphere of 5% (v/v) CO_2 at 37˚C.

RNA isolation and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was isolated from cell lines or tumor samples using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol. miRNA was converted to cDNA using the TaqMan miRNA reverse transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) at 16˚C for 30 min, 42˚C for 30 min and 85˚C for 5 min. The expression levels of miR-27a were determined using the TaqMan miRNA assay kit (Applied Biosystems; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol, and calculated using the $2^{-\Delta\Delta Cq}$ method by normalizing to the signal for U6 expression (25). Complementary DNA synthesis of mRNA was performed using Moloney murine leukemia virus reverse transcriptase kit with oligo dT primers (Promega Corporation, Madison, WI, USA), at 70˚C for 5 min, 42˚C for 60 min and 70˚C for 15 min. The expression levels of FBXW7, Snail, zinc finger E-box binding homeobox 1 (ZEB1), E-cadherin, N-cadherin and Vimentin were evaluated using qPCR with a SYBR green PCR master mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) and calculated using the $2^{-\Delta\Delta Cq}$ method by normalizing to β-actin. The thermocycling conditions were as follows: 95˚C for 10 min, 45 cycles of 95˚C for 15 sec and 60˚C for 1 min, for both miRNA and mRNA PCR. All of the reactions were performed in triplicate and the primer sequences are listed in Table I.

Wound healing assay. MDA-MB-231 and SKBR3 cells were seeded into a 6-well culture plate and grew to 100% confluence in DMEM supplemented with 10% FBS. Subsequently, the cells were starved in serum-free medium overnight. A plastic pipette tip was used to produce a clean wound area across the center of the well. The cell debris were washed away using 1X PBS and the cells were allowed to migrate in DMEM with 10% FBS for 24 h or 48 h. Images of the wound healing process were captured and the gap distance normalized to the control was examined at 0, 24 and 48 h.

In vitro migration assays. For the migration assay, a Transwell system (24 wells; 8-μm pore size with a poly-carbonate membrane; Corning Inc., Corning, NY, USA) was used according to the manufacturer's protocols. The cells were seeded into the upper chambers and cultured in serum-free DMEM. The lower compartment was filled with DMEM with 10% FBS as a chemoattractant. Subsequent to incubation for 24 h, the cells remaining in the upper chamber were removed. The cells at the bottom of the insert were fixed with 70% ethanol for 30 min at room temperature, stained with 0.5% crystal violet for 30 min at room temperature and counted under an inverted fluorescence microscope at x40 (Olympus Corporation, Tokyo, Japan). A total of three independent experiments were performed to determine the mean number of migrated cells.

Oligonucleotide transfection. The hsa-miR-27a mimics, inhibitor, negative control (NC) mimics and NC inhibitor oligonucleotides were obtained from Guangzhou RiboBio Co., Ltd. (Guangzhou, China). The sequences for these oligonucleotides were as follows: 5'-UUCACAGUGGCUAAGUUC CGC-3' (sense) and 5'-GGAACUUAGCCACUGUGAAUU-3'

Table I. Sequences of primers used in reverse transcription-quantitative polymerase chain reaction analysis.

Gene	Sequences
miR-27a	Forward: 5'-TGCGCTTCACAGTGGCTAAGG-3'
	Reverse: 5'-CTCAACTGGTGTCGTGGAGTCCGGCAATTCAGTTGAGGGGAACT-3'
U6	Forward: 5'-CTCGCTTCGGCAGCACA-3'
	Reverse: 5'-AACGCTTCACGAATTTGCGT-3'
FBXW7	Forward:5'-ACTGGAAAGTGACTCTGGGA-3'
	Reverse: 5'-TACTGGGGCTAGGCAAACAA-3'
Snail	Forward: 5'-TCCTCAACCCCACCGCCT-3'
	Reverse: 5'-GCCTTTCCCACTGTCCTCAT-3'
ZEB1	Forward: 5'-ACACGACCACAGATACGGCA-3'
	Reverse: 5'-ATGGGAGACACCAAACCAAC-3'
E-cadherin	Forward: 5'-GCTGGACCGAGAGAGTTT-3'
	Reverse: 5'-TGGAGGTGGTGAGAGAGAC-3'
N-cadherin	Forward: 5'-CAGAATCAGTGGCGGAGA-3'
	Reverse: 5'-GAAGCAAGGAAAGTAGCAT-3'
Vimentin	Forward: 5'-TTTTGAAGAAACTCCACGAAG-3'
	Reverse: 5'-ACGAAGGTGACGAGCCAT-3'
β-actin	Forward: 5'-AGCACAGAGCCTCGCCTTTGC-3'
	Reverse: 5'-CTGTAGCCGCGCTCGGTGAG-3'

ZEB1, zinc finger E-box binding homeobox 1; miR, microRNA; FBXW7, F-box and WD repeat domain containing 7; Snail, snail family transcriptional repressor 1.

(antisense) for miR-27a mimics; 5'-GCGGAACUUAGCCACUGUGAA-3' for miR-27a inhibitor; 5'-UUCUCCGAACGUGUCACGUTT-3' (sense) and 5'-ACGUGACACGUUCGGAGAATT-3' (antisense) for NC mimics; and 5'-CAGUACUUUUGUGUAGUACAA -3' (sense) for the NC inhibitor. The cells (3x10⁵/well) were seeded in a 6-well plate and cultured for 24 h prior to transfection. The cells were transfected with hsa-miR-27a mimics/inhibitor or NC mimics/inhibitor (50 nM) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for induction. After 24 h, the cells were collected to perform the *in vitro* assays.

Western blotting. Following transfection, the cells were lysed in lysis buffer (2.1 µg/ml aprotinin, 0.5 µg/ml leupeptin, 4.9 mM MgCl₂, 1 mM orthovanadate, 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride). The protein concentration was determined using a bicinchoninic acid assay. Subsequently, protein (20 µg/lane) was subjected to electrophoresis on a 12% or 15% SDS-PAGE gel, proteins were transferred onto polyvinylidene difluoride membranes. The membranes were blocked with 5% non-fat milk at room temperature for 2 h and incubated with Snail (cat. no. ab53519; 1:1,000), ZEB1 (cat. no. ab180905; 1:1,000), E-cadherin (cat. no. ab40772; 1:1,000), N-cadherin (cat. no. ab76057; 1:1,000), Vimentin (cat. no. ab8978; 1:1,000) and FBXW7 (cat. no. ab109617;

1:1,000) primary antibodies at 4˚C overnight. The corresponding horseradish peroxidase (HRP)-conjugated secondary antibody was added and incubated at room temperature for 2 h. Signals were visualized using an enhanced chemiluminescence reaction with a HRP substrate (Pierce; Thermo Fisher Scientific, Inc.). All primary antibodies used in the present study, except the β-actin antibody, were purchased from Abcam (Cambridge, UK) and the secondary antibodies [goat anti-mouse IgG-HRP (cat. no. sc-2005; 1:10,000) and goat anti-rabbit IgG-HRP (cat. no. sc-2004; 1:10,000] were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). The antibody against β-actin (cat. no. A8481; 1:5,000) was obtained from Sigma-Aldrich (Merck KGaA) and used as a loading control in clinical specimen western blotting only. GAPDH (cat. no. ab8245; 1:1,000) was used as loading control for all other western blotting. Densitometric analysis of the protein bands was performed using ImageJ software 1.49v (National Institutes of Health, Bethesda, MD, USA).

Plasmid transfection and reporter assay. Next-generation sequencing and TargetScan (www.targetscan.org/vert_71/) was used to predict the target genes of miR-27a. The coding sequences of human FBXW7 mRNA were synthesized and subcloned into the pcDNA3.1 vector (Invitrogen; Thermo Fisher Scientific, Inc.). The integrity of the respective plasmid constructs was confirmed by DNA sequencing. The transfection of the FBXW7 plasmid using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was performed according to the manufacturer's protocol. pGL3-FBXW7 wild-type and pGL3-FBXW7 mutant plasmids were constructed for the 3'-UTR reporter assays. The cells were transfected with 1.2 µg plasmid using Lipofectamine® 2000 or with pGL3 empty vector, which was used as a negative control. A total of 24 ng PRL-CMV (Promega Corporation), encoding *Renilla* luciferase, was included in all transfections to normalize the transfection efficiency. Cells were washed and lysed with the passive lysis buffer from the Dual-Luciferase Reporter Assay system (Promega Corporation), 24 h after transfection. Luciferase activity was measured in each cell lysate using a FLUOstar Galaxy plate reader (BMG Labtech GmbH, Ortenberg, Germany).

Statistical analysis. All data are expressed as the mean ± standard deviation from at least three separate experiments. Histograms were produced using GraphPad Prism 5.0 software (GraphPad Software, Inc., La Jolla, CA, USA). All statistical analyses were performed using GraphPad Prism 5.0 and statistical significance was determined using a two-sided Student's t-test for all data except the basal miR-27a levels in the cell lines, for which one-way analysis of variance followed by the Bonferroni post hoc test was performed to determine the statistical significance. $P<0.05$ was considered to indicate a statistically significant difference. Survival curves based on the Kaplan-Meier method were compared using a log-rank test (Kaplan Meier Plotter; http://kmplot.com/) (26).

Results

miR-27a is positively associated with the migratory activity of human breast cancer cells. To determine whether miR-27a was
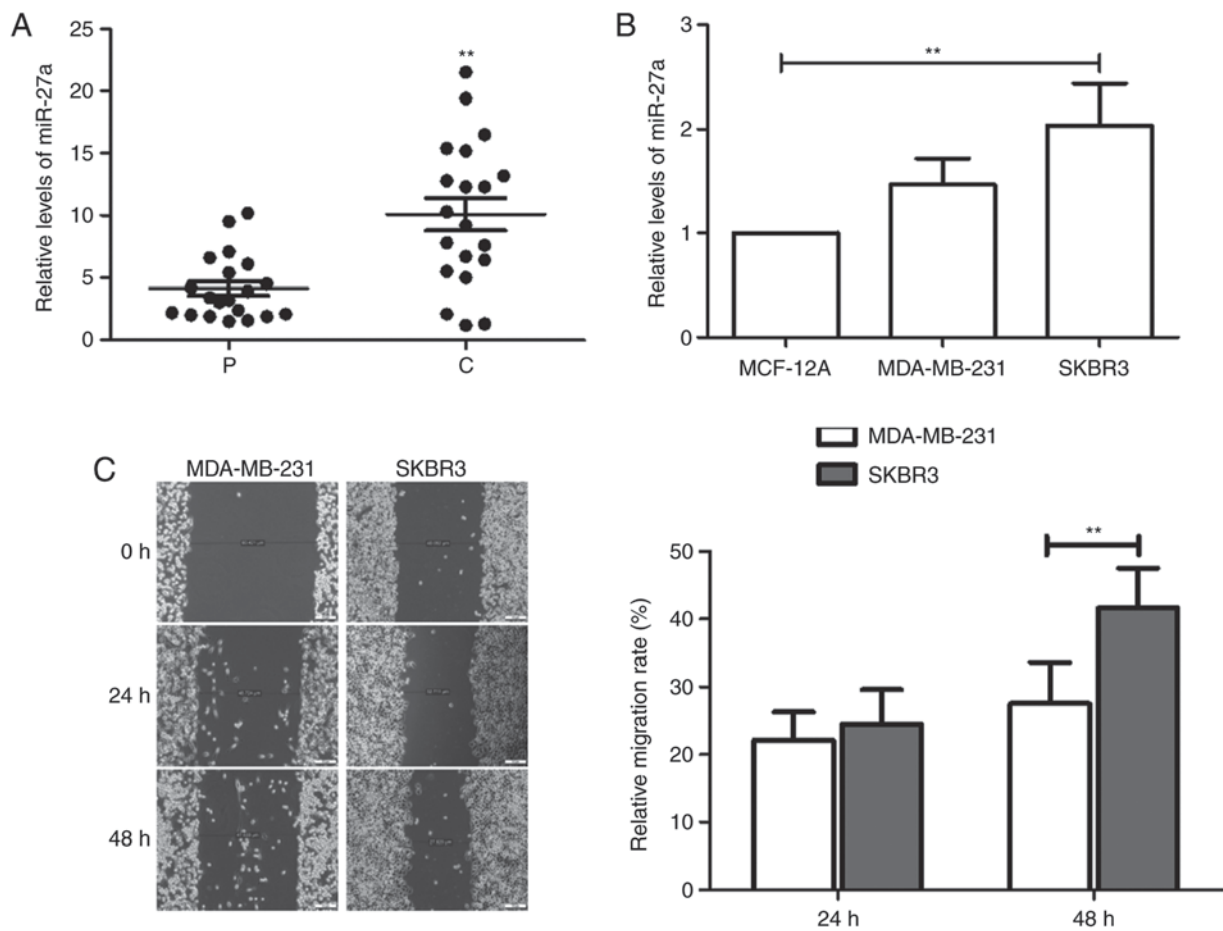
Figure 1. miR-27a expression in human breast cancer tissues and cell lines. (A) miR-27a levels expressed in 20 paired human breast cancer tissues and para-cancerous tissues were examined by RT-qPCR. **P<0.01 vs. P. (B) miR-27a levels expressed in human breast cancer cell lines and normal mammary cells were determined by RT-qPCR. (C) The migration ability of breast cancer MDA-MB-231 and SKBR3 cells at the indicated times was measured by wound healing assay. Magnification, x40. Data are presented as the mean ± standard deviation of three independent experiments. **P<0.01. miR, microRNA; RT-qPCR, reverse transcription-quantitative polymerase chain reaction; P, paracancerous tissues; C, breast cancer tissues.

increased in human breast cancer or not, a total of 20 paired samples of human breast cancer and corresponding paracancerous tissues were obtained and their miR-27a levels were evaluated using RT-qPCR. The results revealed that miR-27a was significantly increased in breast cancer tissues compared with paracancerous tissues (P<0.01; Fig. 1A). In addition, miR-27a expression levels were additionally measured in breast cancer cell lines. As presented in Fig. 1B, miR-27a expression levels were higher in the breast cancer cell lines MDA-MB-231 and SKBR3 compared with the normal mammary cell line MCF-12A. These results indicated that miR-27a was notably overexpressed in breast cancer tissues and cell lines compared with their respective control counterparts.

The basal migratory activity of breast cancer cells was determined using a wound-healing assay in MDA-MB-231 and SKBR3 cells. As presented in Fig. 1C, it was revealed that SKBR3 cells exhibited a significantly accelerated migration rate of cells into the wounded area compared with MDA-MB-231 cells at 48 h (P<0.01). Thus, these data illustrated that SKBR3 cells were more aggressive compared with MDA-MB-231 cells, while expressing a higher level of miR-27a. These results suggested that the upregulation of miR-27a may be associated with the onset of breast cancer migration.

miR-27a may promote cell migration in human breast cancer cells. To identify the physiological function of miR-27a in cell migration, miR-27a mimics or inhibitors were applied in breast cancer cell lines. Firstly, the basal migratory ability of these two cell lines was examined using a Transwell assay. The results revealed that SKBR3 cells possessed substantially increased migratory properties compared with MDA-MB-231 cells (Fig. 2A). Subsequently, MDA-MB-231 or SKBR3 cells were treated with miR-27a mimics or inhibitors. The miR-27a levels were determined by RT-qPCR analysis followed by a Transwell assay to validate the migration capacity of treated cancer cells (Fig. 2B and C). The data from the Transwell assays indicated decreased miR-27a expression significantly inhibited MDA-MB-231 and SKBR3 cell migration (Fig. 2D), while miR-27a overexpression promoted MDA-MB-231 and SKBR3 cell migration (Fig. 2E). These data demonstrated that miR-27a positively regulated breast cancer cell migration.

miR-27a induces EMT in human breast cancer cells through FBXW7. To define the underlying mechanism of miR-27a in regulating cell migration in breast cancer cells, breast cancer cells were treated with a miR-27a inhibitor to detect the expression of EMT-associated proteins. Inhibition of miR-27a suppressed the expression of the EMT-associated transcription
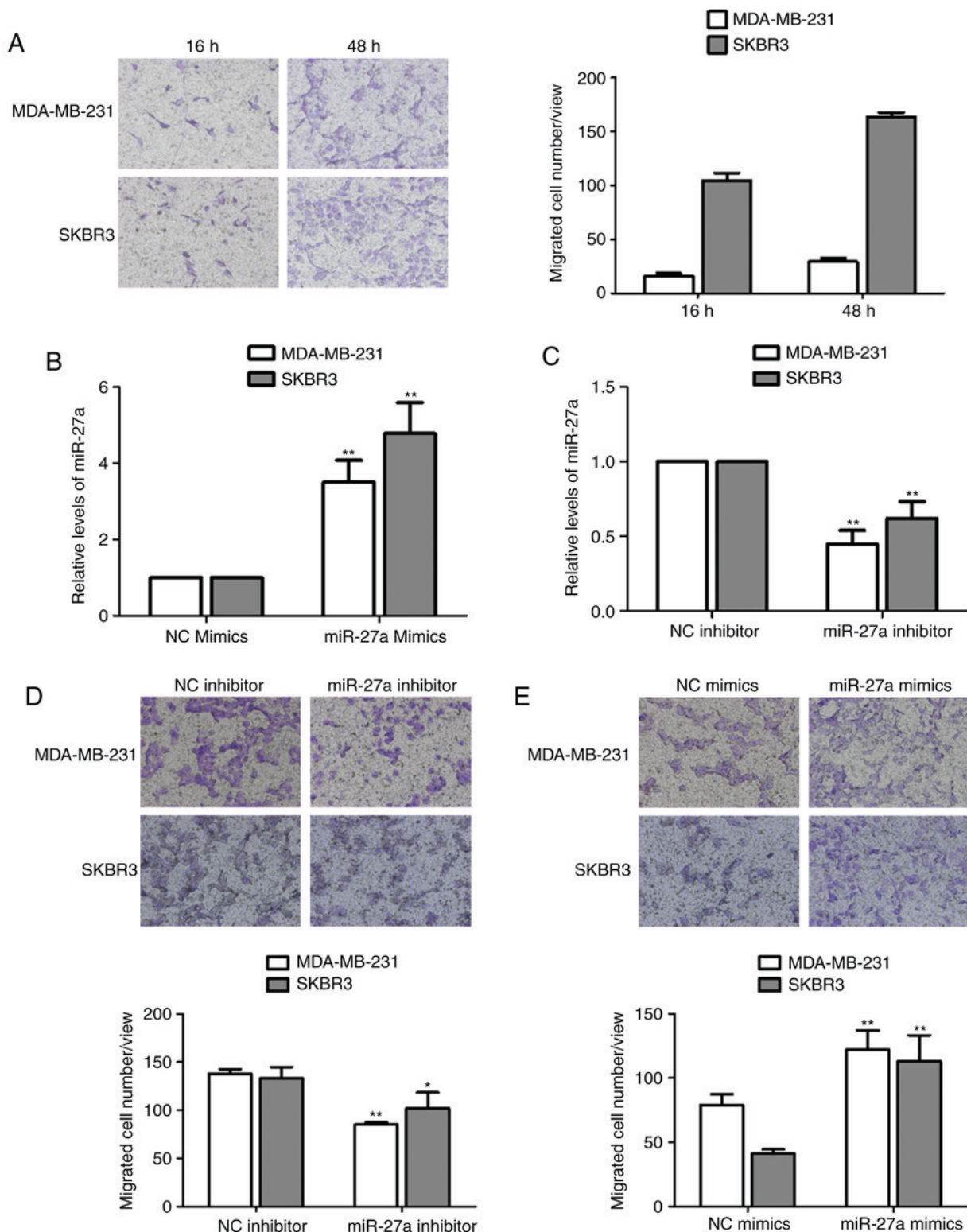
Figure 2. miR-27a promotes cell migration in human breast cancer cell lines. (A) The migration activity of MDA-MB-231 and SKBR3 cells, determined using a Transwell assay. A total of 1x10⁴ cells were seeded in Transwell chambers for 16 h or 48 h. miR-27a levels in MDA-MB-231 and SKBR3 cells transfected with (B) miR-27a mimics or (C) inhibitor for 24 h were assessed using reverse transcription-quantitative polymerase chain reaction analysis. (D) miR-27a inhibitor suppresses cell migration in human breast cancer cells. MDA-MB-231 and SKBR3 cells were transfected with the NC or miR-27a inhibitor for 24 h. A total of 2x10⁴ cells were seeded in upper Transwell chambers for 24 h. (E) miR-27a mimics promote cell migration in human breast cancer cells. MDA-MB-231 and SKBR3 cells were transfected with negative control or miR-27a mimics for 24 h. Then, 1x10⁴ cells were seeded in the upper Transwell chambers for 24 h. Magnification, x100. All data are presented as the mean ± standard deviation of three independent experiments. *P<0.05, **P<0.01 vs. respective NC group. miR, microRNA.

factors Snail and ZEB1, in addition to the mesenchymal markers N-cadherin and Vimentin, while increasing the E-cadherin

protein expression levels in MDA-MB-231 cells (Fig. 3A). All these findings indicated that the EMT of MDA-MB-231 cells
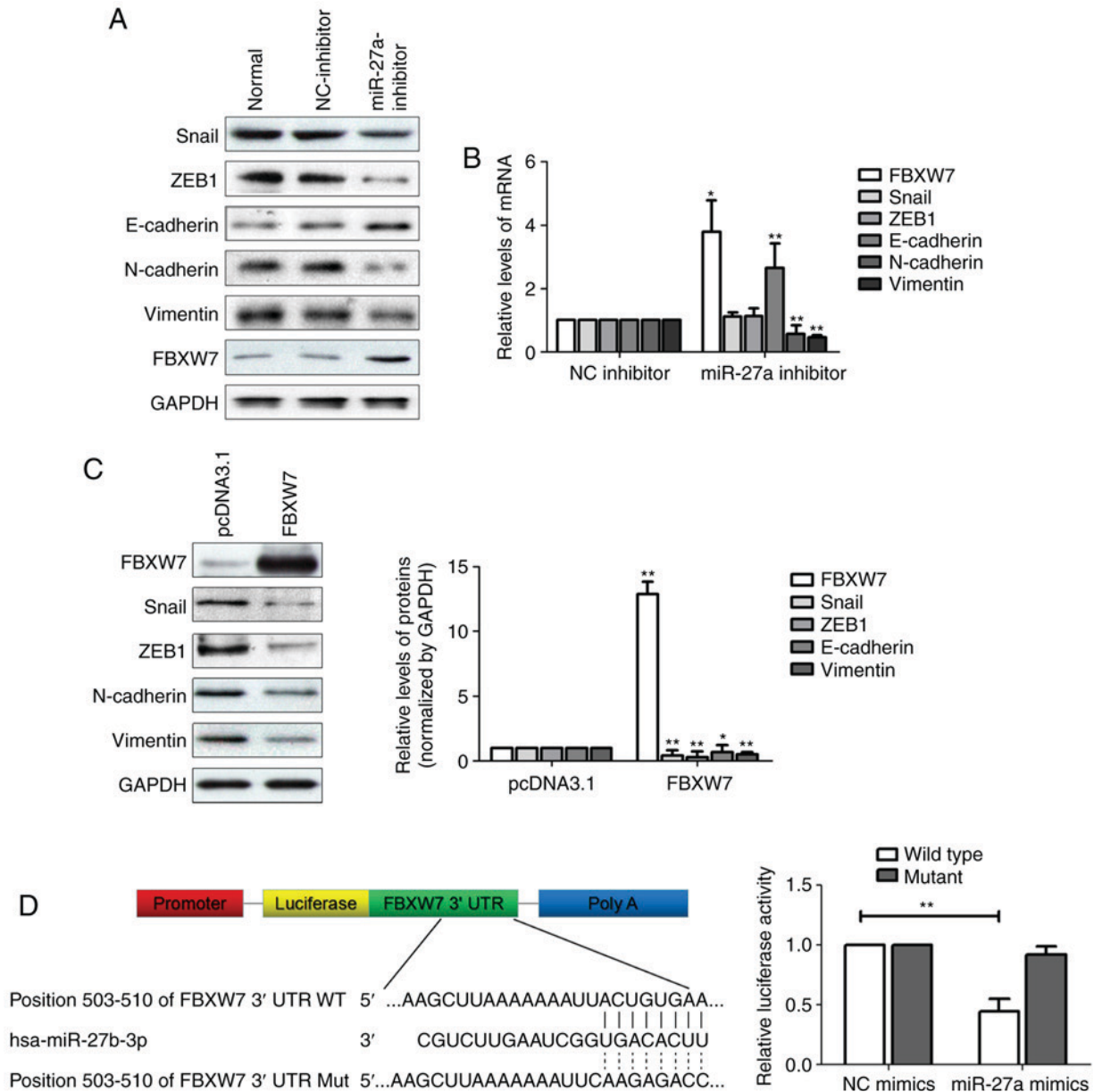
Figure 3. miR-27a induces epithelial-to-mesenchymal transition in human breast cancer cells by inhibiting FBXW7 expression. (A) MDA-MB-231 cells were transfected with negative control or miR-27a inhibitor for 24 h. Western blotting was used to detect the expression of the indicated proteins. GAPDH was used as a loading control. The data are derived from one of three independent experiments. (B) MDA-MB-231 cells were transfected with negative control or miR-27a inhibitor for 24 h. A reverse transcription-quantitative polymerase chain reaction was used to detect the indicated mRNA levels. GAPDH was used as a loading control. Data are presented as the mean ± standard deviation of three independent experiments. (C) MDA-MB-231 cells were transfected with control vector (pcDNA3.1) or FBXW7 expression vector for 24 h. Western blotting was used to detect the expression of the indicated proteins with GAPDH as a loading control (left panel). Data are presented as the mean ± standard deviation of three independent experiments. (D) A 3'-UTR assay was performed to confirm the regulatory association between miR-27a and FBXW7 using WT or Mut PGL3 plasmids. Data are presented as the mean ± standard deviation, n=3. *P<0.05, **P<0.01 vs. respective control miR, microRNA; FBXW7, F-box and WD repeat domain containing 7; UTR, untranslated region; Snail, snail family transcriptional repressor 1; NC, negative control; WT, wild-type; Mut, mutant; ZEB1, zinc finger E-box binding homeobox 1.

was reversed when miR-27a was inhibited (27). However, only the mRNA expression levels of N-cadherin, Vimentin and E-cadherin were significantly increased compared with their respective control, consistent with their protein expression profiles, while the mRNA expression levels of Snail and ZEB1 were not significantly increased.

To further elucidate the underlying mechanism, the mRNA expression profiles in MDA-MB-231 cells treated with miR-27a inhibitor or NC were analyzed by next-generation sequencing (data not shown). Among the mRNAs most notably upregulated by miR-27a inhibition, it was hypothesized that

FBXW7 may be a novel target gene, according to the prediction results from the database TargetScan. In the present study, the mRNA and protein expression of FBXW7 in MDA-MB-231 cells transfected with miR-27 inhibitor was determined. The results revealed that FBXW7 was effectively upregulated at the protein and mRNA levels when miR-27a was inhibited (Fig. 3A and B). Therefore, one explanation for the inconsistency between the mRNA and protein expression levels of Snail and ZEB1 is that these two EMT-associated transcription factors may be regulated at the post-transcriptional level by FBXW7 (22). In addition, to determine whether suppressing
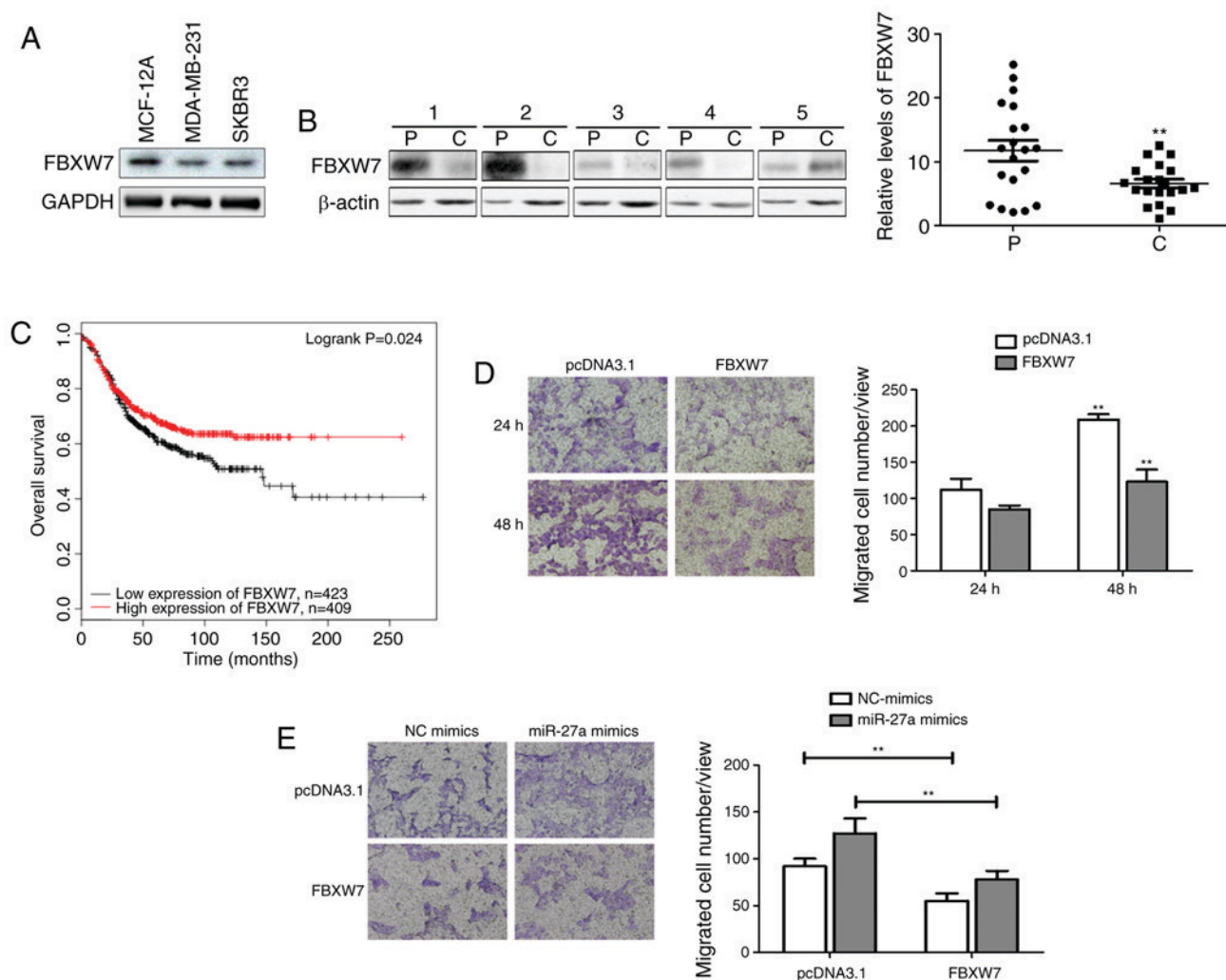
Figure 4. FBXW7 suppresses human breast cancer migration. (A) FBXW7 protein levels expressed in human breast cancer cell lines were determined using western blotting, and GAPDH was used as a loading control. (B) FBXW7 levels expressed in human breast cancer tissues and paracancerous tissues. The protein expression levels of FBXW7 were analyzed by western blotting using an FBXW7 antibody. β-actin was used as a loading control. Representative results from five patients with breast cancer are presented (left panel). The differences between FBXW7 expression levels in human breast cancer tissues and paracancerous tissues from 20 patients with breast cancer were compared (right panel). n=20. **P<0.01 vs. P. (C) Overall survival rate analysis of 832 patients was performed, according to the differential expression of FBWX7 (www.KMplot.com). P=0.024. (D) MDA-MB-231 cells were transfected with control vector (pcDNA3.1) or FBXW7-expression vector for 24 h. A total of 2x10⁴ cells were seeded in upper Transwell chambers for the indicated time courses. Data are presented as the mean ± standard deviation of three independent experiments. (E) MDA-MB-231 cells were transfected with FBXW7 overexpression plasmid or miR-27a mimics alone, or combined for 24 h, and a Transwell assay was used to detect the cell migration. Magnification, x100. **P<0.01 vs. respective control. miR, microRNA; FBXW7, F-box and WD repeat domain containing 7; P, paracancerous tissues; C, breast cancer tissues; NC, negative control.

FBXW7 may induce EMT alone, the present study produced an ectopic expression model by transfecting the FBXW7 plasmid into MDA-MB-231 cells. As presented in Fig. 3C, the ectopic expression of FBXW7 reduced the expression of Snail, ZEB1, N-cadherin and Vimentin in treated MDA-MB-231 cells. Combined results suggested that the downregulation of FBXW7 by miR-27a may promote human breast cancer cell metastatic capacity, potentially by triggering EMT.

It has been reported that FBXW7 is a target gene of miR-27a and serves a negative role in EMT regulation in esophageal cancer cells (28). However, whether FBXW7 was also regulated by miR-27a directly in human breast cancer remained to be confirmed. In the present study, luciferase reporter plasmids containing FBXW7 3'-UTR sequences with either a wild-type miR-27a binding site or the mutant version were constructed. A luciferase assay revealed that the miR-27a mimics were able to effectively repress the transcriptional activity of the wild-type plasmid, although not that of the mutant type. All results suggested that FBXW7 was also a downstream target gene of miR-27a in human breast cancer (Fig. 3D).

miR-27a promotes breast cancer cell migration in an FBXW7 suppression-dependent manner. The present study initially detected the expression levels of FBXW7 protein in human breast cancer cell lines and revealed that FBXW7 expression was lower in MDA-MB-231 and SKBR3 cells compared with MCF-12A cells (Fig. 4A). Similarly, FBXW7 expression was significantly lower in breast cancer tissues compared with paracancerous tissues, as analyzed by western blotting (P<0.01; Fig. 4B). These results supported the idea that FBXW7 was suppressed in human breast cancer, which was negatively associated with the expression profile of miR-27a. More importantly, FBXW7 levels were significantly positively associated with the overall survival rate

of patients with breast cancer, according to public datasets (log-rank P<0.05; Fig. 4C).

Furthermore, the function of FBXW7 in breast cancer migration was also illustrated. As expected, the ectopic expression of FBXW7 significantly inhibited MDA-MB-231 cell migration (P<0.01; Fig. 4D). To determine whether miR-27a-mediated breast cancer cell migration was FBXW7-dependent, the MDA-MB-231 cells were transfected with miR-27a mimics or an FBXW7 overexpression vector alone or in combination. A Transwell assay revealed that the accelerated migration induced by miR-27a was able to be significantly reversed by the ectopic expression of FBXW7 (P<0.01; Fig. 4E). Consequently, the results illustrated that FBXW7 was negatively regulated by miR-27a in human breast cancer tissues and cell lines. Notably, miR-27a may promote breast cancer cell migration by targeting FBXW7.

Discussion

As an important class of non-coding RNAs, miRNAs are reported to be involved in multiple biological events, including carcinogenesis and the progression of cancer in numerous cancer types, such as breast cancer (29-33). As an oncogenic miRNA, miR-27a has been revealed to exert essential effects in a number of cancer types. Myatt *et al* (34) reported that miR-27a suppressed the expression of the tumor suppressor gene forkhead box O1 in endometrial cancer. By targeting SMAD family member (SMAD)2 and SMAD4, miR-27a may regulate transforming growth factor-β signaling pathway activation in lung cancer (35). Furthermore, miR-27a was additionally demonstrated to promote metastasis in human gastric cancer cells by stimulating the expression of vimentin, ZEB1 and ZEB2, thus resulting in the induction of EMT (8). miR-27a was also demonstrated to increase cisplatin resistance and metastasis by promoting EMT in human lung adenocarcinoma cells in a phosphatidylethanolamine binding protein 1-dependent manner (36).

Previously, miR-27a was reported to serve critical roles in radiosensitivity (37), cell differentiation (38), cell proliferation (39) and drug resistance (40) in human breast cancer. More recently, it was demonstrated that miR-27a was able to activate the Wnt/β-catenin signaling pathway by negatively regulating secreted frizzled related protein 1 to promote the proliferation, migration and invasion of breast cancer cells (41). A number of compounds targeting miR-27a have also been revealed to serve anti-oncogenic roles in cell metastasis and various resistance phenotypes in breast cancer. However, to date, functional evidence for the role of miR-27a in breast cancer has not been well documented, particularly in breast cancer metastasis.

The present study attempted to elucidate the roles and the underlying mechanisms of miR-27a in the regulation of cell migration in human breast cancer. Firstly, the present study verified that the expression of miR-27 was increased in breast cancer tissues and cell lines compared with their normal counterparts. Notably, it appeared that the expression level of miR-27a was positively associated with the migration potential of different breast cancer cell lines. Loss- or gain-of-function experiments indicated that miR-27a was able to promote the migratory activity of human breast cancer cells. The present data also revealed that the inhibition of miR-27a substantially decreased the protein expression levels of EMT-associated transcription factors Snail and ZEB1, and their downstream mesenchymal markers N-cadherin and vimentin, while increasing the protein expression levels of the epithelial marker E-cadherin. These results suggested that miR-27a may improve breast cancer cell migration via the EMT pathway, at least partially.

EMT was originally recognized as a process during which epithelial cells present a migratory and invasive mesenchymal phenotype (27). The irregular activation of EMT also contributes to tumor development (3). EMT facilitates the migration and invasion of epithelial tumor cells. The present study attempted to examine how miR-27a was involved in breast cancer cell migration regulation. The expression of FBXW7 in MDA-MB-231 cells with silenced miR-27a was detected, due to the important functions of EMT regulation in cancer cells (20,21). The data revealed that the reduction of miR-27a increased FBXW7 expression in breast cancer cells. The results of the present study also confirmed for the first time, to the best of our knowledge, that FBXW7 is a target gene of miR-27a in human breast cancer, using a luciferase assay. FBXW7 overexpression was induced in MDA-MB-231 cells through transfection with a FBXW7 expression plasmid, and it was revealed that abundant FBXW7 expression suppressed cell migration, and also effectively reversed the positive effect of miR-27a on breast cancer cell migration by inhibiting the EMT process. Additionally, the expression of FBXW7 in breast cancer tissues and cell lines were revealed to be suppressed compared with their normal counterparts. Furthermore, FBXW7 is a positive factor for the overall survival rate of patients with breast cancer. FBXW7 is therefore a functional target gene of miR-27a in human breast cancer.

In conclusion, although other downstream target genes of miR-27a may also be involved in regulating the EMT process, the present data demonstrated that miR-27a was able to promote human breast cancer cell migration, at least in part, by targeting FBXW7, which served a prominent function as a suppressor of EMT. Therefore, the results of the present study demonstrated that the miR-27a/FBXW7 axis is an important regulator in the metastasis of breast cancer and indicated its potential applications in metastatic breast cancer therapy.

Availability of data and materials

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

Authors' contributions

GJ and XZ conceived and designed the experiments. GJ, WS and HF performed the experiments. GJ and WS analyzed the data. GJ and XZ wrote the manuscript.

Ethics approval and consent to participate

All patients provided written informed consent for the use of clinical materials in research, and the project was approved by the Institutional Ethics Committee of the Taizhou Hospital of Zhejiang Province (Taizhou, China).

Patient consent for publication

All patients involved in this study provided written informed consent for the publication of any associated data and accompanying images.

Competing interests

The authors declare that they have no competing interests.

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