

Original Article

KIF11 promotes breast cancer migration and invasion through the Akt signaling pathway and is associated with miR-381-3p downregulation

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Abstract: Objective: To investigate the functional role of kinesin family member 11 (KIF11) in breast cancer cell migration and invasion, and to explore whether miR-381-3p functions as an upstream regulator of KIF11 expression. Methods: Gene set enrichment analysis was performed on data from the TCGA-BRCA database to identify pathways associated with KIF11 expression. KIF11 was stably knocked down in MCF-7 and SK-BR-3 breast cancer cell lines using lentiviral short hairpin RNA. 3D Matrigel invasion, wound healing, and Transwell assays were used to assess cell migration and invasion. Expression of epithelial-mesenchymal transition (EMT) markers and Akt pathway proteins was examined by quantitative polymerase chain reaction (qPCR), western blot, and immunofluorescence. miR-381-3p expression was measured by qPCR, and miR-381-3p mimics were transfected into the cell lines to overexpress miR-381-3p. Results: KIF11 knockdown in breast cancer cell lines reduced cell migration by approximately 60% ($P < 0.001$) and invasion by approximately 75% ($P < 0.001$), decreased vimentin expression (approximately 3-fold), and increased E-cadherin expression (approximately 2.5-fold). KIF11 knockdown also reduced phosphorylation of Akt (Ser473) and GSK-3 β (Ser9) by approximately 50% and 45%, respectively. miR-381-3p expression was decreased in both breast cancer tissues and cell lines. Overexpression of miR-381-3p in the cell lines was associated with reduced KIF11 expression and decreased cell migration and invasion. Conclusions: KIF11 may promote breast cancer cell migration and invasion, possibly through regulation of EMT and Akt signaling. Downregulation of miR-381-3p may contribute to KIF11 upregulation in breast cancer. These findings support further investigation of KIF11/miR-381-3p axis in metastatic breast cancer.

Keywords: KIF11, breast cancer, Akt signaling, miR-381-3p

Introduction

For breast cancer patients, clinical outcomes are critically determined by metastasis. The PI3K/Akt pathway regulates tumor cell migration, invasion, and survival, thereby playing a central role in metastatic progression [1, 2]. In breast cancer, key steps of metastatic dissemination including EMT, cytoskeletal remodeling, and cell migration are promoted by Akt signaling [2, 3].

Kinesin family member 11 (KIF11), a microtubule-associated motor protein, is essential for normal cell function [4-6]. Altered KIF11 structure or expression has been associated with diverse pathologies, including neurodegenerative disorders and developmental defects [7-9]. Using CRISPR activation in human diploid cells, researchers found that raising KIF11 above normal physiologic levels caused centrosome fragmentation, increased microtubule stability, chromosome lagging, and mitotic defects [5].

KIF11 expression is elevated in many human cancers. For example, KIF11 is elevated in Wilms tumor, and KIF11 expression is associated with Wilms tumor clinical outcome [4]. In non-small cell lung cancer, high KIF11 expression also predicts poor prognosis [10]. In hepatocellular carcinoma, KIF11 levels correlate negatively with overall and disease-free survival but positively with tumor size [11]. High KIF11 levels predict worse outcome in adrenocortical carcinoma [12]. Because tumor prognosis is closely linked to the presence of distant metastasis, studies have identified KIF11 as a promoter of tumor invasion and metastasis [13, 14]. In undifferentiated thyroid cancer cells, reduced KIF11 ubiquitination promotes cell migration and invasion [15]. KIF11 expression also independently predicts bone metastasis in prostate cancer and drives invasion, proliferation, and self-renewal in these metastases [16, 17].

Several studies have linked KIF11 to the PI3K/Akt pathway [18]. In gallbladder cancer, KIF11 promotes cell proliferation through the ERBB2/PI3K/Akt pathway [19]. KIF11 has also been shown to participate in PI3K/Akt signaling during tumor cell invasion and the epithelial-mesenchymal transition [19]. Given that KIF11 is a microtubule motor protein, we propose that it may facilitate the intracellular trafficking of Akt or its upstream activators to the plasma membrane, where Akt undergoes activating phosphorylation. Although our previous work showed that KIF11 is elevated in breast cancer and promotes cell proliferation, its specific role in breast cancer metastasis remains unclear [20]. KIF11 is a potential drug target. Thus, its role in breast cancer metastasis remains to be clarified.

MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression [21]. miR-381-3p levels are lower in both breast cancer tissues and cell lines [22]. This miRNA suppresses breast cancer cell proliferation and invasion by targeting multiple genes [23, 24]. Whether miR-381-3p regulates KIF11 expression in breast cancer has not been reported.

In this study, we generated KIF11-knockdown cell lines and evaluated the impact of KIF11 on breast cancer cell migration and invasion. In breast cancer cells, KIF11 knockdown reduces both cell motility and the ability to invade.

Additionally, miR-381-3p expression decreases in breast cancer cells and correlates negatively with KIF11 expression. TargetScan analysis identified two conserved binding sites for miR-381-3p in the KIF11 3'-UTR, suggesting that KIF11 may be a direct target of this miRNA. Overexpression of miR-381-3p reduces KIF11 levels and suppresses cell migration and invasion.

Materials and methods

Cell culture

Normal breast epithelial cells (NBEC1 and NBEC2) were isolated from mammary tissues of healthy donors at the Department of Plastic Surgery, Shenzhen Longgang Maternal and Child Health Hospital, as previously described [20, 25]. The use of human mammary tissues for isolating NBEC1 and NBEC2 was approved by the Ethics Committee of the Maternity & Child Healthcare Hospital of Longgang District, Shenzhen City (approval number: LGFYKYXLL-2024-25). Written informed consent was obtained from all donors prior to tissue collection. NBEC1 and NBEC2 were cultured in serum-free keratinocyte medium (Invitrogen) containing 10 ng/mL epithelial growth factor, 50 µg/mL bovine pituitary extract, and 1% penicillin-streptomycin (Gibco). The breast cancer cell lines T47D, MDA-MB-453, MCF-7, MDA-MB-415, BT-549, ZR-75-30, SK-BR-3, and MDA-MB-231 were obtained from the American Type Culture Collection (ATCC, Manassas). These cells were maintained in DMEM (Thermo Fisher Scientific) supplemented with 10% fetal bovine serum (HyClone) at 37°C in a 5% CO₂ incubator. All cell lines were authenticated within six months of use by short tandem repeat profiling (IDEXX RADIL) and tested negative for mycoplasma using the MycoAlert assay (Lonza). For functional experiments, MCF-7 and SK-BR-3 were selected because they represent the luminal and HER2-positive subtypes, respectively.

Generation of stable KIF11-knockdown cells

Two short hairpin RNA (shRNA) oligonucleotides targeting KIF11 were inserted into pSUPER-retro-puro retroviral vectors as previously described [20]. Retroviral particles were produced and used to transduce the breast cancer cells, followed by selection with 2 µg/mL puromycin

(Sigma) for 72 h. We confirmed knockdown efficiency using qPCR and western blot (see below). [Supplementary Table 1](#) contains the shRNA target sequences.

miR-381-3p overexpression

Cells were transfected with 50 nM miR-381-3p mimics or the corresponding negative control (RiboBio; sequences provided in [Supplementary Table 2](#)) using Lipofectamine 3000 (Thermo Fisher). We evaluated transfection efficiency using qPCR at 48 h after transfection.

qPCR

We isolated total RNA using TRIzol (Invitrogen). Reverse transcription was carried out with PrimeScript RT Master Mix (Takara). For qPCR, we used SYBR Green (Roche) on a QuantStudio 6 platform (Applied Biosystems). The cycling parameters were: 95°C for 10 min; 40 cycles of 95°C for 15 s; 60°C for 1 min. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. We used GAPDH as an internal control for mRNA and U6 for miRNA. [Supplementary Table 3](#) provides the primer sequences.

Western blot

Proteins were separated and transfer were performed according to standard protocols. Primary antibodies against KIF11 (1:800, #14404), E-cadherin (1:800, #3195), vimentin (1:1000, #5741), and α -tubulin (1:5000, #3873) were purchased from Cell Signaling and incubated overnight at 4°C. Secondary antibodies (anti-rabbit [#7074] and anti-mouse [#7076], both 1:5000) were also purchased from Cell Signaling. For total proteins, band intensity was normalized to α -tubulin and expressed relative to the control group. For phosphorylated proteins, the ratio of phosphorylated protein to total protein was calculated after each was independently normalized to α -tubulin.

The cancer genome atlas (TCGA) data analysis

TCGA-BRCA (breast invasive carcinoma) RNA sequencing (RNA-seq) data (RSEM-normalized, $\log_2$ -transformed) and miRNA-seq data (reads per million-normalized) were downloaded from the National Cancer Institute Genomic Data Commons. We ran GSEA (version 4.1.0) on the Hallmark gene sets from MSigDB, applying 1,000 phenotype permutations. A false discov-

ery rate (FDR) below 0.05 was used as the threshold for significance.

Immunofluorescence (IF)

Cells fixed with 4% paraformaldehyde were permeabilized with 0.1% Triton X-100 and blocked with 5% bovine serum albumin. They were then incubated with anti-vimentin (1:500, ab92547, Abcam) or anti-E-cadherin (1:500, ab40772, Abcam) overnight at 4°C. Cells were then incubated for 1 h at room temperature with Alexa Fluor 488- or Alexa Fluor 594-linked secondary antibody staining (1:1000, Invitrogen). Nuclear counterstaining was performed using DAPI (4', 6-diamidino-2-phenylindole) (1:800, D9542, Sigma). A Zeiss Axioskop 2 Plus microscope was used for image capture, and ZEN software was used for analysis.

3D invasion

We mixed 1×10^4 cells with 50 μ L of DMEM containing 1 mg/mL Matrigel (Corning) and seeded the mix into a 24-well plate. After the gel set at 37°C (about 30 min), we added complete DMEM (10% FBS) and changed it every two days. Invasive protrusion length was measured using ImageJ. Images were captured every 2 days over 14 days.

Wound healing

We cultured cells in 6-well plates with complete DMEM until they reached confluence. The medium was then replaced with low-serum DMEM (2% FBS) for 2 h. We made a scratch in the monolayer a 200- μ L pipette tip. The cells were then washed and maintained in low-serum medium. Images were taken at 48 h. The wound area was measured using ImageJ. The percentage of wound closure was determined as follows: (initial area-area at 48 h)/initial area $\times$ 100%.

Transwell invasion

We prepared Matrigel (Corning) at 1.0 mg/mL in serum-free DMEM. Each Transwell insert (8- μ m pores size, Corning) received 50 μ L of this diluted Matrigel on the upper chamber, which was then left to gel at 37°C for 30 minutes. Cells (1×10^4) were resuspended in 200 μ L of serum-free DMEM and placed into the upper chamber. The lower chamber was filled with 600 μ L of complete DMEM to serve as a

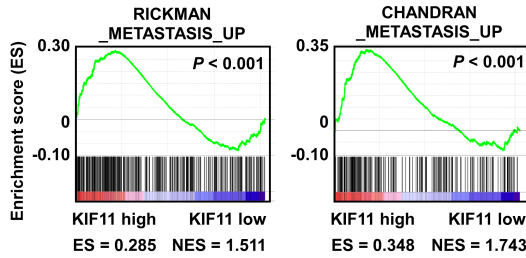


Figure 1. KIF11 overexpression is associated with metastatic gene signatures in breast cancer. Gene set enrichment analysis (GSEA) of data from the TCGA-BRCA dataset showing enrichment of metastasis-related gene sets in KIF11-high breast tumors (false discovery rate [FDR] < 0.05). NES, normalized enrichment score.

chemoattractant. After 24 h, we removed non-invading cells from the upper membrane surface. Invading cells on the lower surface were then fixed (4% paraformaldehyde), stained (0.1% crystal violet), and counted across five random fields per insert.

Statistical analysis

GraphPad Prism 9.0 (GraphPad Software, San Diego, CA) was used for statistics. Data represent mean $\pm$ SD from ≥ 3 independent experiments. Two-tailed Student's t-test was applied for two-group comparisons (paired for matched tumor-normal samples, unpaired for all other comparisons), and one-way ANOVA with Tukey's *post hoc* test was used for multiple-group comparisons. Significance was set at *p*-value < 0.05. No correction for multiple testing was made.

Results

KIF11 overexpression is associated with metastatic gene signatures in breast cancer

As shown in [Supplementary Figure 1](#) and reported previously, KIF11 mRNA levels are higher in breast cancer tissues than in adjacent normal tissues across all subtypes [20]. We used GSEA on the TCGA-BRCA dataset to see whether KIF11 expression relates to metastatic potential. Stratifying tumors by KIF11 expression level revealed that KIF11-high tumors were significantly enriched for metastasis-associated gene signatures (FDR < 0.05, **Figure 1**). Similar associations between KIF11 overexpression and poor prognosis have been reported in other cancers, which led us to investigate its function further.

KIF11 depletion suppresses breast cancer cell invasion and migration

To test whether KIF11 contributes to metastasis, we knocked it down stably in MCF-7 and SK-BR-3 cells using lentiviral shRNA. Two independent shRNAs reduced KIF11 mRNA and protein by more than 70% (**Figure 2A, 2B**). In 3D Matrigel, KIF11-silenced cells formed shorter invasive protrusions than controls, with approximately 50% reduction in protrusion length ($P < 0.001$, **Figure 2C**). Wound healing assays also showed less migration (approximately 60% decrease, $P < 0.001$; **Figure 2D**), and Transwell invasion assays gave similar results (approximately 75% decrease, $P < 0.001$; **Figure 2E**).

KIF11 knockdown alters EMT marker expression

Because migration and invasion were impaired, we asked whether KIF11 affects vimentin and E-cadherin, two EMT-related markers. In MCF-7 and SK-BR-3 cells, KIF11 knockdown lowered vimentin mRNA by about 3-fold and raised E-cadherin mRNA by about 2.5-fold (**Figure 3A**). These changes were confirmed at the protein level by western blot (**Figure 3B**). Immunofluorescence showed that KIF11-depleted cells had more E-cadherin at the membrane and less vimentin in the cytoplasm, consistent with a less mesenchymal state (**Figure 3C**).

KIF11 knockdown reduces Akt phosphorylation

PI3K/Akt signaling was enriched in KIF11-high tumors based on our GSEA (**Figure 4A**). We therefore asked whether KIF11 affects Akt and its downstream target GSK-3 β . In KIF11-knockdown cells, phosphorylated Akt (Ser473) and phosphorylated GSK-3 β (Ser9) were both lower than in controls, whereas total Akt and total GSK-3 β remained unchanged (**Figure 4B**). The p-Akt/t-Akt ratio dropped by about 50%, and the p-GSK-3 β /t-GSK-3 β ratio dropped by about 45% (**Figure 4B**). Thus, lower KIF11 expression correlates with lower Akt pathway activity in breast cancer cells.

miR-381-3p is associated with KIF11 expression and suppresses cell migration and invasion

To identify upstream regulators that may contribute to KIF11 overexpression in breast can-

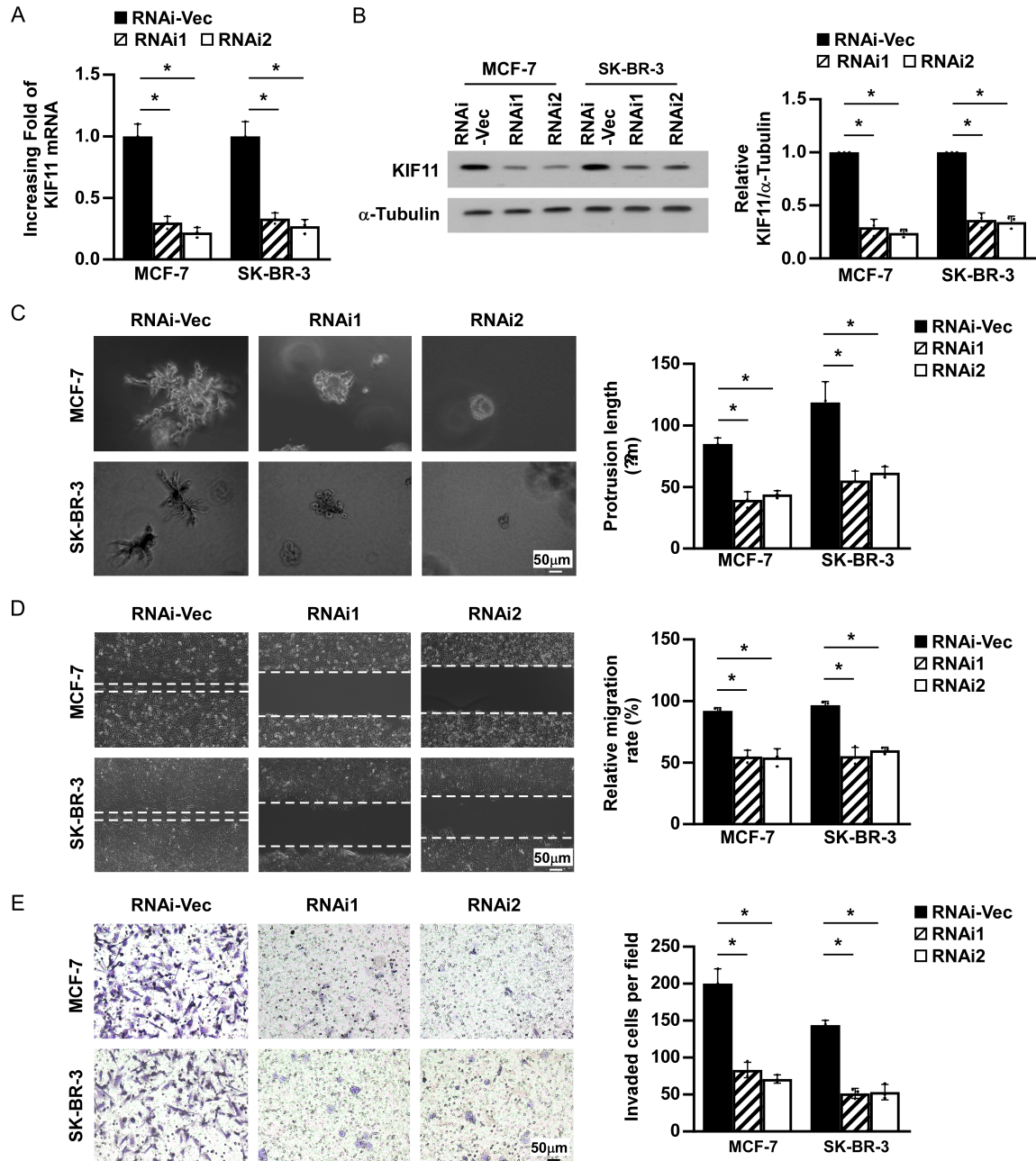


Figure 2. KIF11 depletion suppresses breast cancer cell invasion and migration. Validation of KIF11 knockdown in MCF-7 and SK-BR-3 cells by qPCR (A) and western blot (B). GAPDH and α-tubulin served as loading controls for qPCR and western blot, respectively. (C) 3D Matrigel invasion assay (scale bar: 50 μm). Quantification of the invasive protrusion length is shown in the right panel. Data represent mean protrusion length ± SD of three independent experiments, measured using ImageJ. (D) Wound healing assay (scale bar: 50 μm; 48 h). Relative migration rate was calculated as (wound area at 0 h-wound area at 48 h)/wound area at 0 h × 100%. Data are presented as mean ± SD from three independent experiments. *P < 0.05 vs. shControl. (E) Transwell invasion assay (scale bar: 50 μm; 24 h). Data are presented as mean ± standard deviation (SD) from three independent experiments. *P < 0.05 vs. shControl (two-tailed Student's t-test).

cer, we used TargetScan to screen for miRNAs predicted to target the KIF11 3'-untranslated region. Our analysis identified two con-

served miR-381-3p binding sites on KIF11 (Supplementary Figure 2). Analysis of TCGA miRNA-seq data revealed that miR-381-3p was

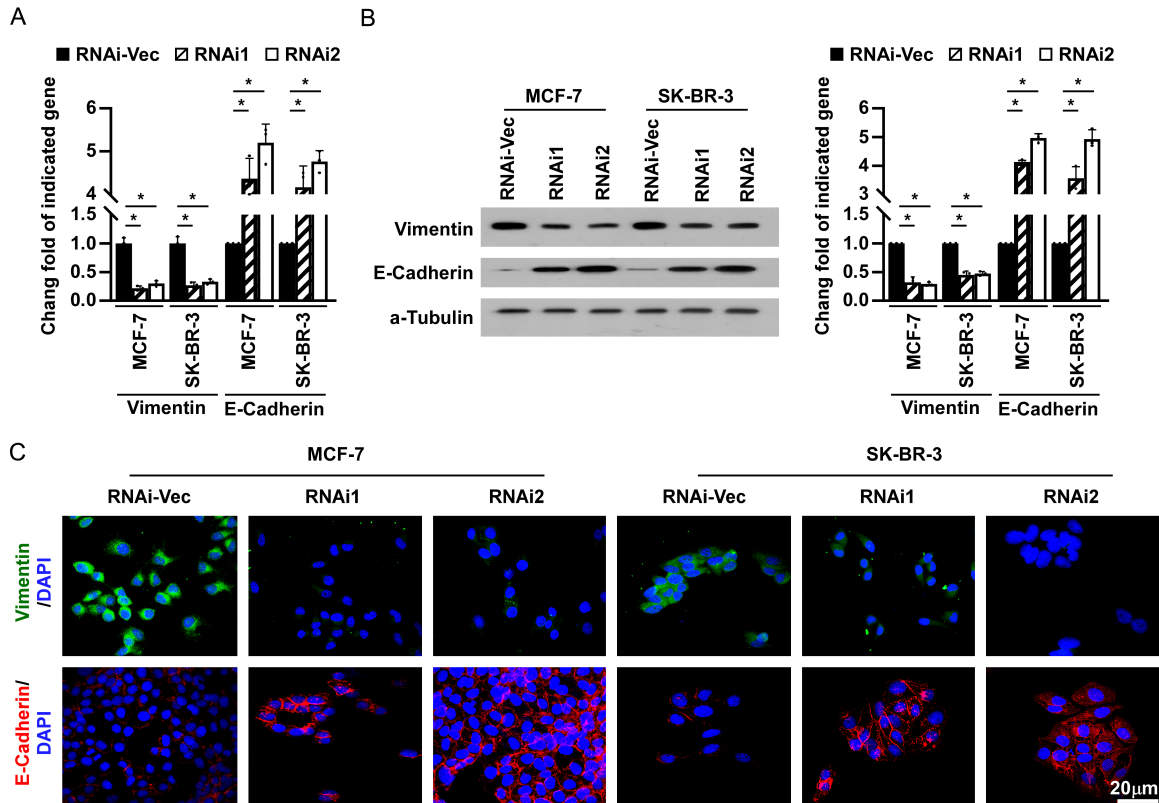


Figure 3. KIF11 modulates EMT marker expression in breast cancer cells. A. qPCR analysis of vimentin (VIM) and E-cadherin (CDH1) mRNA levels in KIF11-knockdown MCF-7 and SK-BR-3 cells. B. Western blot analysis of vimentin and E-cadherin protein expression (left panels) and quantification (right panels). C. Immunofluorescence staining of vimentin (green) and E-cadherin (red) with DAPI (blue; scale bar: 20 μ m). Arrows indicate membrane-localized E-cadherin. Data are presented as mean $\pm$ SD from three independent experiments. *P < 0.05 vs. Control (two-tailed Student's t-test).

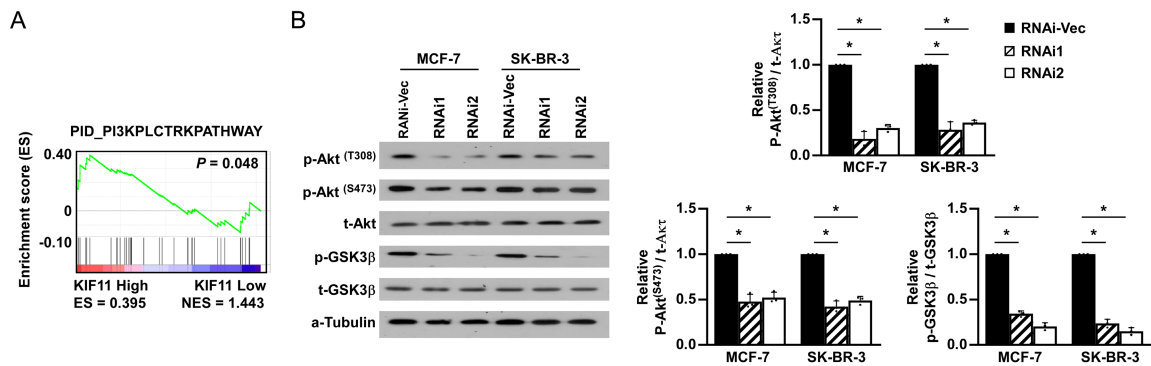


Figure 4. KIF11 knockdown is associated with reduced Akt phosphorylation. A. GSEA of TCGA data showing enrichment of PI3K/Akt signaling genes in KIF11-high tumors (FDR < 0.01). B. Western blot analysis of phosphorylated Akt (Ser473), total Akt, phosphorylated GSK-3 β (Ser9), and total GSK-3 β in KIF11-knockdown MCF-7 and SK-BR-3 cells. Quantification of band intensities normalized to α -tubulin is shown in the right panel. Data are presented as mean $\pm$ SD from three independent experiments. *P < 0.05 vs. shControl (two-tailed Student's t-test).

significantly downregulated in breast tumors (n = 1,077) compared to normal tissues (n = 103; P < 0.001, **Figure 5A**). Paired tumor-normal

samples showed the same pattern (n = 101; **Figure 5B**). Reduced miR-381-3p expression was also seen across several breast cancer cell

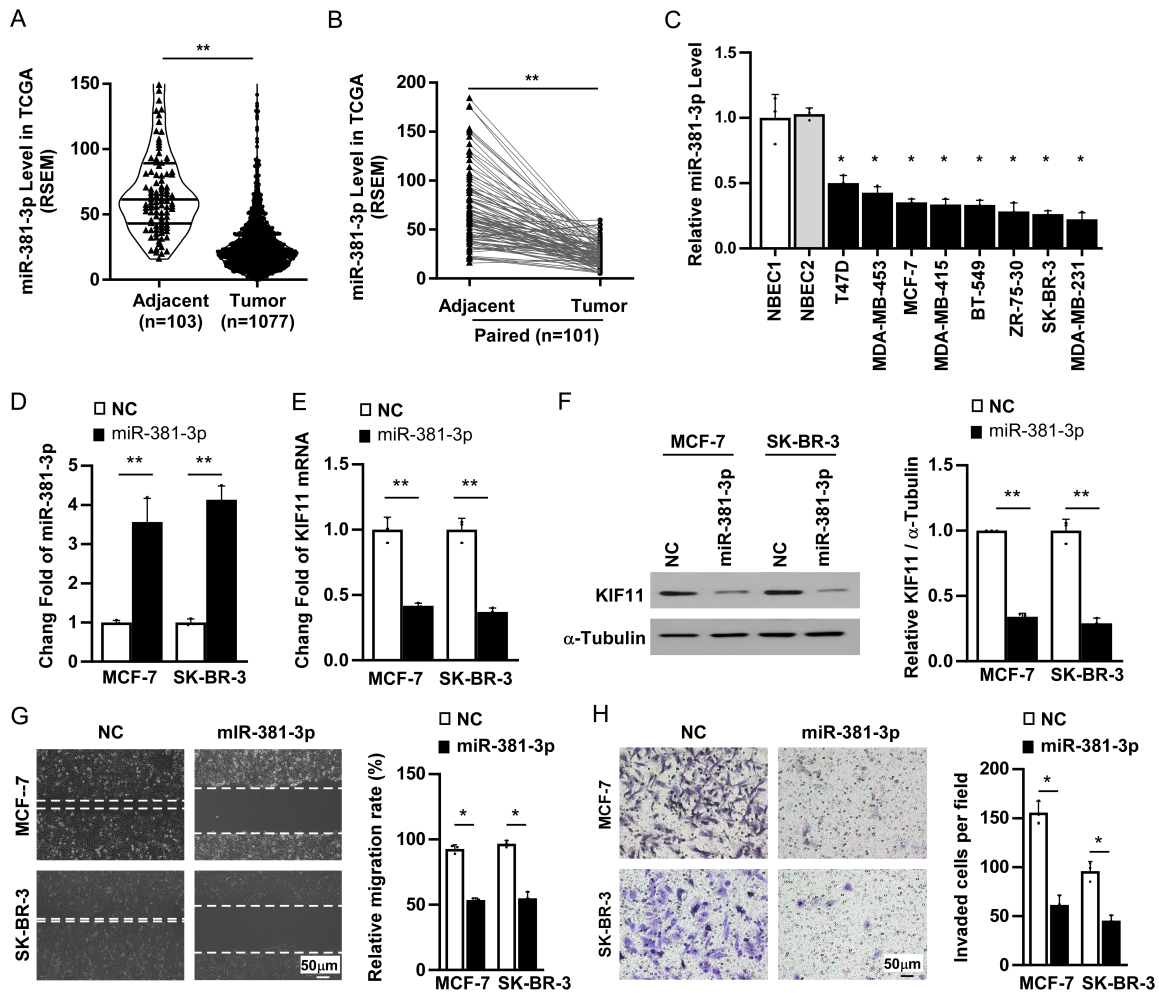


Figure 5. miR-381-3p is associated with KIF11 expression and suppresses breast cancer cell migration and invasion. **A.** miR-381-3p expression levels in TCGA breast tumors (n = 1,077) compared with normal breast tissues (n = 103). **B.** Paired analysis of miR-381-3p expression in breast tumors versus adjacent normal tissues (n = 101). **C.** qPCR analysis of miR-381-3p expression in breast cancer cell lines relative to normal breast epithelial cells (NBEC1 and NBEC2). **D.** qPCR confirmation of miR-381-3p overexpression following transfection of miR-381-3p mimics into MCF-7 and SK-BR-3 cells. **E.** KIF11 mRNA expression in miR-381-3p-overexpressing cells. **F.** Western blot analysis of KIF11 protein expression following miR-381-3p overexpression (left panels) and quantification (right panels). **G.** Wound healing assay showing migration of miR-381-3p-overexpressing cells (scale bar: 50 μ m). **H.** Transwell invasion assay showing invasive capacity of miR-381-3p-overexpressing cells (scale bar: 50 μ m). Data are presented as mean $\pm$ SD from three independent experiments. *P < 0.05 vs. NC mimic (two-tailed Student's t-test). ns, not significant.

lines compared to normal breast epithelial cells (**Figure 5C**).

We then asked whether raising miR-381-3p levels affects KIF11 and cell behavior. Transfecting MCF-7 and SK-BR-3 cells with miR-381-3p mimics led to strong overexpression of the miRNA (**Figure 5D**). Under these conditions, KIF11 mRNA dropped about 2-fold (**Figure 5E**) and KIF11 protein dropped about 60% (**Figure 5F**). Cell migration and invasion

were also reduced (**Figure 5G** and **5H**), similar to what we saw with direct KIF11 knockdown. Thus, miR-381-3p appears to negatively regulate KIF11 and to suppress migratory and invasive behavior in breast cancer cells.

Discussion

This study asked whether KIF11 contributes to breast cancer metastasis and how it might be regulated. We found that KIF11 knockdown

reduced cell invasion and migration, along with changes in EMT markers and lower Akt phosphorylation. We also found that miR-381-3p, which is downregulated in breast tumors, negatively regulates KIF11: raising miR-381-3p lowered KIF11 and suppressed migration and invasion. Together, these data point to a possible link between miR-381-3p loss and KIF11 gain in breast cancer.

Elevated KIF11 has been linked to aggressive features in several cancers. Our previous work established KIF11's role in breast cancer proliferation and stemness [20, 25]. The current study extends these findings by demonstrating that KIF11 also contributes to cellular invasion and migration, two key components of the metastatic cascade. Our observation that KIF11 depletion leads to E-cadherin upregulation and vimentin downregulation suggests that KIF11 helps sustain a mesenchymal-like, invasion-competent state. This finding is consistent with reports in other cancer types linking KIF11 to migratory phenotypes.

Regarding the underlying mechanism, our GSEA and western blot data indicated a potential association between KIF11 expression and Akt pathway activity. KIF11 is a microtubule motor protein that transports cargo along microtubule tracks. Therefore, it is plausible that KIF11 facilitates the intracellular trafficking of Akt or its upstream activators to the plasma membrane, where Akt undergoes activating phosphorylation. This theory represents a testable hypothesis for future mechanistic studies.

Finding that miR-381-3p negatively regulates KIF11 helps explain why KIF11 is often overexpressed in breast cancer. Low miR-381-3p levels are common in breast tumors, and restoring its expression reduces invasion and migration, as we showed. Similar tumor-suppressive functions of miR-381-3p have been reported in colorectal and gastric cancers. Together, these observations raise the possibility that loss of miR-381-3p contributes to KIF11 dysregulation in breast cancer.

Our findings should be viewed with several caveats. First, the functional experiments used MCF-7 and SK-BR-3 cells, which are not highly metastatic models. It is essential to validate our proposed mechanism in highly invasive cell

lines, particularly triple-negative breast cancer-derived lines such as MDA-MB-231. Second, we did not examine other EMT-related transcription factors (Snail, Slug, or ZEB1) or additional epithelial markers (ZO-1 or claudin). Third, our conclusions regarding the KIF11-Akt and miR-381-3p-KIF11 relationships are based on correlative evidence. Definitive causal links can be established with pharmacological rescue experiments (e.g., Akt inhibition in KIF11-overexpressing cells) and dual-luciferase reporter assays to confirm direct miR-381-3p targeting of KIF11. Fourth, all experiments were conducted *in vitro*. *In vivo* metastasis models are critical to validate the therapeutic relevance of targeting the KIF11-miR-381-3p axis. Our current data establish a strong foundation for these future investigations, but cannot be interpreted as confirmation of the complete proposed mechanism.

In conclusion, our study identified a functional association linking KIF11 overexpression to enhanced invasion and migration in breast cancer cells. Additionally, we found that miR-381-3p downregulation drives KIF11 overexpression. Our results implicate Akt pathway activity as a downstream mediator of cell invasion and migration. Thus, the KIF11/miR-381-3p axis merits further study in metastatic breast cancer.

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Disclosure of conflict of interest

None.

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References

- [1] Lambert AW, Pattabiraman DR and Weinberg RA. Emerging biological principles of metastasis. *Cell* 2017; 168: 670-691.
- [2] Deng F, Weng Y, Li X, Wang T, Fan M and Shi Q. Overexpression of IL-8 promotes cell migration via PI3K-Akt signaling pathway and EMT in triple-negative breast cancer. *Pathol Res Pract* 2020; 216: 152902.
- [3] Ali M, Singh N, Yadav M, Nayak S, Bamezai RNK and Singh RP. miR-24-2 targets Akt and inhibits cell survival, EMT, and tumour growth in triple-negative breast cancer. *Biochem Biophys Res Commun* 2025; 775: 152182.
- [4] Luo Y, Liu W, Zhu Y, Tian Y, Wu K, Ji L, Ding L, Zhang W, Gao T, Liu X and Zhao J. KIF11 as a potential cancer prognostic marker promotes tumorigenesis in children with Wilms tumor. *Pediatr Hematol Oncol* 2022; 39: 145-157.
- [5] Dale KL, Armond JW, Hynds RE and Vladimirov E. Modest increase of KIF11 expression exposes fragilities in the mitotic spindle, causing chromosomal instability. *J Cell Sci* 2022; 135: jcs260031.
- [6] Wordeman L and Mitchison TJ. Identification and partial characterization of mitotic centromere-associated kinesin, a kinesin-related protein that associates with centromeres during mitosis. *J Cell Biol* 1995; 128: 95-104.
- [7] Riedl J, Vobetamerbäumer U, Stoffelns B and Elflein H. Total retinal detachment caused by a KIF11 mutation. *Eur J Ophthalmol* 2017; 27: e147-e148.
- [8] Hazan F, Ostergaard P, Ozturk T, Kantekin E, Atlihan F, Jeffery S and Ozkinay F. A novel KIF11 mutation in a Turkish patient with microcephaly, lymphedema, and chorioretinal dysplasia from a consanguineous family. *Am J Med Genet A* 2012; 158A: 1686-1689.
- [9] Bullock JM, Medway C, Cortina-Borja M, Turton JC, Prince JA, Ibrahim-Verbaas CA, Schuur M, Breteler MM, van Duijn CM, Kehoe PG, Barber R, Coto E, Alvarez V, Deloukas P, Hammond N, Combarros O, Mateo I, Warden DR, Lehmann MG, Belbin O, Brown K, Wilcock GK, Heun R, Kölsch H, Smith AD, Lehmann DJ and Morgan K. Discovery by the epistasis project of an epistatic interaction between the GSTM3 gene and the HHEX/IDE/KIF11 locus in the risk of Alzheimer's disease. *Neurobiol Aging* 2013; 34: 1309, e1301-1307.
- [10] Ling J, Wang Y, Ma L, Zheng Y, Tang H, Meng L and Zhang L. KIF11, a plus end-directed kinesin, as a key gene in benzo(a)pyrene-induced non-small cell lung cancer. *Environ Toxicol Pharmacol* 2022; 89: 103775.
- [11] Wu B, Hu C and Kong L. ASPM combined with KIF11 promotes the malignant progression of hepatocellular carcinoma via the Wnt/beta-catenin signaling pathway. *Exp Ther Med* 2021; 22: 1154.
- [12] Zhou Y, Chen X, Li B, Li Y and Zhang B. KIF11 is a potential prognostic biomarker and therapeutic target for adrenocortical carcinoma. *Transl Androl Urol* 2023; 12: 594-611.
- [13] Kang YH, Chen RS, Hu Q, Sun HT, Guo P, Lv SL, Luo LY and Huang DQ. Clinical significance of the S204 phosphorylation of the Smad3 protein in combination with Ki67 for the metastasis and prognosis of gastric cancer. *BMC Gastroenterol* 2025; 26: 37.
- [14] Fang Q, Li Q, Qi Y, Pan Z, Feng T and Xin W. ASPM promotes migration and invasion of anaplastic thyroid carcinoma by stabilizing KIF11. *Cell Biol Int* 2023; 47: 1209-1221.
- [15] Han Y, Chen J, Wei D and Wang B. KIF11 is a promising therapeutic target for thyroid cancer treatment. *Comput Math Methods Med* 2022; 2022: 6426800.
- [16] Tallman MM, Zalenski AA, Stabl I, Schrock MS, Kollin L, de Jong E, De K, Grubb TM, Summers MK and Venere M. Improving localized radiotherapy for glioblastoma via small molecule inhibition of KIF11. *Cancers (Basel)* 2023; 15: 3173.
- [17] Wang H, Li S, Liu B, Wei S, Wang T, Li T, Lin J and Ni X. KIF11: a potential prognostic biomarker for predicting bone metastasis-free survival of prostate cancer. *Oncol Lett* 2022; 24: 312.
- [18] Dundar MS, Belanova I, Bonetti G, Gelanova V, Kozacikova R, Veselenyiova D, Macchia A, Micheletti C, Medori C, Donato K, Ricci M, Cestari M, Amato B, Micheletti C, Boccardo F, Dundar M, Bertelli M and Michelini S. Genetic variants in genes correlated to the PI3K/AKT pathway: the role of ARAP3, CDH5, KIF11 and RELN in primary lymphedema. *Lymphology* 2023; 56: 152-159.
- [19] Wei D, Rui B, Qingquan F, Chen C, Ping HY, Xiaoling S, Hao W and Jun G. KIF11 promotes cell proliferation via ERBB2/PI3K/AKT signaling pathway in gallbladder cancer. *Int J Biol Sci* 2021; 17: 514-526.
- [20] Pei YY, Li GC, Ran J, Wan XH, Wei FX and Wang L. Kinesin family member 11 enhances the self-renewal ability of breast cancer cells by participating in the Wnt/beta-Catenin pathway. *J Breast Cancer* 2019; 22: 522-532.
- [21] Ambros V. The functions of animal microRNAs. *Nature* 2004; 431: 350-355.
- [22] Xue Y, Xu W, Zhao W, Wang W, Zhang D and Wu P. miR-381 inhibited breast cancer cells proliferation, epithelial-to-mesenchymal transition and metastasis by targeting CXCR4. *Biomed Pharmacother* 2017; 86: 426-433.

- [23] Yu YZ, Mu Q, Ren Q, Xie LJ, Wang QT and Wang CP. miR-381-3p suppresses breast cancer progression by inhibition of epithelial-mesenchymal transition. *World J Surg Oncol* 2021; 19: 230.
- [24] Mi H, Wang X, Wang F, Li L, Zhu M, Wang N, Xiong Y and Gu Y. miR-381 induces sensitivity of breast cancer cells to doxorubicin by inactivation of MAPK signaling via FYN. *Eur J Pharmacol* 2018; 839: 66-75.
- [25] Pei YY, Li GC, Ran J and Wei FX. Kinesin family member 11 contributes to the progression and prognosis of human breast cancer. *Oncol Lett* 2017; 14: 6618-6626.

KIF11 and miR-381-3p in breast cancer migration

Supplementary Table 1. shRNA target sequences used for KIF11 knockdown

shRNA name	Target sequence (5'→3')	Species
KIF11-RNAi1	UAUGGUGUUUGGAGCAUCUACUAAA	Human
KIF11-RNAi2	CAGUACACAACAAGGAUGAAGUCUA	Human
KIF11-RNAi-Vec (scramble)	UAAAGCUACUAGGUGUUUGGAGCA	Human

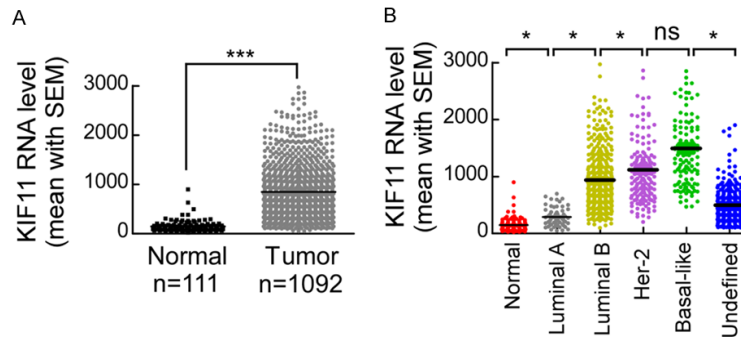
Supplementary Table 2. Sequences of miR-381-3p mimic and negative control

Name	Target sequence (5'→3')	Type
hsa-miR-381-3p mimic	UAUACAAGGGCAAGCUCUCUGU	Mature miRNA sequence
Negative control (NC)	CAGUACUUUUGUGUAGUACAA	Scramble control

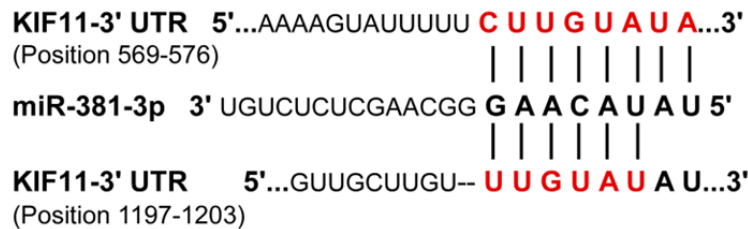
Supplementary Table 3. Primer sequences for quantitative PCR (qPCR)

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Amplicon (bp)
KIF11	TCTAGTCGTCCTCACTCAGTTT	GAGGTGTTCTTTCTACAAGGGCAGT	~200
Vimentin (VIM)	TCCAGCAGCTTCTCTGTAGGT	CCCTCACCTGTGAAGTGGAT	~150
E-cadherin (CDH1)	GCTGAGCTGGACAGGGAGGA	ATGGGGGCGTTGTCATTAC	~120-180
GAPDH	CACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA	~250
U6	CTCGCTTCGGCAGCATATACT	ACGCTTCACGAATTTGCGTGTC	~90

Note: For miR-381-3p detection, stem-loop RT-qPCR was performed as described previously.



Supplementary Figure 1. KIF11 mRNA is upregulated in breast cancer tissues and across all molecular subtypes. A. KIF11 mRNA expression levels in 1,092 breast cancer tissues (Tumor) compared to 111 adjacent normal breast tissue samples (Normal) from the TCGA-BRCA dataset. B. KIF11 mRNA expression levels were upregulated in every molecular subtype of breast cancer (Luminal A, Luminal B, HER2-enriched, Basal-like, and Normal-like). Data are presented as mean $\pm$ SD. *P < 0.05. This figure is adapted from our previous publication (J Breast Cancer. 2019 Dec;22(4):522-532).



Supplementary Figure 2. Putative miR-381-3p binding sites in the KIF11 3'-untranslated region (UTR). Schematic representation of the human KIF11 3'-UTR showing two conserved binding sites for miR-381-3p. The binding sites were predicted using TargetScan (targetscan.org).