Structural Maintenance of Chromosomes 1A (SMC1A) regulated by KIAA1429 in m6A-independent manner promotes EMT progress in breast cancer

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Research

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Abstract

Background

As a component in the m6A ‘writers’, KIAA1429 was reported to promote breast cancer proliferation and growth in m6A-independent manners. However, the related mechanism of KIAA1429 in breast cancer metastasis have not been reported.

Methods

Western blots and quantitative real-time PCR were carried out to verify the expression of KIAA1429 in breast cancer cells SUM1315 and ZR-75-1 after KIAA1429 knockdown or overexpression. Transwell and in vivo metastasis assay were conducted to investigate the effects of KIAA1429 on migration and invasion of breast cancer cells. RIP and REMSA assay was performed to explore the direct correlation between KIAA1429 and SMC1A mRNA. ChiP assay combined with luciferase reporter assay were apply to explore the direct binding between SMC1A and SNAIL promoter region.

Results

KIAA1429 could significantly promote the migration and invasion of breast cancer cells. Knockdown of KIAA1429 could impede breast cancer metastasis in nude mice in vivo. The level of SNAIL expression and EMT progress was positively related with KIAA1429. Knockdown of KIAA1429 induced cell migration, invasion and EMT progress could be reversed by the upregulation of SNAIL. However, SMC1A, not KIAA1429 bound with SNAIL promoter region directly and promoted the transcription of SNAIL. Then, KIAA1429 could bind to the motif in the 3′-UTR of SMC1A mRNA directly and enhanced SMC1A mRNA stability.

Conclusions

In conclusion, our study revealed a novel mechanism of the KIAA1429/SMC1A/SNAIL axis in the regulation of invasion and metastasis of breast cancer, which may provide a potential biomarker and therapeutic target for breast cancer. Moreover, it firstly provided compelling evidences that KIAA1429 could regulate the targeted gene expression at posttranscriptional levels as an RNA-binding protein, unrelated the m6A modification.

Background

Breast cancer, originated from breast tissue, is the most commonly diagnosed malignant tumor and represents the leading cause of cancer-associated deaths among women worldwide [1, 2]. Although dramatic advancement has been made in the early diagnosis, and complex treatment such as surgical operation, radiotherapy, endocrine therapy, and immunotherapy in the past decades, the prognosis of breast cancer patients is still poor due to the high rate of lethal distant metastasis [3]. Metastasis is a multi-step process by which cancer cells spread from the primary tumor to colonize distant sites. EMT is defined as a biologic procedure wherein cells lose their epithelial features and acquire mesenchymal characteristics, which enables them to migrate further and invade the underlying mesenchyme [4]. Although recent studies have raised concerns about the truly contribution of EMT to metastasis in pancreatic cancer [5] and lung cancer [6], the experimental results acquired in breast cancer still support the idea that EMT plays a vital role in breast cancer metastasis [7–10].

Up to now, more than 100 kinds of RNA modifications have been confirmed [11]. Among them, N6-methyladenosine (m6A) has been considered as the most pervasive, abundant and dynamic eukaryotic RNA modifications, which affects RNA transcription, processing, translation and metabolism [12]. m6A RNA modification is regulated by methyltransferases (‘writers’), demethylases (‘erasers’) and RNA binding proteins (‘readers’) and the m6A ‘writers’ complex includes the methyltransferase like 3 (METTL3), METTL14, WT1 associated protein (WTAP), RNA binding motif protein 15/15B (Rbm15/15B), KIAA1429 (or VIRMA) [13]. It was found that the components in m6A ‘writers’ complex were involved in many cancers metastasis and invasion via regulating the RNAs fate of many oncogenes or tumor suppressor genes [14–16]. In the m6A methyltransferase complex, KIAA1429 serves as a scaffold in bridging the catalytic core components METTL3 and METTL14 and acts as a positive regulator of oncogenesis. For instance, KIAA1429 facilitated the migration and invasion of hepatocellular carcinoma (HCC) through m6A modification of ID2 mRNA [17]. It act as an oncogenic factor in gastric cancer by stabilizing c-Jun mRNA in an m6A-independent manner [18]. Furthermore, circ_KIAA1429, which came from KIAA1429, could accelerate HCC advancement, maintained the expression of Zeb1 through the mechanism of m6A–YTHDF3-Zeb1 in HCC [19]. KIAA1429 also act as independent prognostic factor to classify lung cancers for patient stratification [20]. In our previous studies, KIAA1429 could promote breast cancer cells proliferation in an m6A-independent manner as RNA binding protein [21]. We also found that KIAA1429 could increase the metastasizing ability of breast cancers, but the detailed mechanism was still unknown.

In this study, we explored the deeper mechanisms that KIAA1429 participated in the invasion and metastasis of breast cancer. We firstly demonstrated that KIAA1429 was associated with breast cancer invasion and metastasis both in vivo and in vitro. Then we investigated the role of KIAA1429 in regulating cellular morphology and EMT markers and found that KIAA1429 could affect snail family transcriptional repressor 1 (SNAIL) expression obviously in the EMT regulator. However, we proved that KIAA1429 could not regulate SNAIL directly. So we found the structural maintenance of chromosomes 1A (SMC1A) was the potential targeting genes of KIAA1429 in breast cancer identified by RNA immunoprecipitation (RIP). Moreover, SMC1A could regulate SNAIL as a transcription factor, which promoted the process of EMT. We then confirmed that KIAA1429 could regulate SMC1A by an m6A-independent manner. Here we discovered a specific mechanism for KIAA1429 in the invasion and metastasis of breast cancer.

Methods

Cell cultures
The human breast cancer cell lines ZR-75-1 were obtained from the American Type Culture Collection (ATCC, USA), and the SUM-1315 cell lines was kindly provided by Dr. Stephen Ethier University of Michigan. The cells were cultured in complete high glucose DMEM (Wisent, China), supplemented with 10% fetal bovine serum, 100 μg/ml penicillin-streptomycin (Hyclone, USA). All cells were incubated in a humidified atmosphere containing 5% CO₂ at 37 °C.

**Lentivirus transfection and Adenovirus transfection**

The breast cancer cells were transfected with lentivirus (Obio, Shanghai, China) to repress KIAA1429 and METTL3 expression (termed as shKIAA1429-1, shKIAA1429-2 and shMETTL3). And pLKDCMV-G&PR-U6 negative control vectors was termed as shRNA-NC. Then, we transfected the breast cancer cells with adenovirus (Obio, Shanghai, China) to overexpress KIAA1429 protein (termed as KIAA1429), and pLenti-CMV-3FLAG negative control vectors was termed as Vector. Cells were plated in 6 wells dishes overnight at 30%~40% confluence and infected with the retroviruses. Meanwhile, we add the polybrene (5 μg/ml) to enhance the target cells infection efficiency. Then puromycin (3 μg/ml) was used to select the stable cells for two weeks.

To explore the further relationship between KIAA1429, SNAIL and SMC1A in breast cancer cells, these cells were seeded in 6-well plates overnight and then transfected with SMC1A-siRNA (GenePharma, Shanghai, China) and the nonspecific siRNA control, using Lipofectamine® 3000 transfection agent (Invitrogen, USA). The sequences of the siRNAs are as follows:

- si-SMC1A-1 sequences: 5′-GCAGCAGAAAGGCAGAGAUATT-3′
- si-SMC1A-2 sequences: 5′- GCUAUGAGCCACCUCAUAUTT-3′

In addition, the KIAA1429 negative control and knockdown breast cancer cells were transfected stably with SNAIL negative control vectors and SNAIL overexpression vectors pLenti-CMV-SNAIL-2A-mCherry3FLAG-PGK-Zeo (termed as shRNA-NC + Vector, shKIAA1429 + Vector, shRNA-NC + SNAIL and shKIAA1429 + SNAIL). All plasmids were verified by sequencing (GenePharma, Shanghai, China).

**RNA extraction, reverse transcription and qRT-PCR**

Total RNA was extracted using Trizol reagent (Takara, Japan), and about 1000 ng RNA was reverse-transcribed into cDNA using Primescript RT Reagent (Takara, Japan). The qRT-PCR were carried out as described [22]. The following PCR primers were used:

- β-actin forward, 5′-TCACCCACACTGTGCCCCATCTGCA-3′
- β-actin reverse, 5′-CAGCGAGCCCGCTATTGGCCTGG-3′
- KIAA1429 forward, 5′- CAACGATGGCACGAATTAC-3′
- KIAA1429 reverse, 5′- TGTTGCTTCATATTCCGACA-3′
- SMC1A forward, 5′- TGATGCTGCTTGGATAACA-3′
- SMC1A reverse, 5′-GGGTACTTGGTGAGGTCGAA-3′
- SNAIL forward, 5′- GCTCCACAAGACCAAGAGTG-3′
- SNAIL reverse, 5′- CAGGCAGAGGCACGACGACC-3′
- E-cadherin forward, 5′- GAACGCATTGCCACATACAC-3′
- E-cadherin reverse, 5′- CAGTAGATCTCCGCCCCCC-3′
- N-cadherin forward, 5′- ATGGAAGGCAATCCCACATA-3′
- N-cadherin reverse, 5′- CAGTAGATCTCCGCCCCCC-3′

**Western blot analysis**

Western blot analysis was conducted as previously described [23]. Briefly, cells were ruptured with RIPA (P0013C, Beyotime, China) buffer containing 1% PMSF, 1% phosphatase inhibitor, and 0.1% protease inhibitor. Cell lysates were resolved by SDS-PAGE and transferred to a PVDF membrane (Millipore, USA). After incubated with the primary antibodies, the membranes were then incubated with secondary antibodies (CST, USA). The primary antibodies included anti-mouse β-actin (3700S, CST, USA), anti-rabbit KIAA1429 (8835B, CST, USA), SMC1A (ab243875, Abcam, UK), SNAIL(3879S, CST, USA), E-cadherin (3195S, CST, USA), N-cadherin (13116S, CST, USA). The dilutions of antibodies were according to the product usage information.

**Wound healing assay**

Breast cancer cells were seeded in a 6-well plate and grown to full confluence. The monolayer was scratched with a sterile plastic tip, washed twice with PBS to remove loose cells and replace the media with serum free media. The wounded areas were photographed under a microscope. Image J was used to measure the area between the invading fronts of cells in three random microscopic fields (×100) for each condition and time point (0, 24 h).

**Transwell migration and invasion assay**
The migration and invasion assay of breast cancer cells were conducted as described previously [24]. Briefly, 5 × 10^4 cells were seeded into the upper chambers of each transwell, which was coated with or without Matrigel (BD Biosciences, USA) for the invasion and migration assays, and 500 μl of medium with 10% FBS was added to the lower chamber. After incubation at 37 °C for 48 h, non-invading cells were wiped from the upper side of the membrane. Then cells adhered to the lower membrane were fixed in methanol and stained with 0.1% crystal violet. Images of three random fields (×100) were captured from each membrane, and the number of invading or migrating cells was counted.

Experimental metastasis assay

The balb/c female nude mice (4-week-old) were obtained from Animal Research Center of Nanjing Medical University (Nanjing, China) and the use of animal was approved by Institutional Animal Care and Use Committee of Nanjing Medical University. For the mouse lung metastasis model, SUM-1315 cells (2×10^6 /0.2 ml) expressing NC, shKIAA1429, or shKIAA1429+SNAIL were injected into the nude mice through the tail vein. After 8 weeks, mice were sacrificed, and the lungs were excised, imaged, and examined for lung metastases using hematoxylin-eosin staining (H&E staining).

RNA stability assays

KIAA1429-knockdown cell lines, KIAA1429-overexpressing cell lines and their control cell lines were cultured in 6-well plates. Then added actinomycin D (ActD) 5 μg/ml at 0 h, 1h, 2 h, 4 h, 6 h and 8 h before cell scraping collection. Total RNA was isolated using Trizol reagent and qRT-PCR was performed to quantify the relative levels of SMC1A.

 Luciferase reporter assay

Transcription factor prediction was obtained from Cistrome (https://www.cistrome.org/). Luciferase reporter assay was performed as previously described [25]. Briefly, the breast cancer cells were seeded into 24-well plate and transfected with Renilla luciferase vector and pGL3 reporter. After two days, the cells were harvested and the luciferase activity was tested by the Dual-Luciferase reporter assay system (Promega, USA).

RNA immunoprecipitation (RIP)

RIP assay was conducted as previously described [26]. Cell lysates were prepared with RNA immunoprecipitation lysis buffer (Magna RIP Kit, Millipore, USA) and then incubated with 5 μg of anti-KIAA1429 or rabbit IgG at 4°C overnight. The RNA-protein immunocomplexes were collected by protein A/G magnetic beads. After eluted and purification, the purified RNA was analyzed by RT-PCR and qRT-PCR.

The m6A RIP was performed as previously described with some modifications [27]. Total RNA were isolated from SUM-1315 stable KIAA1429/METTL3 knockdown and control cells with DNase I (Sigma Aldrich, USA). RNAs were fragmented by RNA fragmentation reagents. Immunoprecipitations were performed using an anti-m6A antibody (1:1000, Abcam, USA) previously bound to magnetic Dynabeads (Life Technologies, USA) in the RIP Immunoprecipitation Buffer (Magna RIP Kit, Millipore, MA) and incubated with DNA-free fragmented RNAs. RNAs was extracted by miRNeasy® Mini kit (QIAGEN, Germany) and subjected to qRT-PCR and normalizing to input.

RNA electrophoretic mobility shift assay (REMSA)

To generate RNA electrophoretic mobility shift assay probes, various regions (A and B) in SMC1A mRNA were amplified by PCR with T7 promoter sequence (5′-TAATACGACTCACTATAGGG-3′). Biotin-labelled RNA probes were made from in vitro transcription with a MEGA shortscript Kit (Ambion, Waltham, MA, USA) in the presence of biotin-16-UTP (Roche) according to the manufacturer's instructions. The primers for probes A and B are listed following:

Probe A, 5′-GGGGCTAACAATATTACCTACCTCATAGGATTTAATGATGTCAAGCTCCTCACTGGAGGCCTTATCCCTTCGTGGAGCCCACTAGGTGCCGACCCCTCAGAATATAACCCTCATGCCTGGACCCCTGAGAGCTTCTGATCCCAGCTATTAGGGACAGAAGAA3′

Probe B, 5′-AGGACTTCACCTTACAGGGGTGCGATGTATCAAATGGCAAATGTATGAAACAACCAGATCTTTCAGGGAGGCAGAATGTGAGCTATTCAGAAGAAGTGAACGTTAATTAG3′

The REMSA was performed with a LightShift Chemiluminescent RNA EMSA Kit (20158, Thermo, USA) following the manufacturer's instruction. Briefly, crude extracted protein from SUM-1315 cell lines and bio-labelled, unlabelled or mutant DNA probe was mixed in REMSA binding buffer and incubated for 25 min at room temperature. The RNA/protein complexes were then electrophoreticed by 4% native polyacrylamide gel and transferred to nylon membrane (77015, Thermo, USA). The RNA was UV crosslinked to the membrane, and the membrane was blocked in blocking buffer and then replaced for the blocking buffer with conjugate/blocking buffer. After washing with 1× wash buffer for 3 times, the membrane was incubated in substrate equilibration buffer. Then, the membrane was incubated in working solution and exposed.

Statistical analysis

All experiments were performed at least three times, unless otherwise specified. The data were analyzed using the Graphpad Prism 7.0 Software (GraphPad, La Jolla, CA, USA). Students t-test was used to research the statistical significance of the differences between groups, and P<0.05 was considered statistically significant.
Results

KIAA1429 could promote breast cancer migration and invasion both in vitro and in vivo

To investigate the effect of KIAA1429 on the metastasis of breast cancer, SUM-1315 and ZR-75-1 cells were transfected with lentivirus or adenovirus to repress or overexpress the expression of KIAA1429. KIAA1429 knockdown lentivirus constructs were generated and obtained as previously described [21]. The mRNA and protein levels of KIAA1429 were verified by qRT-PCR (Fig. 1a and 1b) and western blot (Fig. 1c and 1d). As shown in Fig. S1a and S1b, the migrated area of KIAA1429 knockdown increased, compared to the control cells (*P<0.05) in SUM-1315 and ZR-75-1 cells. On the contrary, the migrated area of KIAA1429 overexpression decreased, compared to the control in SUM-1315 and ZR-75-1 cells (Fig. S1c and S1d, *P<0.05). In addition, KIAA1429 knockdown showed significant decreasing ability of migration and invasion in SUM-1315 and ZR-75-1 cells (Fig. 1e and 1f, *P<0.05), while KIAA1429 overexpression showed significant increasing ability of migration and invasion in SUM-1315 and ZR-75-1 cells (Fig. 1g and 1h, *P<0.05).

To investigate the effects of KIAA1429 on breast cancer metastasis in vivo, the SUM-1315 cells were injected into tail veins of nude mice. The tumor presence was validated by histological examination (Fig. 1i-k). The results demonstrated that mice injected with sh-KIAA1429 cells produced less lung colonization, compared to those with the control cells (Fig. 1g and 1h). Moreover, we found that knockdown of KIAA1429 could prolong the survival time of mice, compared with the control group (Fig. 1i). These data strongly proved that KIAA1429 could promote breast cancer metastasis in vitro and in vivo.

KIAA1429 promoted EMT of breast cancer cells

The morphological change in breast cancer cells was widely used for assessing EMT and evaluated by microscopy. Significant morphological changes (from round-shaped to fibroblast-like cells) were observed in KIAA1429 overexpression cells, compared to the control cells after 48 h of transfection in the SUM-1315 and ZR-75-1 cell lines. (Fig. 2a and 2b). We also revealed upregulation of the mesenchymal phenotype marker, N-cadherin as well as down-regulation of the epithelial phenotype marker E-cadherin in KIAA1429 knockdown cells when compared to the control cells. Reversed expression of E-cadherin and N-cadherin was observed in KIAA1429 overexpression cells compared to the control cells (Fig. 2c-f). To explore the mechanism that KIAA1429 regulate EMT process in breast cancer cells, western blot and qRT-PCR was applied to show EMT regulator in KIAA1429 knockdown breast cancer cells and we found SNAIL was mostly associated with KIAA1429 knockdown (Fig. S4). SNAIL has a pivotal role in the regulation of EMT, which involves regulation of related biomarkers [28]. These results imply that KIAA1429 might affect EMT progress by regulating SNAIL expression.

SNAIL reversed the suppression of migration and metastasis induced by KIAA1429 knockdown in vitro and in vivo

SNAIL is known as a significant transcription factor which can promote the EMT progress in breast cancer cells. To explore the effect of KIAA1429 on SNAIL-inducing EMT progress, KIAA1429 knockdown and the control groups of SUM-1315 and ZR-75-1 cells were transfected to overexpress SNAIL. The transfection efficiency was confirmed by qRT-PCR and western blot (Fig. 3a-c). In transwell migration and invasion assay, SNAIL overexpression group exhibited a stronger ability of metastasis in SUM-1315 cells, while cell migration and invasion were significantly inhibited by the knockdown of KIAA1429. Similar results were also found in ZR-75-1 cells (Fig. 3d-g). Furthermore, NC, shKIAA1429 and shKIAA1429+SNAIL groups of SUM-1315 cell lines were injected into tail veins of nude mice. Figure 3h-i indicated that overexpression of SNAIL obviously increased lung metastases formed in numbers, while knockdown of KIAA1429 strongly decreased the formation of metastases. All the results indicated that KIAA1429 could promote SNAIL-inducing EMT progress both in vitro and in vivo.

SMC1A might be the potential target which was regulated by KIAA1429, and regulated the expression of SNAIL

Figure S2a also demonstrated a positive correlation between the expression of KIAA1429 and SNAIL in 54 breast cancer patients in our hospital (*P<0.05). According to the previous study, the m6A level of total RNAs reduced obviously in the sh-KIAA1429 and sh-METTL3 cells, comparing with the control groups in breast cancer cells [21]. To explore the mechanism whether KIAA1429 would regulate SNAIL expression directly by m6A-dependent manner, we immunoprecipitated RNAs of control, KIAA1429 knockdown and METTL3 knockdown SUM-1315 cells using anti-m6A antibody. The results indicated that KIAA1429 knockdown did not decrease the m6A level of SNAIL mRNA (Fig. S2b). Meanwhile METTL3 knockdown decreased the m6A level of SNAIL mRNA obviously (Fig. S2c). These results indicated that KIAA1429 could not regulate SNAIL by m6A-dependent manner. To investigate whether KIAA1429 regulated SNAIL in m6A-independent manner, KIAA1429 knockdown and control cells were treated with actinomycin-D (Act-D, 5 mg/ml) at different time points in both SUM-1315 and ZR-75-1 cells lines. However, the half-life of the SUM-1315 and ZR-75-1 cells showed no difference (Fig. S2d and S2e). Another RNA immunoprecipitation (RIP) assay followed by RT-PCR was used and found that SNAIL mRNA transcript was not present in KIAA1429 (Fig. S2f). Our former RIP-seq of KIAA1429 indeed found SNAIL or other EMT markers were not bound with KIAA1429 [21]. According to the above results, KIAA1429 might not regulate SNAIL mRNA directly. So we speculated that there were intermediate links between KIAA1429 and SNAIL.

To identify potential mRNAs which were associated with KIAA1429 protein, we selected 201 genes which were different expression between KIAA1429 and IgG group from former RIP-seq of KIAA1429 as described previously [21]. It is known from the above that knockdown or overexpression of KIAA1429 would affect the EMT progress. Thereinto, SNAIL was together increased or decreased with KIAA1429, which was the best characterized EMT effector. Then we predicted the potential target which was regulated by KIAA1429, and regulated the expression of SNAIL. Then we found about 125 target genes from the Cistrome which regulated the EMT progress. Thereinto, SNAIL was together increased or decreased with KIAA1429, which was the best characterized EMT effector. Then we predicted the potential target which was regulated by KIAA1429, and regulated the expression of SNAIL. Then we found about 125 target genes from the Cistrome which regulated the EMT progress. Then we predicted the potential target which was regulated by KIAA1429, and regulated the expression of SNAIL. Then we found about 125 target genes from the Cistrome which regulated the EMT progress. Then we predicted the potential target which was regulated by KIAA1429, and regulated the expression of SNAIL. Then we found about 125 target genes from the Cistrome which regulated the EMT progress. Then we predicted the potential target which was regulated by KIAA1429, and regulated the expression of SNAIL. Then we found about 125 target genes from the Cistrome which regulated the EMT progress.
analysis of the SNAIL gene revealed the presence of DNA recognition sequence of SMC1A [29] (sequence: 5′-AGGGGGGC-3′) with the potential sites to be bound specifically by SNAIL in its promoter region. ChiP assays showed that SMC1A bound to this recognition sequence of SNAIL gene in SUM-1315 and ZR-75-1 cells (Fig. 5d and 5e). β-actin, which represented a negative control, was not bound by SMC1A. Luciferase activity assays found that luciferase activity for a reporter carrying the SMC1A recognition sequence in SNAIL promoter region was prominently increased in the absence of SMC1A in SUM-1315 (Fig. 5g) and ZR-75-1(Fig. 5h) cells. These indicated that SMC1A could regulate SNAIL expression by binding to the recognition sequence in the promoter region of the SNAIL gene in breast cancer cells.

**KIAA1429 regulated SMC1A mRNA expression in an m6A-independent manner**

To explore the mechanism that KIAA1429 regulate SMC1A and whether KIAA1429 would influence the m6A level of SMC1A RNAs directly, MeRIP were conducted and the results indicated that KIAA1429 knockdown did not decrease the m6A level of SMC1A mRNA (Fig. 6a). On the country, METTL3 knockdown decreased the m6A level of SMC1A mRNA obviously (Fig. 6b). Moreover, the SMC1A mRNA interacted with KIAA1429 did not change, while METTL3 knockdown changed (Fig. 6c). These results showed that KIAA1429 could not disturb the m6A level of SMC1A mRNA, implying the m6A modification could not affect the interaction between KIAA1429 and SMC1A mRNA. All the results indicated that KIAA1429 regulated the SMC1A mRNA expression in an m6A-independent manner.

To investigate whether KIAA1429 regulate the expression of SMC1A in an m6A-independent manner, KIAA1429 knockdown and the control breast cancer cells were treated with 5 mg/ml ActD at different time points. Figure 6d indicated that down-regulation of KIAA1429 decreased the half-life of SMC1A mRNA from 3.4 to 2.2 h. Overexpression of KIAA1429 increased the half-life of SMC1A mRNA from 3.8 to 6.6 h in SUM-1315 cells. Similar results were confirmed in ZR-75-1 cells (Fig. 6e). These results suggested that KIAA1429 could increase SMC1A expression by regulating its mRNA stability. Furthermore, we investigated whether KIAA1429 bound to the SMC1A mRNA directly, RIP assay was performed in SUM-1315(Fig. 6f) and ZR-75-1(Fig. 6g) cell lines. The results showed that SMC1A mRNA was detected in KIAA1429 and Input group, whereas not in IgG group. It suggested that KIAA1429 could bind physically to SMC1A mRNA. To further explore if KIAA1429 could specifically bind to the motif in the 3′-UTR of SMC1A mRNA, luciferase reporter assay was carried out with pGL3 reporter containing A and B regions of the 3′-UTR. As the schematic diagram suggested (Fig. 7a), 3′-UTR-A and B contained the motif while 3′-UTR-A-mut and B-mut did not. The results indicated that the luciferase activity of a reporter carrying 3′-UTR-A and B was greatly repressed in KIAA1429 knockdown of SUM-1315 and ZR-75-1 cells than that of 3′-UTR-A-mut and B-mut (Fig. 7b and 7c).

REMSA was performed to demonstrate the direct binding sites of KIAA1429 in SMC1A mRNA. Using bio-Utp-labelleled probes (probes A and B), REMSA was performed containing various fragments of in the 3′-UTR of SMC1A mRNA (Figure 7d) to find out potential KIAA1429 binding site in SMC1A mRNA. The KIAA1429 protein was able to form RNA-protein complexes (RPCs) with probe A and B. (Fig. 7e). These results proved that KIAA1429 could directly bind to the motif in the 3′-UTR of SMC1A mRNA to stabilize SMC1A expression.

**Discussion**

Recent studies have demonstrated that KIAA1429 is associated with tumorigenesis and development in breast cancer, gastric cancer[18], HCC [30], and osteosarcoma [31]. However, reports about the effect of KIAA1429 on breast cancer metastasis are rare. In this study, we firstly provided compelling evidences that KIAA1429 could promote breast cancer metastasis and invasion both in vitro and in vivo. We found that KIAA1429 could enhance SMC1A mRNA stability by targeting the motif of SMC1A mRNA directly, and increased the SMC1A protein expression. Moreover, SMC1A promoted SNAIL expression by binding the promoter region of the SNAIL gene directly, and therefore promoted breast cancer cell migration and invasion.

As the largest known component in the m6A methyltransferase complex, KIAA1429 is identified as a scaffold that orchestrates the core components, which consist of METTL3, METTL14 and WTAP. In recent years, more and more evidences indicated that these components were involved in many cancer metastasis and invasion via regulating many oncogenes and tumor suppressor genes [27, 32, 33]. For instance, METTL3 has been identified as an oncogene which could promote HCC tumorigenicity and metastasis by repressing the expression of SOCS in an m6A-dependent manner [34]. METTL14 is an anti-metastatic factor and serves as a favorable factor in HCC by regulating m6A-dependent miRNA processing[35]. In the present study, we found overexpression of KIAA1429 could increased breast cancer cells migration ability, while knockdown of KIAA1429 repressed breast cancer cell migration and invasion in vitro and inhibited lung metastases in vivo. Moreover, we found breast cancer cells reveal an EMT-like morphological change after changing the expression of KIAA1429, which verified that KIAA1429 was involved in EMT progress. Accordingly, qrt-PCR proved that SNAIL regulated this process, instead of other EMT regulators. However, RIP and MeRIP assay proved that KIAA1429 could not regulate SNAIL directly. So we speculated that there were intermediate links between KIAA1429 and SNAIL. We then combined these 125 genes from the Cistromeur which would promote the transcription of SNAIL with our former RIP-seq of KIAA1429 and verified SMC1A as the primary potential target of KIAA1429 in breast cancer. SMC1A encodes a subunit of the cohesin-core complex that tethers sister chromatids together to ensure correct chromosome segregation in both mitosis and meiosis and takes part in gene transcription regulation and genome organization [36]. We did find SMC1A could bind directly to the DNA recognition sequence in the promoter region of SNAIL gene in breast cancer by ChiP experiment.

In the previous studies, KIAA1429 exhibited its activities by an m6A-dependent or m6A-independent manner [17, 21]. In this study, MeRIP experiment found that KIAA1429 could not disturb the m6A level of SMC1A mRNA, implying the m6A modification could not affect the interaction between KIAA1429 and SMC1A mRNA. It seemed that KIAA1429 exhibited its activities in breast cancer by an m6A-independent manner. RNA stability, RIP and luciferase reporter assay suggested that KIAA1429 could stabilize SMC1A mRNA dependent of the motif sites in 3′-UTR of SMC1A. REMSA is commonly employed in a primary characterization of protein–RNA interactions [37]. In this study, REMSA containing two fragments of SMC1A mRNA 3′-UTR (probes A and B) was performed and the results proved that KIAA1429 could bind to the motif in the 3′-UTR of SMC1A mRNA directly as an RNA-binding protein and regulate SMC1A
expression. It seemed that KIAA1429 could regulate the targeted gene expression at posttranscriptional levels, unrelated to its m6A modification. Overall, our study revealed that KIAA1429 could promote EMT progress by regulating SMC1A in m6A-independent manner in breast cancer.

Conclusions

In total, we demonstrated that the KIAA1429 promoted breast cancer migration and invasion in vitro and in vivo. We provided a novel mechanism of the KIAA1429/SMC1A/SNAIL axis in the regulation of breast cancer invasion and metastasis, which may be promising therapeutic target for human breast cancer. Moreover, it firstly provided detailed investigation how KIAA1429 regulated the targeted gene expression at posttranscriptional levels as an RNA-binding protein, unrelated to its m6A modification.

Abbreviations

KIAA1429: Vir Like M6A Methyltransferase Associated
SMC1A: Structural Maintenance of Chromosomes 1A
SNAIL: Snail Family Transcriptional Repressor 1
N-cadherin: Cadherin 2
E-cadherin: Cadherin 1
Act D: Actinomyclin D

Declarations

Acknowledgements

Not applicable

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Availability of data and materials

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

Authors’ contributions

QD and JW designed the study; XZ, XD, JQ carried out the experiments; FX and ZW performed the statistical analysis; TX, XZ, XL participated in the clinical specimens detection; XZ and JW wrote and revised the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The nude mice, purchased from Animal Core Facility of Nanjing Medical University (Nanjing, China), was approved by Institutional Animal Care and Use Committee for animal use. All samples were used according to the ethical guidelines of the 1975 Declaration of Helsinki.

Consent for publication

Not applicable

Conflict of interests

The authors declare no competing interests.

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