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Research article

LncRNA HIF1A-AS2 promotes triple-negative breast cancer progression and paclitaxel resistance via MRPS23 protein

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ABSTRACT

Dysregulation of lncRNAs is a critical factor in the migration and invasion of tumors. Here our study reveals that lncRNA HIF1A-AS2 is highly expressed in breast cancer tissues and various TNBC cell lines. Moreover, we present compelling evidence supporting the role of HIF1A-AS2 in promoting TNBC cell proliferation, metastasis, invasion, and resistance to paclitaxel treatment. Additionally, our transcriptome sequencing analysis identifies MRPS23 as a potential downstream target protein regulated by HIF1A-AS2 and knockdown of HIF1A-AS2 leads to decreased expression of MRPS23 in TNBC cells. Moreover, MRPS23 exhibits similar effects on enhancing cell proliferation, metastasis, invasion, and paclitaxel resistance in TNBC cells. Furthermore, down-regulating HIF1A-AS2 suppresses the enhanced functionality observed in TNBC cells due to upregulated MRPS23 expression. These findings suggest that modulation of MRPS23 protein expression by HIF1A-AS2 may influence cellular processes and paclitaxel sensitivity in TNBC cells.

1. Introduction

According to the "Global Cancer Statistics 2020", Female breast malignancy has become the leading cause of new cancer cases globally in 2020, accounting for 11.7 % of all cancer cases with approximately 2.3 million new cases [1]. Triple-negative breast cancer (TNBC) is a unique subtype of breast cancer that is deficient in the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [2]. TNBC is highly invasive and has poorer survival rates at each stage compared to other breast cancer subtypes [3]. Due to the unique molecular phenotype, TNBC is imperviable to endocrine therapy and molecular target therapy, and is limited primarily to cytotoxic chemotherapy, such as paclitaxel and docetaxel [4]. Although manifold immunotherapy and targeted treatments are under exploration, those treatments are effective for only a few patients [5–7]. Therefore, identifying genes crucial to TNBC progression is essential for advancing precision medicine.

With the swift progress in genomics and transcriptomics, abundant evidence shows that noncoding RNAs (ncRNAs) participate in

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List of abbreviations

HIF1A-AS2	HIF1A antisense RNA 2
MRPS23	mitochondrial ribosomal protein S23
TNBC	Triple-negative breast cancer
ER	estrogen receptor
PR	progesterone receptor
HER2	human epidermal growth factor receptor 2
LncRNAs	Long noncoding RNAs
EMT	epithelial-mesenchymal transformation
ARF4	ADP ribosylation factor 4
MEST	mesoderm specific transcript
RCN1	reticulocalbin 1
COPB2	COPI coat complex subunit beta 2

various cellar processes. Long noncoding RNAs (lncRNAs), a subtype of ncRNAs, are characterized as RNAs that are over 200 bases in length, usually lack coding potential, and have been revealed to engage in various tumor processes [8,9]. Recent research shows that lncRNAs affect multiple aspects of breast cancer, including ferroptosis, apoptosis, cell cycle, proliferation, migration, and invasion [8,10,11]. For example, Jiang et al. found that the lncRNA DDIT4-AS1 activates autophagy to facilitate the migration and proliferation of TNBC cells and high H3K27 acetylation caused DDIT4-AS1 increase in TNBC cells. Additionally, silencing DDIT4-AS1 could make TNBC cells more sensitive to paclitaxel [12]. Therefore, exploring the underlying mechanisms of lncRNAs in TNBC metastasis could provide a potential therapeutic strategy and serve as an efficient diagnostic target.

In this study, we found that HIF1A-AS2 was abnormally expressed in breast cancer tissues and TNBC cells. Furthermore, silencing of HIF1A-AS2 in TNBC cells significantly suppresses cell proliferation, migration, and invasion and also make TNBC cells more sensitive to paclitaxel. Additionally, we used RNA sequencing approach to identify MRPS23 as a potential target of HIF1A-AS2. We further demonstrated that MRPS23 regulates TNBC cell progression and sensitivity to paclitaxel. Rescue experiments confirmed that HIF1A-AS2 regulates TNBC cell progression and sensitivity to paclitaxel via MRPS23. In conclusion, this study suggests that lncRNA HIF1A-AS2 could be a promising therapeutic target for treating TNBC.

2. Materials and methods

2.1. Patient specimens

Ten samples of breast cancer tissues and corresponding adjacent normal tissues were collected from patients at the Third Hospital of Nanchang City (Nanchang, China). The breast cancer tissues were staged according to the TNM classification system (American Joint Committee on Cancer, 8th edition) [13]: two were classified as T1N0M0, three as T2N1M0, and five as T3N2M0. Histological grading was performed based on the Nottingham grading system. All specimens were promptly frozen in liquid nitrogen immediately after surgical excision. This study was approved by the Ethics Review Committee of the Third Hospital of Nanchang (Jiangxi, China) (approval no. K-ky2020116). All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from all participants.

2.2. Cell culture

The human TNBC cell lines MDA-MB-453 and MDA-MB-474 were a gift from First Hospital of Nanchang, while MDA-MB-231, BT-549, and the normal mammary epithelial cell line, MCF10A, were purchased from Procell (Wuhan, China). MDA-MB-453, MDA-MB-474, and MDA-MB-231 cells were maintained in DMEM (Gibco, USA), and BT-549 cells were maintained in RPMI 1640 (Gibco, USA) with 0.01 mg/ml insulin (Procell, China). All cell culture media were added with 10 % fetal bovine serum (Gibco, USA). MCF10A cells were cultured in a specific growth medium (Procell, China).

2.3. Reagents and antibodies

Primary antibodies against E-cadherin (CAS:60355, 1:5000), N-cadherin (CAS:22018, 1:5000), Vimentin (CAS:60330, 1:20000), MRPS23(CAS:18345, 1:3000), and GAPDH (CAS:60004, 1:20000) were purchased from Proteintech (Chicago, USA)

2.4. si-RNA and plasmid transfection

si-RNAs were purchased from GenePharma (Shanghai, China), and plasmids were purchased from MIAOLING BIOLOGY (Wuhan, China). Cells were seeded in six-well plates (1×10^5/well) during the exponential growth phase for siRNA or Plasmid transfection.

After 24 h of culture, cells were transfected with si-RNA or plasmid with lipofectamine 2000 (Invitrogen, USA) in accordance with the manufacturer's protocol. The list of siRNA sequences is now provided in Table 1 for better clarification.

2.5. Total RNA extraction and RT-qPCR

Total RNA was extracted using the Trizol reagent (Thermo Fisher, USA). The concentration and purity of the extracted RNA were determined using a NanoDrop spectrophotometer (Thermo Fisher, USA). The RNA samples showed A260/A280 ratios between 1.8 and 2.0, indicating high purity. The First Strand cDNA Synthesis kit (TaKaRa, Japan) was employed to compound complementary DNAs (cDNAs) from 1 μg of the extracted RNA. For quantitative polymerase chain reaction (qPCR), SYBR Mix (Thermo Fisher, USA) was utilized. To achieve relative quantification, the data was normalized to GAPDH using the 2–ΔΔCt method. The primer sequences utilized for gene expression analysis are now provided in Table 2.

2.6. MTT assay

For drug-sensitivity assays, TNBC cells (3×10^3/well) were seeded into a 96-well plate with four replicates per group. The cells were incubated overnight and then exposed to drugs at specified concentrations for 72 h. For cell viability assays, 3×10^3 cells were seeded into each well of a 96-well plate with four replicates per group, and then cells were bred for 24, 48, and 72 h. To each well, 20 μL of MTT solution (5 mg/mL) was added and incubated for 4 h. After removing the supernatant, 150 μL of DMSO was added to each well and agitated to dissolve the formazan crystals. Optical density (OD) at 490 nm was measured using a multifunctional microplate reader.

2.7. Colony formation assays

Cells were seeded in 6-well plates (1×10^3/well) during the exponential growth phase and incubated for 12–15 days. After incubation, the cells were fixed with 4 % paraformaldehyde for 20 min and stained with 0.1 % crystal violet for 10 min. Subsequently, PBS was used to wash away any excess stain, and then the colonies present were enumerated.

2.8. EDU cell proliferation assay

The EDU cell proliferation assay (Beyotime, China) was used to measure cell proliferation. Briefly, after transfecting with si-RNA or plasmid for 48h, cells were incubated with 10 μm EDU solution for 6 h in the atmosphere to label them. Then, cells were fixed using a 4 % paraformaldehyde for 20 min, followed by permeabilization with 0.5 % Triton X-100 (Beyotime, China) at room temperature. After removing the supernatant, 3 % BSA solution in PBS was used to wash the cells. Subsequently, cells were incubated in Click Additive Solution under light protection and stained with Hoechst 33342. Fluorescence images of EDU incorporation samples were captured and photographed using OLYMPUS-CKX53 (Olympus, Japan).

2.9. Wound healing assay

Cells were seeded to full clustering in 6-well plates after transfection with siRNAs or plasmids. A serum-free stripping medium was used to maintain cell growth. A vertical scratch was made on the bottom of each plate using a 20 μL pipette tip. Three images were captured per well after washing away cellular debris with PBS. The initial wound area size was measured and recorded as S0 using image analysis software. Subsequently, cells migrated and re-entered into the wound area. After 48 h, images were taken to calculate the final wound area size, which was recorded as S1. Cell migration was quantified as percentage wound occlusion using the formula: $=(S0–S1/S0) \times 100$ %.

2.10. Transwell migration and invasion assays

Cells transfected with siRNA or plasmids were seeded into the upper compartment of 8 μm transwell chambers (Corning, USA) with serum-free media (4×10^4/well). Subsequently, they migrated towards the bottom compartment containing complete media. Invasion assays were performed by coating the polycarbonate filters with a 300 μg/mL matrigel layer (Thermo Fisher, USA) before seeding the cells. After incubating for 48 h, the inserts were washed with PBS three times and fixed using a solution containing 4 % paraformaldehyde for 30 min. Following, the 0.1 % crystal violet solution was used to stain cells for 10 min and extensively rinsed with

Table 1
List of siRNA Sequences.

siRNA	Sequence (5'→3')
si-NC	UUCUCCGAACGUGUCACGUTT
si-HIF1A-AS2	AAGAGAUCUGUGGCUCAGUUCCUUU
si-MRPS23#1	CCCGGAAAUCCGAACACUUTT
si-MRPS23#2	GCGAAGCAAGGACUCAACATT

Table 2
List of primer sequences.

Primer	Sequence (5'→3')
Forward primer for GAPDH	GGAGCGAGATCCCTCCAAAAT
Reverse primer for GAPDH	GGCTGTTGTCATACTTCTCATGG
Forward primer for HIF1A-AS2	TCTGTGGCTCAGTTCCTTTTGT
Reverse primer for HIF1A-AS2	ATGTAGGAAGTGCCAGAGCC
Forward primer for MRPS23	TCTGTGGCTCAGTTCCTTTTGT
Reverse primer for MRPS23	ATGTAGGAAGTGCCAGAGCC

water to remove unstained cells from the lower compartment by gently wiping them away using a cotton swab. Migrated or invaded cells were photographed and quantified.

2.11. Western blot

The total cellular proteins were extracted using RIPA buffer containing protease inhibitor (Beyotime, China). All samples underwent separation on SDS-PAGE gel with 12 % acrylamide and then transferred to PVDF membranes. They were blocked with 5 % skim milk for 1.5 h. Primary antibodies were then incubated at 4 °C overnight, followed by washing three times with TBST solution for 10 min each. Secondary antibodies were then incubated for 2 h, and protein bands were visualized using the ECL reagent. The gray values

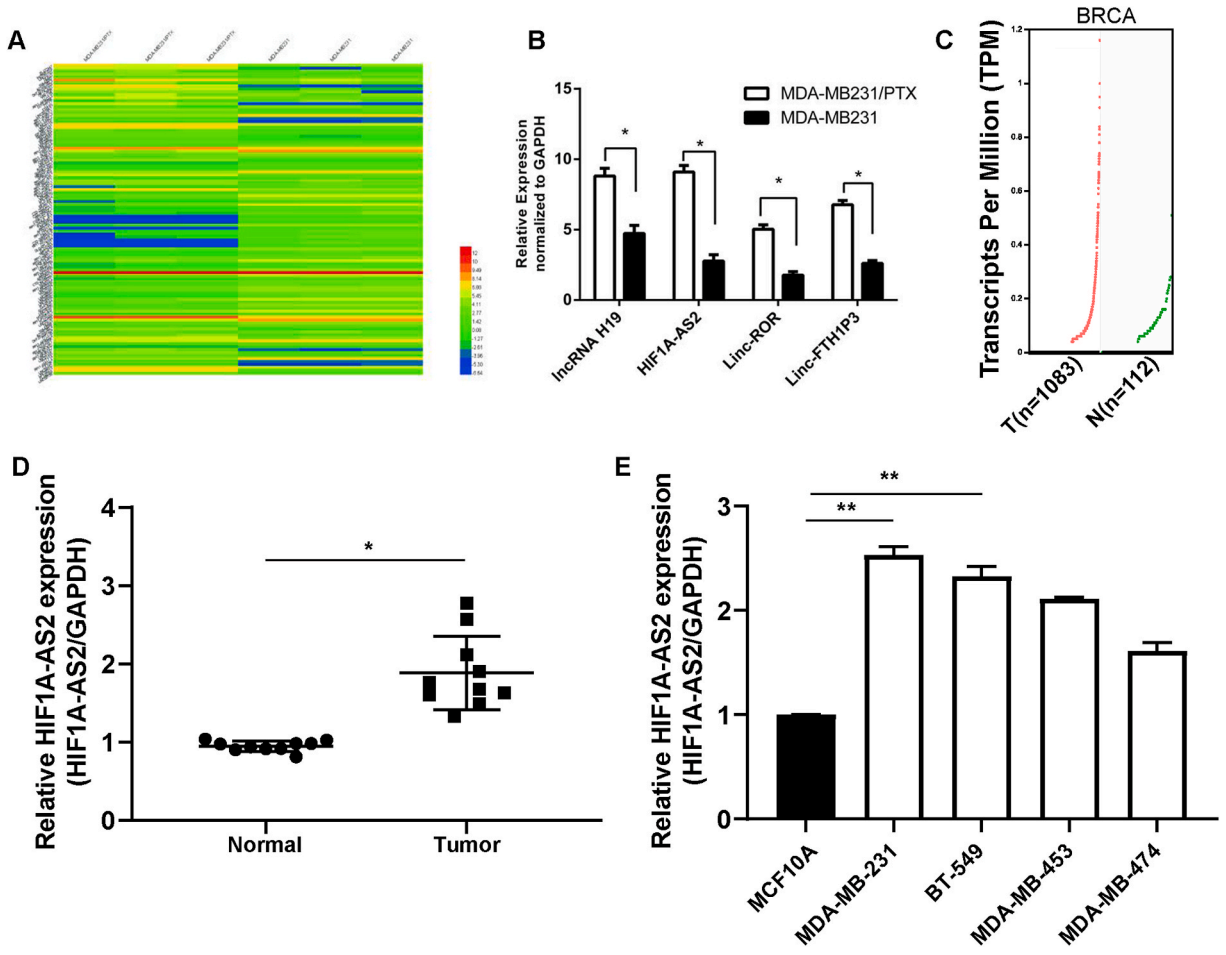

Fig. 1. Lnc RNA HIF1A-AS2 levels are higher in breast cancer and TNBC cell lines. (A) Heatmap of differentially expressed lncRNAs in MDA-MB-231/PTX and MDA-MB-231 cells. (B) LncRNA expression levels were measured using RT-qPCR in both MDA-MB-231/PTX and MDA-MB-231 cells. (C) The GEPIA2 database was applied to obtain HIF1A-AS2 expression levels in breast tumors and normal tissue. Red represents tumor tissues, and green represents normal tissues (http://gepia2.cancer-pku.cn). (D) Relative expression of HIF1A-AS2 was measured in both breast tumor tissues and normal tissues using RT-qPCR. (E) RT-qPCR analysis was performed to assess HIF1A-AS2 expression levels in several TNBC cells and MCF10A cells. *$p < 0.05$, **$p < 0.01$. $n = 3$ biological replicates.

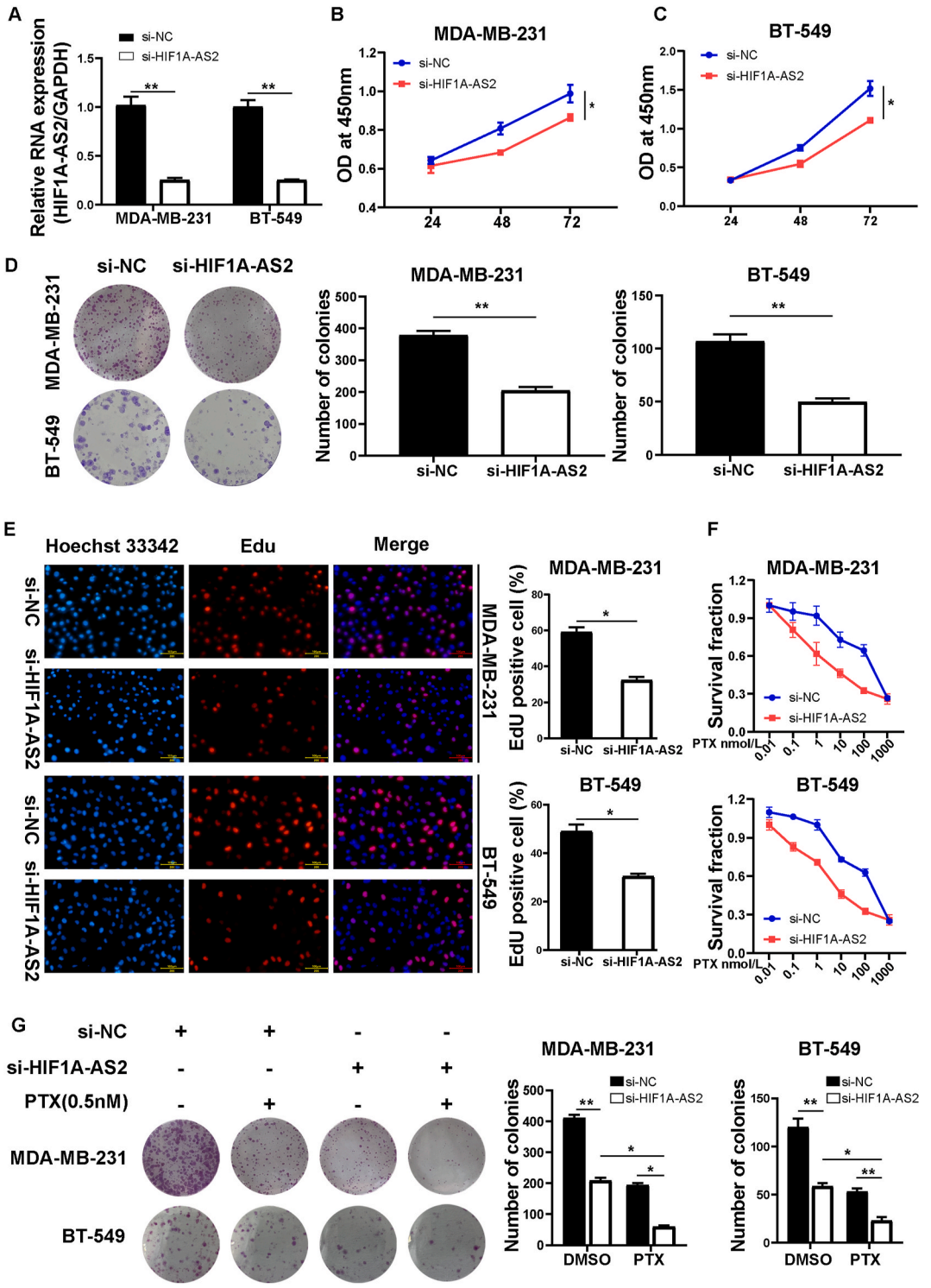

Fig. 2. Silencing of LncHIF1A-AS2 inhibited TNBC cells proliferation and enhanced the chemosensitivity of cells to paclitaxel. (A) TNBC cells were transfected with siRNAs targeting HIF1A-AS2(si-HIF1A-AS2) and control siRNA (si-NC) for 48 h. The effect of HIF1A-AS2 knockdown was detected using RT-qPCR. (B–D) MTT and colony formation assays evaluated the impact of HIF1A-AS2 knockdown on TNBC cells proliferation. (E) The proliferation rate of TNBC cells was estimated using EDU assays following the knockdown of HIF1A-AS2. Scale bar, 100um. (F) Cell viability of TNBC cells was detected using the MTT assays after HIF1A-AS2 knockdown and treatment with equipotential increased dosages of paclitaxel (PTX). (G). Number of cell clones formed after HIF1A-AS2 knockdown in TNBC cells which then were treated with manifested concentrations of paclitaxel. *p < 0.05, **p < 0.01. n = 3 biological replicates.

of the bands were analyzed by Image J software.

2.12. Cellular immunofluorescence staining

After transfection with si-RNA or plasmid in a 24-well plate for 48 h, cells were washed three times with PBS and fixed with 4 % paraformaldehyde to fix for 15 min. After rinsing thrice with PBS, immunofluorescence permeabilization was achieved by treating each well with a 0.5 % Triton X-100 solution (Beyotime, China) for 20 min. Next, 3 % BSA solution was used to block with 500 μL per well. The blocking step lasted for 30 min after rinsing the wells thrice with PBS. After removing the blocking solution, anti-E-cadherin antibodies (CAS:60335, Proteintech, 1:300) and anti-N-cadherin antibodies (CAS:22018, Proteintech, 1:300) were added into each well and incubated at 4 °C for overnight. The next day, samples were washed with PBS three times before being treated with corresponding fluorescent secondary antibodies (either CoraLite488 or CoraLite594, Proteintech) at 200 μL per well. Incubation was carried out at 37 °C for 1 h. After two times PBS washes, cells were counterstained with DAPI (Beyotime, China) for 10 min. Following two more rounds of PBS washing, cells were imaged using OLYMPUS-CKX53 (Olympus, Japan).

2.13. RNA-sequence

Total RNA was isolated from MDA-MB-231 cells transfected with either si-NC or si-HIF1A-AS2 using Trizol reagent. A ribosomal RNA depletion kit was used to remove rRNA. Then, mRNA was fragmented to synthesize cDNA; after the repair, add A and adaptor ligation in cDNA and the PCR reaction system and program were configured and set up to amplify the product. Libraries were sequenced by the BGISEQ platform at The Beijing Genomics Institute (Shenzhen, China).

2.14. Statistical analysis

GraphPad Prism software was performed to analyze statistics and generate charts. Results are presented as the mean ± standard error of the mean (mean ± SEM). Differences between two groups were assessed using a Student's t-test, while one-way ANOVA was used for multiple group comparisons. Statistical significance was defined as a P value < 0.05.

3. Results

3.1. LncHIF1A-AS2 levels are abnormally increased in breast cancer

In our previous study, we observed a significant upregulation of LncRNA HIF1A-AS2 in paclitaxel-resistant MDA-MB-231 cells compared to non-resistant MDA-MB-231 cells (Fig. 1A and B). This special lncRNA sparked our interest. To investigate the impact of HIF1A-AS2 on breast cancer progression, we initially assessed HIF1A-AS2 RNA levels in 10 fresh primary breast cancer tissues and their adjacent normal breast tissues. The breast cancer tissues were staged according to the TNM classification system: two were classified as T1N0M0, three as T2N1M0, and five as T3N2M0. Histological grading was performed based on the Nottingham grading system, resulting in three cases classified as grade 1 (G1), four as grade 2 (G2), and three as grade 3 (G3). HIF1A-AS2 RNA levels were extraordinarily upregulated in breast cancer tissues (Fig. 1D). Moreover, the GEPIA2 database indicated higher HIF1A-AS2 RNA levels in breast cancer tissues compared to normal breast tissues (Fig. 1C). Furthermore, we comprehensively examined HIF1A-AS2 expression across various breast cancer cell lines and MCF10A cells. The results demonstrated higher expression of HIF1A-AS2 in four TNBC cell lines in comparison with MCF10A, particularly in MDA-MB-231 and BT-549 cells (Fig. 1E). These findings indicate abnormal upregulation of HIF1A-AS2 in breast cancer and TNBC cell lines.

3.2. Silencing of LncHIF1A-AS2 inhibited TNBC cell proliferation and enhanced the chemosensitivity of TNBC cells to paclitaxel

Subsequently, we aim to investigate the impact of elevated HIF1A-AS2 expression on the progression of TNBC cells. siRNAs were used to knockdown HIF1A-AS2 expression in TNBC cells, resulting in a significant reduction in RNA levels of HIF1A-AS2 (Fig. 2A). The MTT assay results revealed that silencing HIF1A-AS2 significantly suppressed TNBC cell viability (Fig. 2B and C). Furthermore, the knockdown of HIF1A-AS2 decreased the colony-forming ability of TNBC cells (Fig. 2D). Additionally, suppression of HIF1A-AS2 resulted in a decrease in the number of EDU-positive cells (Fig. 2E). These findings indicate that suppression of HIF1A-AS2 expression inhibited TNBC cell proliferation.

With the deepening of research on lncRNAs, several studies have implicated their role in paclitaxel resistance in breast cancer [12, 14]. To explore the involvement of HIF1A-AS2 participates in paclitaxel resistance in TNBC cells, MTT assays and colony formation assays were designed. Cells depleted of HIF1A-AS2 exhibited increased susceptibility to paclitaxel compared to control cells (Fig. 2F). Similar trends were seen in colony formation assays (Fig. 2G). These findings suggest that HIF1A-AS2 may contribute to paclitaxel resistance in TNBC cells.

3.3. LncHIF1A-AS2 enhances the migration and invasion of TNBC cells

Recurrence and metastasis pose significant challenges in TNBC treatment. Compared to other forms of breast carcinoma, TNBC is characterized by its enhanced invasiveness and greater propensity distant metastasis. Patients with recurrent or metastatic TNBC

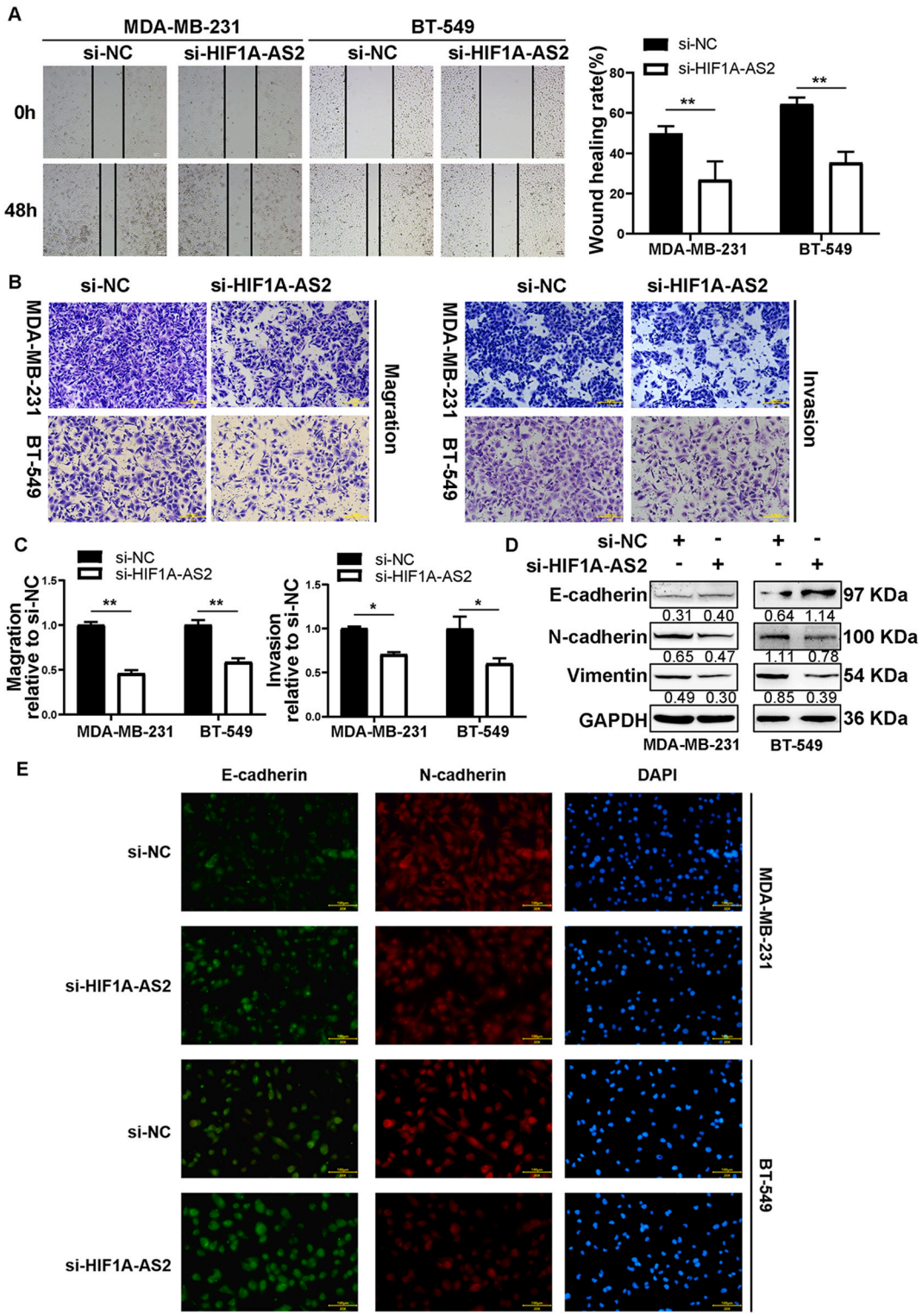

Fig. 3. LncHIF1A-AS2 enhances TNBC cells migration and invasion. (A) Cells migration ability was measured using the wound healing assay to assess the effect of HIF1A-AS2 knockdown. Scale bar, 100um. (B&C) Cells migration and invasion were evaluated by Transwell assay. Quantitative analysis revealed that HIF1A-AS2 silencing inhibited cell migratory and invasive ability. Scale bar, 200um. (D) EMT-related proteins expression in TNBC cells after HIF1A-AS2 knockdown was determined by Western blot. (E) Immunofluorescence images of E-cadherin (red) and N-cadherin (green) expression in TNBC cells after HIF1A-AS2 knockdown. DAP1 staining was used for nuclei visualization (blue). *$p < 0.05$, **$p < 0.01$. $n = 3$ biological replicates.

typically have an overall survival time for only 13–18 months [15]. To investigate the functional roles of HIF1A-AS2 in promoting the migration and invasion of TNBC cells, TNBC cells were transfected with HIF1A-AS2 siRNA, and their migration and invasion abilities were measured. As shown in Fig. 3A, the results of wound healing assays demonstrated that suppression of HIF1A-AS2 significantly affected cell mobility. The Transwell assay indicated a significant decrease in the number of migrating and invading cells in TNBC cells following downregulation of HIF1A-AS2 expression (Fig. 3B and C).

Metastasis in cancer cells is primarily driven by the process of epithelial-mesenchymal transformation (EMT), which enhances cell migration and invasion by acquiring mesenchymal characteristics from epithelial cells. Western blot and immunofluorescence assays were conducted to assess the expression of EMT-related proteins. Following HIF1A-AS2 knockdown, an increase in E-cadherin expression, an epithelial marker, was observed. Conversely, the expression of two mesenchymal cell markers, N-cadherin and Vimentin, was observed to decrease in TNBC cells (Fig. 3D). Similar results were also observed in immunofluorescence (Fig. 3E). Based on the data collected, it can be inferred that HIF1A-AS2 plays a role in enhancing cell migration and invasion abilities in TNBC cells.

3.4. MRPS23 was the target protein of LncHIF1A-AS2

Consequently, our objective was to identify the downstream effectors that regulate TNBC progression through HIF1A-AS2. The RNA transcriptome sequencing was performed after HIF1A-AS2 knockdown in MDA-MB-231 cells. Following HIF1A-AS2 knockdown, a total of 158 mRNAs exhibited differential expression compared to control, with 128 showing up-regulation and 30 displaying down-regulation (fold change<0.5 or >1.5, $P < 0.05$), as indicated by the Heatmap (Fig. 4A). According to recent research, we identified five significantly downregulated mRNAs (ARF4, MEST, MRPS23, RCN1, and COPB2) that play a pivotal role in tumors progression. To confirm the expression of these mRNAs after HIF1A-AS2 knockdown in MDA-MB-231 and BT-549 cells, RT-qPCR assays were performed, revealing significant downregulation of MRPS23 (Fig. 4B).

To examine the biological characteristics of MRPS23, we investigated its correlation with prognosis in breast cancer patients. GEPIA2 database results indicated a significant elevation of MRPS23 in breast cancer tissues, with high MRPS23 expression correlating with reduced overall survival (Fig. 4C and D). Additionally, MRPS23 expression levels were assessed in collected breast cancer tissues, revealing abnormally high expression (Fig. 4E). Then, we assessed MRPS23 RNA levels in various breast cancer cell lines and MCF10A cells, finding high expression in MDA-MB-231 and BT-549 cells (Fig. 4F). Overall, these findings demonstrated that MRPS23 may significantly impact on breast cancer.

3.5. MRPS23 promotes the TNBC cell progression and resistance to paclitaxel

To investigate the role of MRPS23 in TNBC progression, a series of biological function experiments was measured. Knockdown of MRPS23 resulted in a significant decrease in cell viability in MDA-MB-231 and BT-549 cells (Fig. 5A–D), which was consistent with results from EDU assays (Figs. S1A and C). Clonogenicity and EDU-positive cell numbers were markedly reduced after MRPS23 silencing. These findings indicate that MRPS23 facilitates cell proliferation in TNBC cells. Furthermore, we investigated the impact of MRPS23 on TNBC cell migration and invasion, finding that MRPS23 suppression significantly reduced cell motility, migration, and invasion (Fig. 5E–F and S1B&D). Conversely, after transfected with MRPS23 plasmid, which contains 3 × flag, the overexpression of MRPS23 had the opposite effect on TNBC cells (Fig. 6A–H). Overall, these observations indicate that MRPS23 acts as an oncogene in TNBC by promoting cell proliferation, migration and invasion.

Furthermore, we investigated the impact of MRPS23 on the sensitivity to paclitaxel of TNBC. Cells with reduced MRPS23 exhibited increased sensitivity to paclitaxel compared to control cells. Consistent results were obtained from MTT assay and colony formation assays (Figs. S1F–G). Conversely, overexpression of MRPS23 reduced the susceptibility of TNBC cells to paclitaxel treatment (Fig. 6I). Overall, our findings suggest that MRPS23 promotes TNBC cell progression and resistance to paclitaxel.

3.6. LncHIF1A-AS2 regulates the proliferation, metastasis, and drug resistance of TNBC through MRPS23

Based on the aforementioned experimental results, we hypothesize that HIF1A-AS2 potentially regulates the progression of TNBC by interacting with MRPS23. Experiments were designed to validate this hypothesis. As shown in Fig. 7A, we found that co-transfection of MRPS23 plasmid and HIF1A-AS2 siRNA in MDA-MB-231 cells markedly reduced the protein level of MRPS23 compared to transfection with MRPS23 plasmid alone. The expression of HIF1A-AS2 in each group was measured by RT-qPCR (Fig. S2). Moreover, MTT and colony assays indicated that the enhanced proliferative capacity induced by MRPS23 overexpression can be attenuated through siHIF1A-AS2 inhibition (Fig. 7B and C). Furthermore, additional investigations on migration and invasion have shown that the upregulation of MRPS23 significantly boosts migratory and invasive potentials, which can be effectively inhibited by reducing HIF1A-AS2 expression in TNBC cells (Fig. 7D and E). These findings suggest a potential role of lncHIF1A-AS2 in TNBC proliferation and metastasis.

The sensitivity experiments in MDA-MB-231 and BT-549 cells were performed to evaluate the effect of HIF1A-AS2 downregulation on paclitaxel resistance revealed its potential to effectively suppress MRPS23-mediated paclitaxel resistance (Fig. 7F). Together, our findings suggest that HIF1A-AS2 regulates TNBC cell progression and paclitaxel resistance through MRPS23.

4. Discussion

Recent cancer statistics indicate that breast cancer now has the highest incidence rate among women compared to other

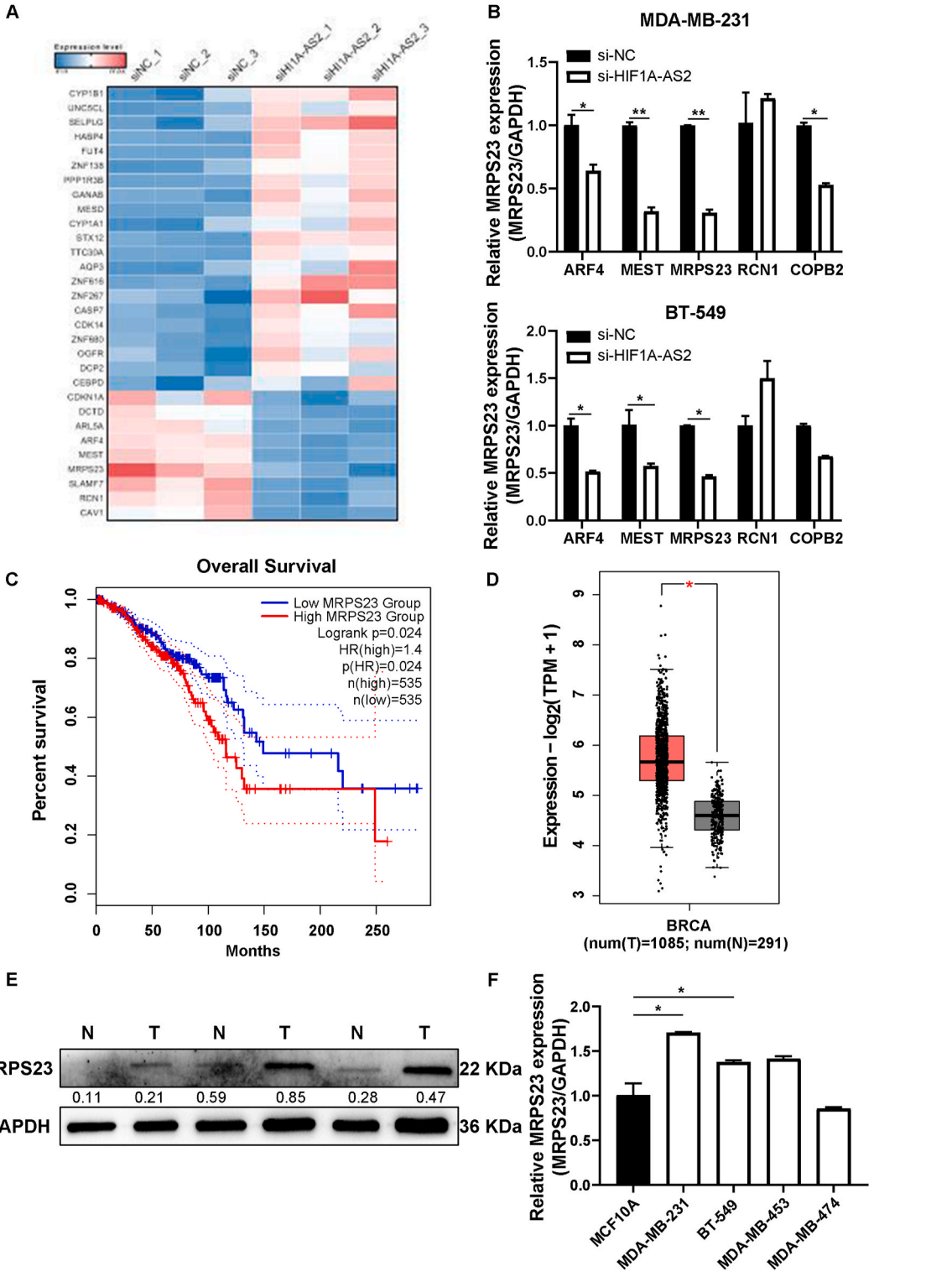

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Fig. 4. Identification of MRPS23 as a crucial downstream effector of HIF1A-AS2. (A) Heatmaps of the differentially expressed mRNAs between HIF1A-AS2 downregulated MDA-MB-231 cells and control cells. (B) RT-qPCR measured the expression levels of mRNAs in TNBC cells. (C) Kaplan-Meier survival curve comparing the overall survival of patients with breast cancer which with high (n = 535) MRPS23 expression or low (n = 535) MRPS23 expression in tumor tissues. (D) The GEPIA2 database was used to obtain relative MRPS23 expression in breast tumors and adjacent normal tissues. Red color represents cancer tissues, and gray color represents normal tissues(http://gepia2.cancer-pku.cn). (E) MRPS23 levels were measured in breast tumors and adjacent normal tissues by western blotting. (F) The levels of MRPS23 expression in multiple TNBC cell lines and MCF10A cells were assessed by RT-qPCR analysis. *$p < 0.05$, **$p < 0.01$. n = 3 biological replicates.

malignancies [1]. TNBC is particularly challenging to treat due to limited treatment options. Therefore, it is urgent to explore highly effective therapeutic approaches exclusively for TNBC. Increasing evidence suggests that lncRNAs significant influence on various cellular processes. Several researches have highlighted the regulatory potential of lncRNAs in TNBC, indicating promising prospects for targeting lncRNA as a therapeutic approach [16].

HIF1A-AS2 was initially discovered by Thrash-Bingham and Tartof in human renal cancer. It is categorized as an antisense non-coding RNA, known as aHIF, because it is a spontaneous antisense transcript that originates from the 3′ region of the HIF1A gene and can bind to the 3' non-coding region of HIF1A mRNA [17,18]. With the progress of research on HIF1A-AS2, it has been disclosed to exert regulatory functions in manifold malignancies, including lung cancer, kidney cancer, and colorectal cancer, and these findings encompass different aspects of tumor progression [19–21]. However, its involvement in breast cancer has been less studied. In this study, we discovered that levels of HIF1A-AS2 were significantly increased in breast tumor tissues and several TNBC cell lines. Subsequent analysis revealed that reducing HIF1A-AS2 significantly inhibited the proliferative capacity of TNBC cells. Moreover, our experiments showed that downregulation of HIF1A-AS2 effectively suppressed TNBC cell invasion and migration. Additionally, it modulates the expression levels of EMT markers by suppressing N-cadherin and Vimentin and increasing E-cadherin. Overall, these findings suggest a potential oncogenic function for HIF1A-AS2 in TNBC, but further in vivo studies are necessary to confirm these results.

Chemotherapy with paclitaxel, a standard treatment for TNBC, can effectively inhibit tumor progression and benefit certain patients when combined with other chemotherapy drugs. However, drug resistance remains a significant challenge. Latest research has shown that lncRNAs are involved in paclitaxel resistance through various mechanisms. For instance, the downregulation of lncRNA H19 in paclitaxel-resistant TNBC restores chemosensitivity by mediating the Akt signaling pathway [22]. Additionally, lncRNA FTH1P3 acts as a competing endogenous RNA with miR-206 to upgrade the level of drug-resistant protein ACBC1, leading to paclitaxel resistance in TNBC [23]. In this study, we founded that silencing of HIF1A-AS2 significantly increased the susceptivity of TNBC cells to paclitaxel. Combined treatment with si-HIFA-AS2 and paclitaxel significantly reduced the number of clonogenic cells compared to the control group. Additionally, MTT assay results showed that increasing paclitaxel concentrations more significantly inhibited the cell viability in the si-HIF1A-AS2 group. Furthermore, considering our previous findings (refer to Fig. 1A and B), these results imply that HIF1A-AS2 might be a regulatory factor contributing to paclitaxel resistance in TNBC.

Interestingly, in our investigation into the potential mechanism underlying the regulation of HIF1A-AS2 in TNBC, we discovered an intriguing protein, MRPS23. MRPS23, a member of the mitochondrial ribosomal small subunit proteins, has been reported to correlate with prognosis and drug sensitivity in patients with liver cancer [24,25]. In breast cancer research, MRPS23 has been shown to influence proliferation, propagation, and response to CDK1 inhibitors [26,27]. Our experimental data confirms the regulatory function of MRPS23 in TNBC. Elevated levels of MRPS23 expression are found in breast cancer tissues and TNBC cells. Additionally, reducing HIF1A-AS2 significantly suppresses MRPS23 expression in TNBC cells. Our experiments consistently show that MRPS23 promotes cell growth, migration, and invasion in TNBC cells. Furthermore, silencing HIF1A-AS2 can suppress the functional enhancement of TNBC cells induced by MRPS23 overexpression. These findings suggest that HIF1A-AS2 influences various biological functions in TNBC cells by controlling MRPS23 expression.

However, there are still limitations in this study that need to be addressed. Further investigation is required to elucidate molecular mechanisms underlying the association between HIF1A-AS2 and MRPS23, as well as other downstream targets of HIF1A-AS2. Additionally, in vivo experiments are need to demonstrate the effect of HIF1A-AS2 in breast cancer. Therefore, it is essential to validate these findings with additional experiments.

5. Conclusions

In conclusion, our findings reveal elevated levels of lncRNA HIF1A-AS2 in breast cancer and its role in promoting paclitaxel resistance, proliferation and metastasis of TNBC cells. The underlying mechanism may involve the regulation of MRPS23 protein expression. MRPS23 overexpression promotes TNBC cell progression and paclitaxel resistance, while HIF1A-AS2 knockdown reversed these phenotypes.

Funding statement

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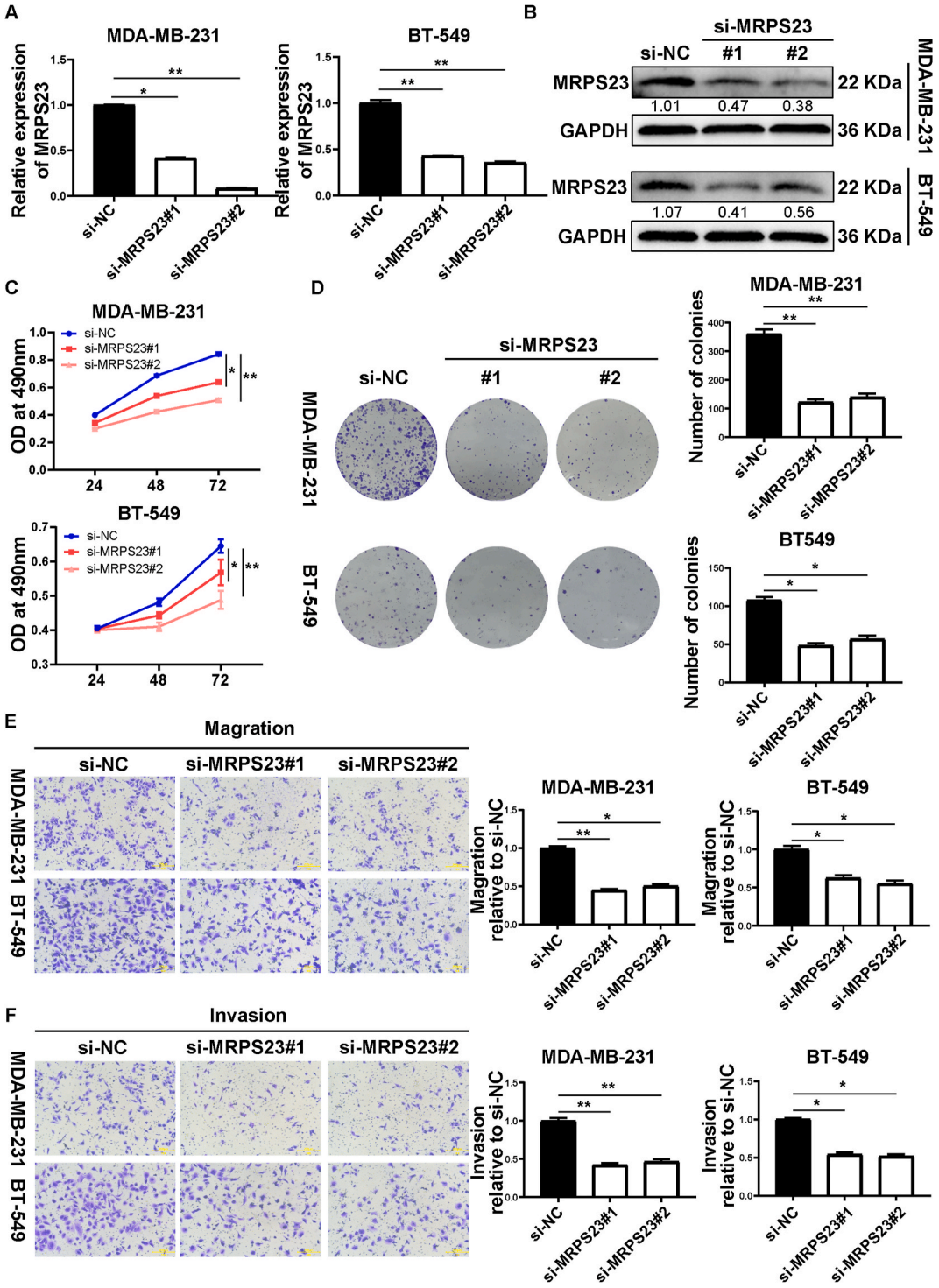

Fig. 5. MRPS23 inhibition significantly suppressed TNBC cell proliferation, migration, and invasion. (A&B) TNBC cells were transfected with two independent siRNAs targeting MRPS23(si-MRPS23#1 and si-MRPS23#2) or si-NC, and the silence effect of siRNAs were measured by RT-qPCR and Western blot. (C&D) The effect of MRPS23 knockdown on the proliferation of TNBC cells was assessed through MTT assay and colony formation assay. (E&F) Transwell assay were performed to evaluate TNBC cells migration and invasion ability after MRPS23 knockdown Scale bar, 200um. *p < 0.05, **p < 0.01. n = 3 biological replicates.

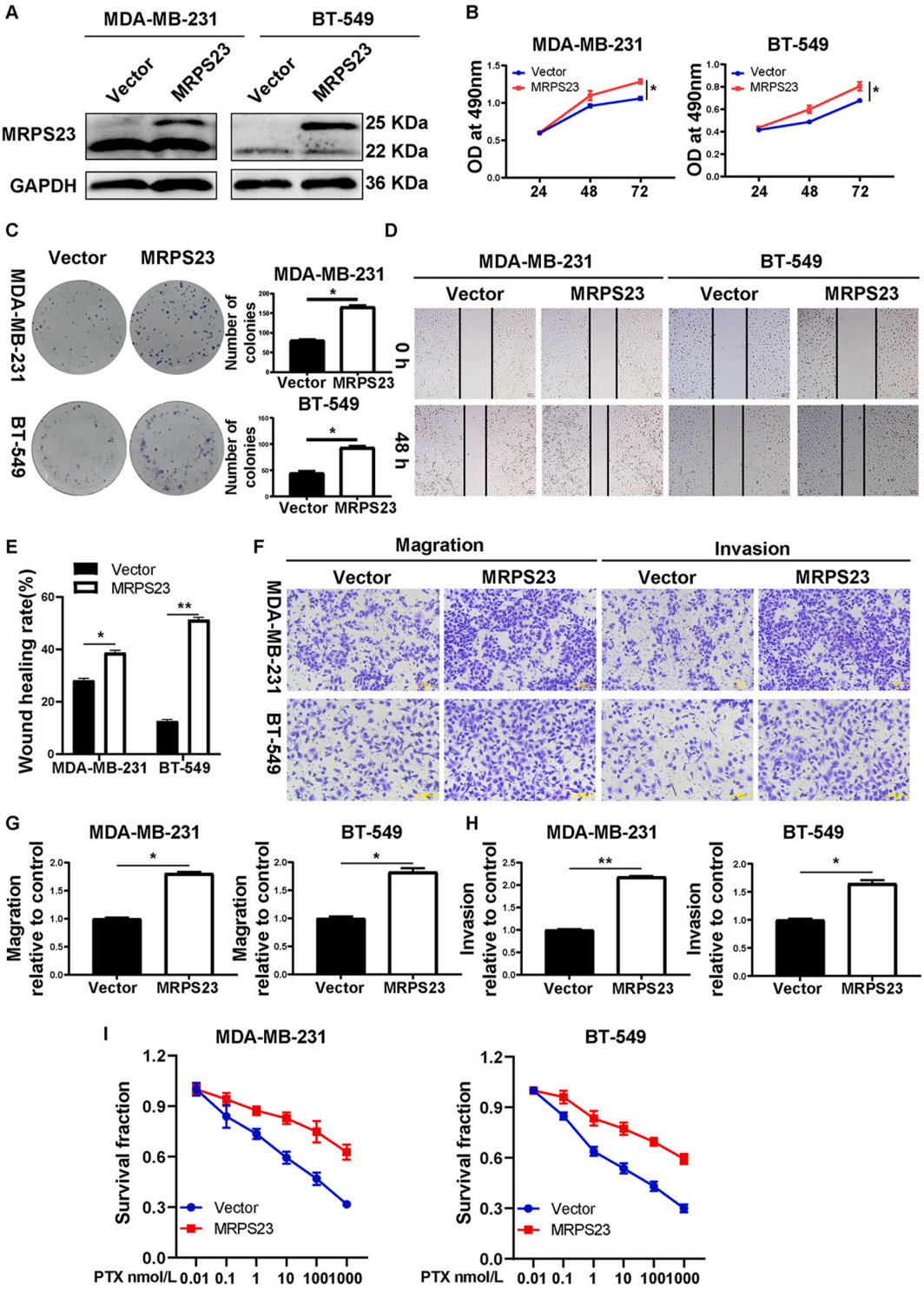

Fig. 6. MRPS23 promotes the TNBC cell progression and resistance to paclitaxel. (A) Western blot of MRPS23 overexpression efficiency in TNBC cells. (B&C) MTT and colony formation assays were implemented to evaluate the affection of MRPS23 overexpression in the proliferation of TNBC cells. (D&E) Cells migration after MRPS23 overexpression was evaluated by wound healing assays. (F–H) The Transwell assays was used to evaluate TNBC cells migration and invasion after overexpression of MRPS23. (I) Cell viability after MRPS23 overexpression and treatment with equipotential increasing dosages of paclitaxel (PTX) was detected by MTT assay. *$p < 0.05$, **$p < 0.01$. $n = 3$ biological replicates.

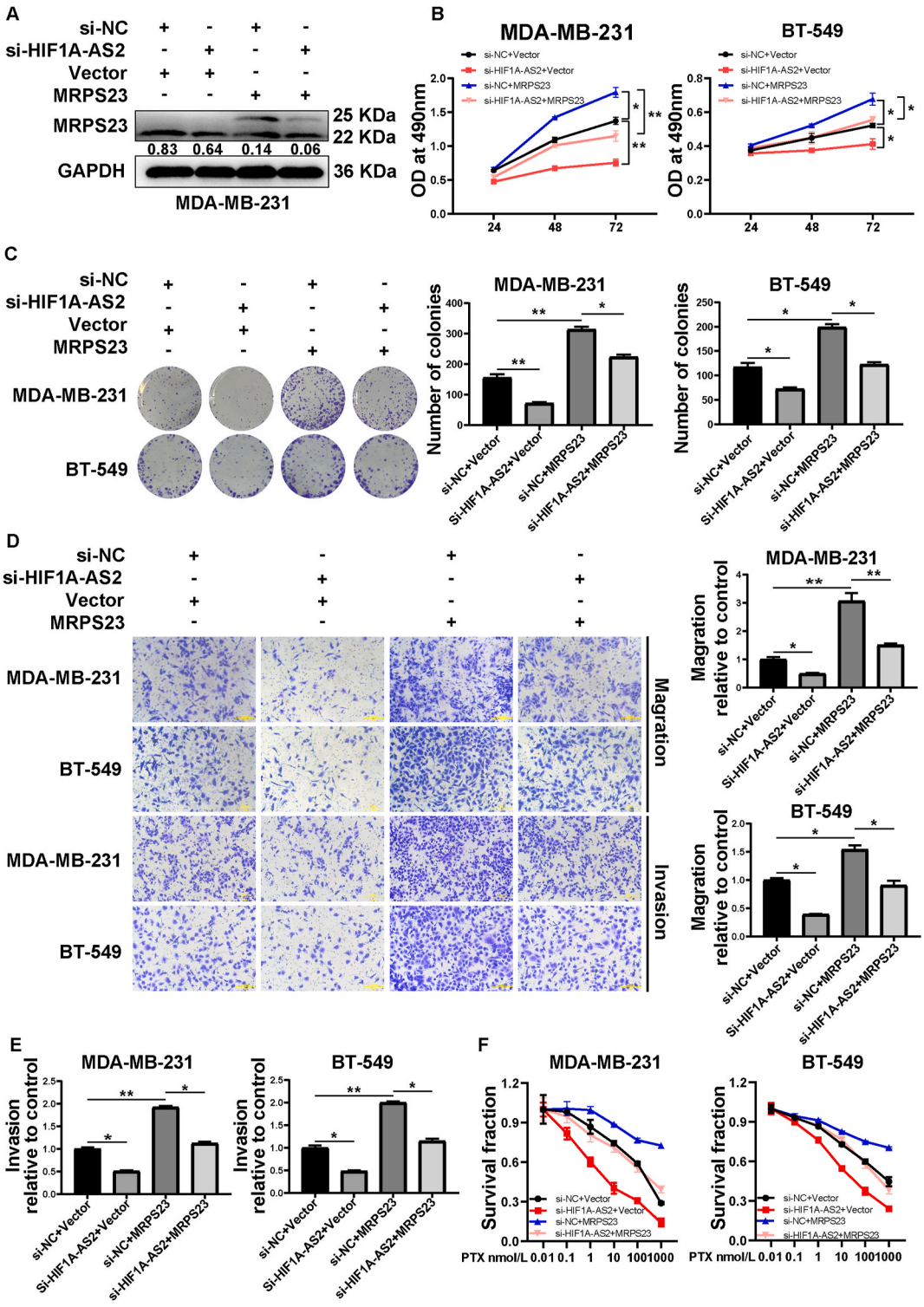

Fig. 7. LncHIF1A-AS2 regulates TNBC cells proliferation, metastasis, and drug resistance through MRPS23. (A) The protein levels of MRPS23 were measured in MDA-MB-231 cells after co-transfected with control siRNA/HIF1A-AS2-targeting siRNA and control vector/MRPS23 overexpression plasmid. (B&C) Proliferation of TNBC cells with the referential treatments was measured via MTT and colony formation assays. (D&E) Cell migration and invasion with the indicated treatments were evaluated by Transwell assay. (F) Cell viability was assessed by MTT assay after co-transfection with control siRNA or HIF1A-AS2-targeting siRNA and control vector or MRPS23 overexpression plasmid, followed by treatment with escalating doses of paclitaxel (PTX). *$p < 0.05$, **$p < 0.01$. n = 3 biological replicates.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Liangliang Min: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lu Chen:** Methodology, Formal analysis, Data curation. **Da Huang:** Resources, Methodology, Investigation. **Yulu Zhang:** Resources, Methodology, Investigation. **Aihua You:** Resources, Investigation, Data curation. **Xiaohua Yan:** Project administration, Conceptualization. **Zhi-hua Li:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e36469.

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