



Long Non-coding RNA LINC01224 Promotes the Malignant Behaviors of Triple Negative Breast Cancer Cells via Regulating the miR-193a-5p/NUP210 Axis

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Abstract

Triple negative breast cancer (TNBC) is a prevalent malignant tumor in women and is characterized by high incidence and mortality. Current evidence has suggested that multiple long noncoding RNAs (lncRNAs) play regulatory roles in TNBC, while the specific mechanism of LINC01224 in TNBC remains unclear. In this study, LINC01224 was highly expressed in TNBC cells. Moreover, LINC01224 downregulation inhibited TNBC cell proliferation, migration, and invasion, and promoted cell apoptosis. Additionally, LINC01224 stabilized NUP210 mRNA through interaction with miR-193a-5p, thereby aggravating the malignant phenotypes of TNBC. Overall, LINC01224 functions as a tumor promoter for TNBC.

Keywords LINC01224 · miR-193a-5p · NUP210 · TNBC

Introduction

Breast cancer is a frequently diagnosed malignant tumor that adversely threatens the health of women. It is a leading factor for cancer-related deaths among females under 45 years old [1]. It is estimated that in 2022, there will be approximately 290,560 new cases diagnosed as breast cancer, and 43,780 deaths resulting from this disease in the USA [2]. Although great improvements have been achieved in therapeutic options such as radiotherapy, chemotherapy, targeted therapy, and traditional surgery during recent years [3], the

prognosis of patients with breast cancer, remains poor due to tumor recurrence and distant metastasis [4]. Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer [5], accounting for about 15% of all cases [6]. Patients with TNBC are insensitive to trastuzumab or endocrine treatments [7] due to the absence or low expression of human epidermal growth factor receptor 2 (HER2), progesterone receptor (PR), and estrogen receptor (ER) [8]. Recently, following the development of immune checkpoint inhibition (ICI) in solid tumors and validation of the immunogenicity in TNBC, immunotherapy has been emerged as a promising therapeutic strategy in TNBC [9]. However, TNBC is a highly heterogeneous disease and only a proportion of TNBC is immunotherapy-responsive [10, 11]. Thus, probing the molecular mechanisms under TNBC and finding out effective biomarkers for this disease are of great significance.

Long noncoding RNAs (lncRNAs) refer to a subset of transcripts with over 200 nucleotides in length with no or limited capacity for protein coding, which are involved in various cellular biological processes, such as chromatin dynamics and gene regulation [12]. An increasing number of lncRNAs have been reported to play an extensive regulatory role in malignancies and modulate gene expression at the phase of transcription, modification, and post-transcriptional regulation [13]. Moreover, it has also been widely proposed that a subset of lncRNAs mediate the tumorigenesis of

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numerous cancers via competitively interacting with downstream miRNAs to upregulate the expression of target genes, which is called the competing endogenous RNA (ceRNA) pattern [14]. Emerging evidence has illustrated that lncRNAs can act as a tumor promoter or a tumor suppressor in different types of cancers. For example, lncRNA prostate gene expression marker 1 (PCGEM1) expression is abundant in prostate cancer cells and facilitates cell proliferation while suppresses doxorubicin-induced apoptosis [15]. lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) modulates the miR-374b-5p/SRSF7 pathway to positively regulate lung cancer cell proliferation, migration, and invasion [16]. lncRNA colon cancer associated transcript 1 (CCAT1) facilitates colorectal cancer cell growth by regulating miR-181a-5p level [17]. Moreover, lncRNA maternally expressed 3 (MEG3) inhibits gastric cancer cell proliferation and metastasis by regulating p53 signaling pathway [18]. lncRNA phosphatase and tensin homolog pseudogene 1 (PTENP1) works as a tumor suppressor in cervical cancer by downregulating miR-106b [19]. Previous studies have indicated that lncRNA LINC01224 promotes tumorigenesis in a number of cancers such as hepatocellular carcinoma [20] and epithelial ovarian cancer [21]. Importantly, it was reported that LINC01224 was associated with the prognosis of breast cancer patients and might play a part in the carcinogenesis of breast cancer [22]. However, the specific functions and potential mechanism of LINC01224 in TNBC are still undefined.

In this study, the potential role and the underlying molecular mechanism of LINC01224 in TNBC were investigated. It was hypothesized that LINC01224 might affect the malignant behaviors of TNBC cells by regulating its downstream molecules. Our findings implied that LINC01224 might be a novel and effective biomarker to improve the prognosis of patients with TNBC.

Materials and Methods

Cell Culture

Human normal breast epithelial cell line (MCF-10A) and six human breast cancer cell lines (MCF-7, T-47D, BT-20, HCC1937, MDA-MB-453, and MDA-MB-231) were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were incubated in DMEM (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum and 1% streptomycin in a humidified incubator at 37°C with 5% CO₂.

Cell Transfection

Specific short harpin RNA (shRNA) targeting LINC01224 together with negative control shRNA were termed

sh-LINC01224#1/2 and sh-NC. To enhance endogenous miR-193a-5p function, miR-193a-5p mimics was used, with scrambled NC mimics as the control. Overexpression vectors pcDNA3.1/LINC01224 and pcDNA3.1/NUP210 (nucleoporin 210) were used for enhancing functions of endogenous LINC01224 and NUP210, along with the empty pcDNA3.1 vector as the control. All the above-mentioned products were commercially provided by Genechem (Shanghai, China). Finally, 6-well plates were applied to culture TNBC cells, which were transfected with abovementioned products for 2 days using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

qRT-PCR

Extraction of total RNA from TNBC cells was conducted using TRIzol reagent (Invitrogen). To determine total RNA concentration and purity, the Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used. LINC01224 and NUP210 expression was calculated standardized to GAPDH, and miR-193a-5p expression was calculated standardized to U6 with the 2^{-ΔΔC_t} method. Primer sequences (5′–3′) are listed as follows:

LINC01224, Forward (F): TGATCAAAGCTACTCCGGG,
Reverse (R): GAGTCTCACAATGTCGCCT.
NUP210, F: GACCATCTACAACCACCCT,
R: GTAACCTGAGCCTTCCCTG.
GAPDH, F: TCATTTCTGGTATGACAACGA,
R: GTCTTACTCCTTGGAGGCC.
miR-193a-5p, F: ACGCTGGGTCTTTGCGG,
R: TATGGTTGTTACGACTCCTTCAC.
U6, F: ATACAGAGAAAGTTAGCACGG,
R: GGAATGCTTCAAAGAGTTGTG.

5-Ethynyl-2'-deoxyuridine (EdU) Assay

Cells (4 × 10³ cells/well) with different transfections were seeded into 96-well plates and incubated with a Cell-Light EdU Apollo488 In Vitro Kit (RiboBio, Guangzhou, China) at 37 °C for 2 h. Afterward, cells were stained in DAPI solution for 5 min. At last, these cells were observed in 5 randomly selected fields under a BX63 Automated Fluorescence Microscope (Olympus, Tokyo, Japan).

Flow Cytometry Analysis

Flow cytometry analysis was conducted using an FITC Annexin V Apoptosis Detection kit I (BD Biosciences, San Jose, CA, USA) for the detection of cell apoptosis rate. In brief, cells were pretreated for the indicated time for a quarter in the dark and harvested after centrifugation at $300\times g$ in the 5-min time limit. Later, the binding buffer was utilized to resuspend the cells, and then propidium iodide (PI) and Annexin V-FITC were employed for staining these cells in the dark. Lastly, a FACScan flow cytometer (BD Biosciences) was used to analyze cell apoptosis.

Colony Formation Assay

Colony formation assay was utilized for evaluation of proliferation of TNBC cells. Cells (1×10^3 cells/well) were inoculated into 24-well plates with 1 mL of cultured medium to each well and cultured for 14 days at 37 °C. Afterward, treated cells were fixed with 4% paraformaldehyde for half an hour and subsequently stained with crystal violet (both from Sigma-Aldrich, St. Louis, MO, USA) for 20 min. Finally, we employed a microscope (Nikon, Tokyo, Japan) to count the visible colonies from five randomly selected fields.

Western Blotting

Total protein of the cells was obtained using RIPA lysis buffer (Solarbio Life Science, Beijing, China). Next, a BCA detecting kit (Beyotime, Haimen, China) was employed to quantify the proteins. After being isolated with 10–12% SDS-PAGE, proteins were transferred onto polyvinylidene fluoride (PVDF) membranes (Thermo Fisher Scientific). Then primary antibodies were added into membranes which were blocked with 5% skim milk. The following were the primary antibodies used in this assay: anti-Bax (ab182733, Abcam, Cambridge, MA, USA), anti-Bcl-2 (ab182858, Abcam), anti-NUP210 (ab200677, Abcam), anti-NLN (ab119802, Abcam), and anti-GAPDH (ab181602, Abcam). Next, the PVDF membranes were rinsed and subjected to hybridization for 1 h at room temperature with HRP-conjugated secondary antibodies (Abcam). In this assay, GAPDH was used as a loading control. The protein bands were visualized using an enhanced chemiluminescent (ECL) system (Beyotime) and quantified using ImageJ software (National Institutes of Health, Baltimore, MD, USA).

Wound Healing Assay

Wound healing assay was applied to assess cell migration capacity. Treated cells (1×10^5 cells/well) were seeded into

6-well plates and cultured until 80% confluency. Later, a single wound in the cell monolayer was created using a 200- μ L pipette tip. At 0 and 24 h, a microscope (Leica, Wetzlar, Germany) was used to capture the images of the migratory cells.

Transwell Assay

A Transwell chamber (Corning, Maine) containing a mixture of 90% serum-free medium and 10% Matrigel was used to analyze TNBC cell invasion. Serum-free DMEM (200 μ L) containing 5×10^4 cells were added into the upper chamber, and DMEM (600 μ L) with 10% FBS was added to the bottom chamber. After incubation for one day at 37 °C, the chamber was rinsed and fixed with 4% paraformaldehyde for 30 min. Subsequently, cells were stained with 0.1% crystal violet for 15 min. Thereafter, we gently cleaned the chamber and swabbed to remove cells that have not passed through the membrane. Finally, we counted the number of invasive cells with an inverted microscope (Olympus).

Subcellular Fractionation

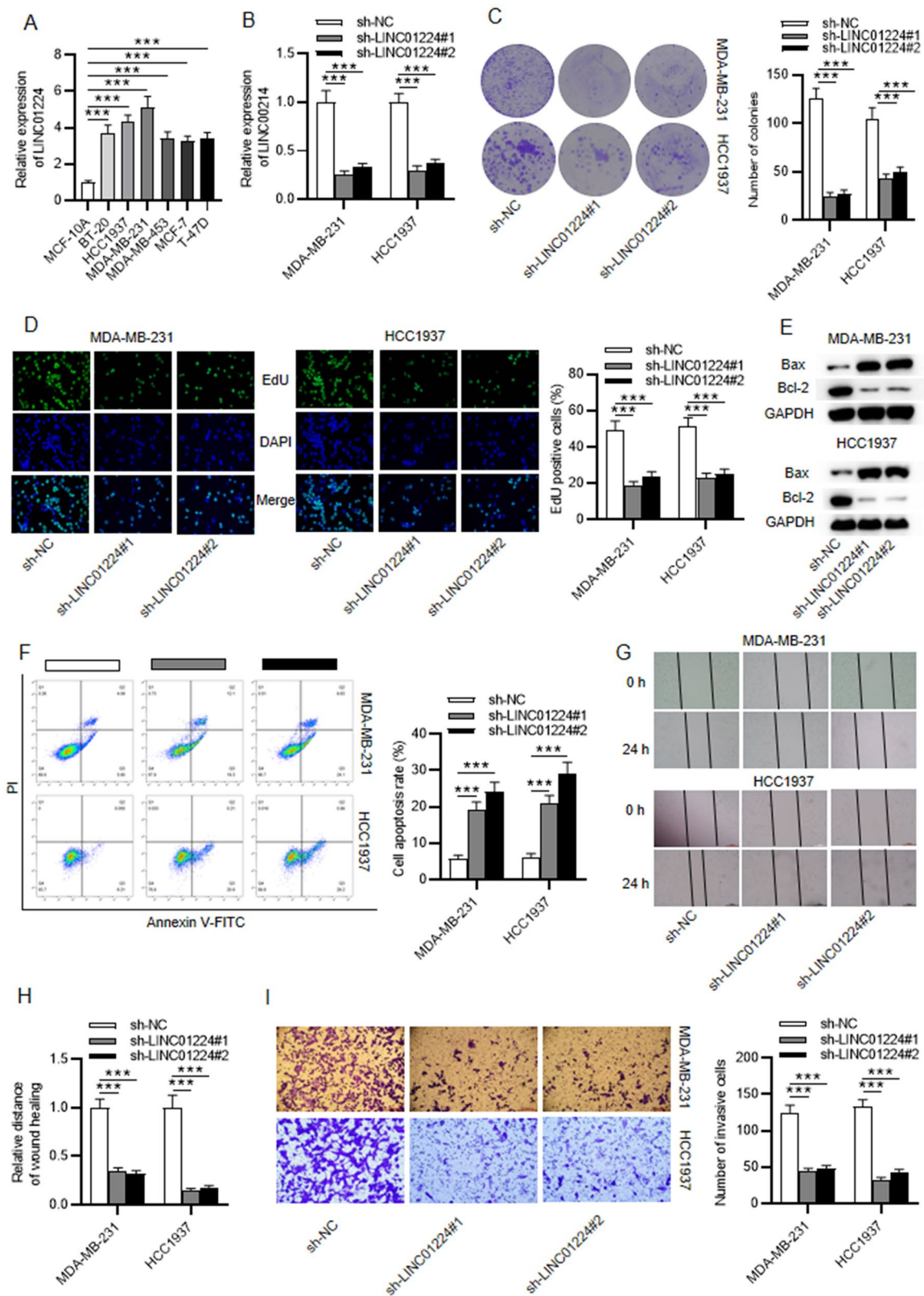
The localization of RNAs in cancer cells was measured by subcellular fractionation. The extractions of nucleus and cytoplasm were separated from TNBC cells using NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific). In order to analyze RNAs in nucleus and cytoplasm, qRT-PCR was applied. U6 (nucleus control), GAPDH (cytoplasm control) and LINC01224 expression levels were measured, respectively.

RNA Immunoprecipitation (RIP) Assay

A Magna RIP™ RNA-binding protein immunoprecipitation kit (Millipore) was used for RIP assay according to the manufacturer's protocols. Briefly, TNBC cells were lysed in 100 μ L RIP lysis buffer containing protease inhibitor cocktail and RNase inhibitors. Next, cell lysates (200 μ L) were incubated with magnetic beads coated with anti-Ago2 (ab186733, Abcam) or anti-IgG (ab190475, Abcam) at 4 °C overnight. Afterward, proteinase K was used to remove the protein in the immunoprecipitate and precipitated RNA was isolated and purified by TRIzol reagent (Takara, Dalian, China). qRT-PCR analysis was then performed to assess RNA level.

Luciferase Reporter Assay

Putative binding sites between LINC01224 and miR-193a-5p or NUP210 and miR-193a-5p were predicted by starBase (<https://starbase.sysu.edu.cn/index.php>) [23].



Wild-type (Wt) or mutant-type (Mut) binding sequences of LINC01224 or NUP210 3'UTR were sub-cloned into pmir-GLO vectors (Promega, Madison, WI, USA) to construct

the LINC01224-Wt, LINC01224-Mut, NUP210-Wt, or NUP210-Mut plasmids. These plasmids were co-transfected with NC mimics, miR-193a-5p mimics, or miR-193a-5p

Fig. 1 LINC01224 was upregulated in TNBC cells and facilitated cellular progression of TNBC. **A** The qRT-PCR evaluated LINC01224 expression in MDA-MB-453, BT-20, HCC1937, MDA-MB-231, MCF-4, T-47D cells, and control MCF-10A cells. **B** Knock-down efficiency of LINC01224 in TNBC cells, as evaluated by qRT-PCR. **C, D** Proliferative ability of TNBC cells transfected with sh-LINC01224#1/2, as evaluated by EdU (**C**) and colony formation assays (**D**). **E, F** Effects of LINC01224 inhibition on cell apoptosis rate was assessed by Annexin V-FITC/PI staining on a flow cytometry (**E**) and western blotting of Bax and Bcl-2 (**F**). **G–I** Migration and invasion of TNBC cells under silencing of LINC01224 were evaluated by wound healing (**G–H**) and Transwell assays (**I**). *** $P < 0.001$

mimics + pcDNA3.1/LINC01224 for 48 h. The luciferase activities were determined using the Luciferase Reporter Assay System (Promega).

RNA Pull-Down Assay

The binding of LINC01224 and miRNAs was detected by an RNA pulldown assay. LINC01224 biotinylated (biotin) probe and LINC01224 no-biotin probe were obtained from GenePharma (Shanghai, China). TNBC cells were incubated with LINC01224 biotin probe or control no-biotin probe for 6 h. Later, TNBC cells were lysed on ice using lysis buffer and then subjected to centrifugation. The supernatants were harvested and incubated with M-280 streptavidin magnetic beads (Invitrogen) overnight at 4 °C. Afterward, the beads were collected and washed with ice-cold phosphate buffer saline. Subsequently, the pull-down samples were purified using TRIzol reagent (Takara) and the expression levels of miRNAs were analyzed through qRT-PCR.

Statistical Analysis

Data derived from three independent assays (3 biological repeats and 3 technical repeats) were exhibited as means \pm standard deviation. IBM SPSS Statistics V22.0 (IBM Corp, Armonk, NY, USA) was used for statistical analysis. Differences between 2 groups or among more than 2 groups were evaluated by *t* test or ANOVA with Tukey's post hoc test. *P* values less than 0.05 were regarded as statistical significance.

Results

LINC01224 was Upregulated in TNBC Cells and Facilitated Cell Proliferation and Migration as Well as Suppressed Cell Apoptosis

To investigate whether LINC01224 is involved in the tumorigenesis of TNBC, at first, we detected LINC01224 level in human breast cancer cell lines and control MCF-10A cell line using qRT-PCR, which demonstrated that

LINC01224 level in human breast cancer cell lines was significantly higher than that in MCF-10A cell line (Fig. 1A). We selected two TNBC cell lines (HCC1937 and MMDA-MB-231) with relatively higher LINC01224 expression for further analysis. Subsequently, qRT-PCR revealed that LINC01224 expression was strongly down-regulated in sh-LINC01224#1/2 transfected TNBC cells (Fig. 1B). Thereafter, as presented by EdU assay and colony formation assay, LINC01224 silencing restrained the proliferative ability of TNBC cells (Fig. 1C, D). Through western blotting analysis and flow cytometry assay, we found that LINC01224 depletion increased the apoptosis rate of TNBC cells (Fig. 1E, F). Furthermore, the migratory and invasive capacities of TNBC cells were inhibited after LINC01224 knockdown (Fig. 1G–I).

LINC01224 Acted as a Sponge for miR-193a-5p

Subsequently, bioinformatics analysis was performed to probe the expression of LINC01224 in breast invasive carcinoma (BRCA). As revealed by starBase database, LINC01224 expression was higher in 1104 BRCA samples than that in 113 normal control samples (Fig. 2A). The results of subcellular fractionation displayed that LINC01224 was mainly distributed in the cytoplasm of TNBC cells (Fig. 2B). Therefore, we predicted that LINC01224 might act as a ceRNA targeting miRNAs. Afterward, we searched the potential miRNAs that might bind to LINC01224, and six miRNAs were screened from the database of starBase with the screening condition of CLIP Data ≥ 1 (Fig. 2C). Moreover, RNA pull-down assay was performed, suggesting that three miRNAs were abundantly enriched in LINC01224 biotin-probe group, while other miRNAs had no significant changes (Fig. 2D). Next, qRT-PCR was conducted to investigate expression levels of predicted miRNAs, suggesting that among the predicted miRNAs, only miR-193a-5p was significantly downregulated in TNBC cells (Fig. 2E). Consistently, the data from starBase database confirmed the lower miR-193a-5p expression in 1085 BRCA samples than in 104 normal control samples (Fig. 2F). To further identify the interaction between LINC01224 and miR-193a-5p, we overexpressed LINC01224 with pcDNA3.1/LINC01224 vectors and the efficiency was validated by qRT-PCR (Fig. 2G). As displayed in Fig. 2H and I, overexpressing LINC01224 significantly inhibited miR-193a-5p expression, while knocking down LINC01224 induced the upregulation of miR-193a-5p. Thereafter, miR-193a-5p mimics was used to overexpress miR-193a-5p (Fig. 2J). The binding site between LINC01224 and miR-193a-5p was predicted by starBase (Fig. 2K). As shown by luciferase reporter assay, in comparison to that in the control group, the luciferase

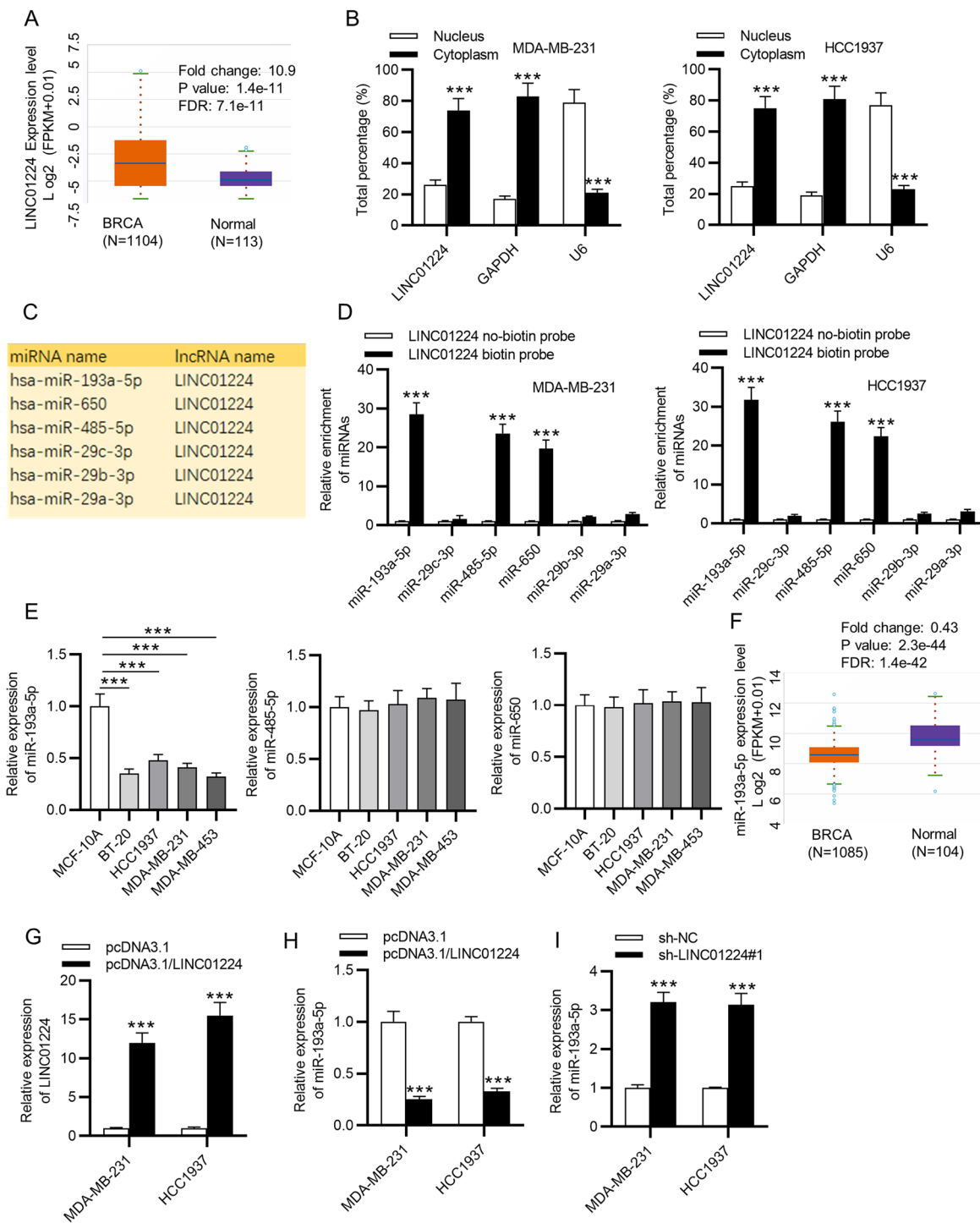


Fig. 2 LINC01224 bound with miR-193a-5p. **A** starBase predicts relative expression profile of LINC01224 in 1104 BRCA samples, normalized to that in 113 control samples. **B** The distribution of LINC01224 in TNBC cells was evaluated by subcellular fractionation analysis. **C** The potential miRNAs binding to LINC01224 were revealed from starBase database. **D** Relative enrichment of candidate miRNAs pulled down by LINC01224 biotin-probe in TNBC cells. **E** qRT-PCR analysis for miRNA expression in TNBC cells. **F** starBase online tool predicts miR-193a-5p expression in 1085 BRCA samples

and in 104 control samples. **G** qRT-PCR analysis for assessing the efficiency of LINC01224 overexpression. **H–I** qRT-PCR analysis for evaluating miR-193a-5p expression in LINC01224-overexpressed/depleted TNBC cells. **J** qRT-PCR analysis for measuring the efficiency of miR-193a-5p overexpression. **K** Binding site between LINC01224 and miR-193a-5p, as revealed from starBase online tool. **L, M** Relative luciferase activities of TNBC cells after co-transfection of LINC01224-Wt/Mut and miR-193a-5p (or NC mimics) were revealed by luciferase reporter assay. *** $P < 0.001$

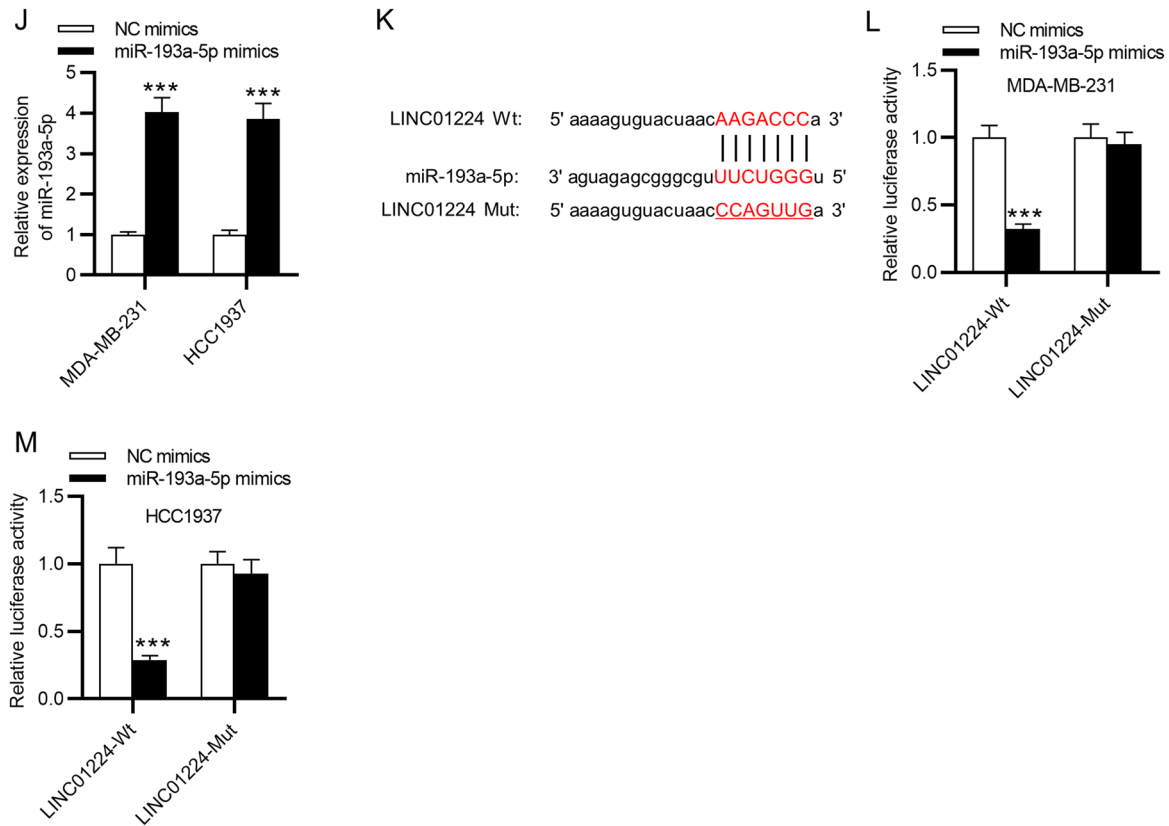


Fig. 2 (continued)

activity of LINC01224-Wt was significantly reduced in miR-193a-5p mimics-treated group, but had no significant changes after mutation (Fig. 2L, M).

NUP210 was a Target of miR-193a-5p

Based on the ceRNA hypothesis, we investigated the downstream mRNAs of miR-193a-5p through bioinformatics analysis. Through TargetScan [24], miRDB [25], and miRTarBase [26], two common potential target genes, NUP210 and NLN were presented using Venn diagram (Fig. 3A). Next, we conducted western blotting and qRT-PCR for detecting the expression of these two genes at both mRNA and protein levels. Results suggested that NUP210 was downregulated in miR-193a-5p-overexpressing TNBC cells (Fig. 3B, C). Data from starBase revealed the higher NUP210 expression in 1104 BRCA samples than in 113 control samples (Fig. 3D). Subsequently, NUP210 was found to be overexpressed in TNBC cells (Fig. 3E). Furthermore, the binding site between NUP210 3'UTR and miR-193a-5p was shown by starBase (Fig. 3F). NUP210 mRNA expression and protein level were decreased by the silence of LINC01224 in TNBC

cells (Fig. 3G, H). Next, it was found that miR-193a-5p mimics had a suppressing effect on the luciferase activity of NUP210-Wt, which was rescued by overexpressing LINC01224, while no significant difference was demonstrated in that of NUP210-Mut (Fig. 3I). Additionally, RIP assay depicted that LINC01224, miR-193a-5p, and NUP210 coexisted in the same RNA-induced silencing complexes (RISCs) (Fig. 3J), further confirming the binding relation between the three molecules.

LINC01224 Promoted the Malignant Behaviors of TNBC

To further explore whether LINC01224 induced the malignant phenotypes of TNBC cells via the miR-193a-5p/NUP210 pathway, we conducted several rescue experiments. In the first place, NUP210 mRNA and protein levels were increased in TNBC cell lines under transfection of pcDNA3.1/NUP210, as shown by qRT-PCR analysis and western blotting (Fig. 4A, B). Through the EdU assay and colony formation assay, we discovered that NUP210 overexpression counteracted the LINC01224 silencing-inhibited proliferative ability in TNBC cells (Fig. 4C, D). Besides, the

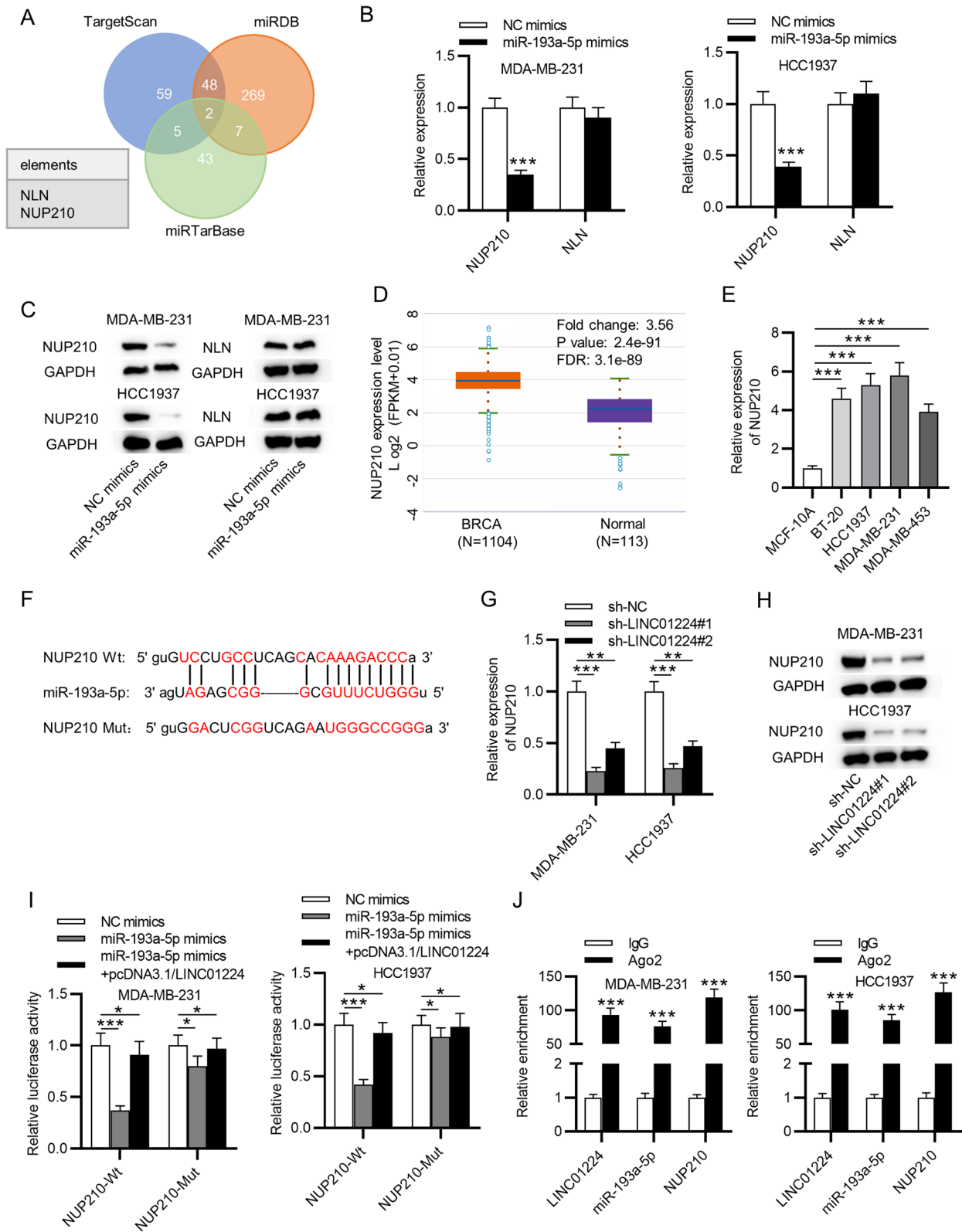


Fig. 3 LINC01224 upregulated NUP210 by absorbing miR-193a-5p. **A** Two targets (NLN, NUP210) of miR-193a-5p, as predicted by miRDB, TargetScan, and miRTarBase. **B, C** Levels of NLN, NUP210 in miR-193a-5p overexpressing cells were determined by qRT-PCR and western blotting. **D** starBase predicts NUP210 expression in 1104 BRCA samples and 104 control samples. **E** qRT-PCR analysis of NUP210 expression in TNBC cells. **F** Binding site between miR-193a-5p and NUP210 3'UTR, as predicted from TargetScan database. **G, H** Effects of LINC01224 downregulation on NUP210 expression according to qRT-PCR and western blotting results. **I** Relative luciferase activities of TNBC cells under the co-transfection of NUP210-WT or NUP210-Mut plasmids and miR-193a-5p mimics or miR-193a-5p mimics + pcDNA3.1-LINC01224 were revealed by luciferase reporter assay. **J** The co-existence of LINC01224, miR-193a-5p, and NUP210 in RISCs formed by Ago2 was explored through RIP assay. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

cell apoptosis increased by silencing of LINC01224 was rescued with upregulated NUP210 as shown by flow cytometry assay and western blotting analysis (Fig. 4E, F). Moreover, Transwell assay and wound healing assay revealed that the LINC01224 depletion-induced suppressive effects on cell invasion and cell migration were rescued by NUP210 upregulation in TNBC cells (Fig. 4G–I).

Discussion

As one of the most common tumors in women, TNBC is characterized by high aggressiveness, poor prognosis, and high rates of metastasis and recurrence [27]. Although emerging therapies appear promising for breast cancer treatment, clinical outcomes for TNBC remain unsatisfactory [28]. Thus, to research the molecular mechanism underlying TNBC is in urgent need, and it is also important to offer theoretical foundation for future treatment options.

LncRNAs, nonprotein-coding RNAs, have been regarded as potential regulators or biomarkers in various tumors [29, 30]. In the past few years, accumulating studies have manifested that multiple lncRNAs exert regulatory functions in TNBC progression [31]. For example, lncRNA MNX1 antisense RNA 1 (MNX1-AS1) promotes cell growth of TNBC by enhancing signal transducer and activator of transcription 3 (Stat3) phosphorylation [32]. LncRNA H19 regulates the p53/TNFAIP8 axis to facilitate TNBC cell migration and invasion [33]. Moreover, lncRNA X inactive specific transcript (XIST) inhibits TNBC cell proliferation, epithelial mesenchymal transition, and promotes apoptosis by interacting with miR-454 [34]. LncRNA zinc finger NFX1-type containing 1 antisense RNA 1 (ZFAS1) suppresses the progression of TNBC by targeting Stat3 [35]. The evidence indicates that lncRNAs can function as an oncogene or a tumor suppressor in TNBC. Nonetheless, the role of LINC01224 in TNBC has not been specified. In this study, we found LINC01224 was overexpressed in TNBC cells.

Additionally, silenced LINC01224 suppressed cell proliferation, migration, and invasion, while at the same time, facilitated cell apoptosis in TNBC. Our findings suggested the oncogenic function of LINC01224 in TNBC.

MicroRNAs refer to small RNAs containing 21–25 nucleotides [36] and are highly linked with the modulation of cancer progression, containing cell differentiation [37], proliferation [38], and apoptosis [39]. Furthermore, more and more research highlighted the hypothesis of ceRNA pattern referring to that lncRNA binds with specific miRNA to release miRNA-targeted mRNA, consequently regulating the development of cancers [40]. For example, lncRNA LOXL1 antisense RNA 1 (LOXL1-AS1) binds with miR-423-5p to stabilize MYB proto-oncogene like 2 (MYBL2), facilitating the tumorigenesis and development of lung cancer [41]. LncRNA mall nucleolar RNA host gene 22 (SNHG22) promotes the malignant phenotypes of TNBC through sponging miR-324-3p and upregulating SDS3 homolog, SIN3A corepressor complex component (SUDS3) [42]. Previous studies have demonstrated that lncRNAs exert different regulatory functions based on their subcellular localization. In the cytoplasm, lncRNAs can act as ceRNAs to regulate mRNA stability [43]. Here, we validated the cytoplasmic localization of LINC01224 in TNBC cells, indicating its role at the post-transcriptional level. Using bioinformatics and a series of assays, we identified that LINC01224 bound with miR-193a-5p in TNBC, which has been found to participate in the progression of ovarian cancer [44] colorectal cancer [45] and prostatic cancer [46] to suppress tumor development. Interestingly, the interaction between LINC01224 and miR-193a-5p was also reported in other tumors, including gastric cancer [47] and melanoma [48], further validating the credibility of the results in this study. Our findings further revealed that miR-193a-5p level was underexpressed in TNBC cells.

Thereafter, to further understand the mechanism of LINC01224 in TNBC, we screened the downstream target of miR-193a-5p using bioinformatics analysis and experiment validation. As a result, NUP210, located in chromosome 3, NC_000003.12 (13316235–13420322), was identified to be targeted by miR-193a-5p in TNBC cells. Previous research has suggested that NUP210, as the main component of the nuclear pore complex, participates in the regulation of macromolecular transport between nucleus and cytoplasm [49]. NUP210 was shown to act as a tumor promoter in lung cancer [50]. It was also reported that downregulation of NUP210 induced cell cycle arrest and apoptosis in cervical cancer by interacting with miR-22 and regulating Fas expression [49]. NUP210 is considered as a metastasis susceptibility gene and NUP210 loss reduces expression of cell adhesion/migration-related genes [51]. Intriguingly, higher NUP210 protein level was reported to be significantly associated with the poorer distant metastasis-free survival of

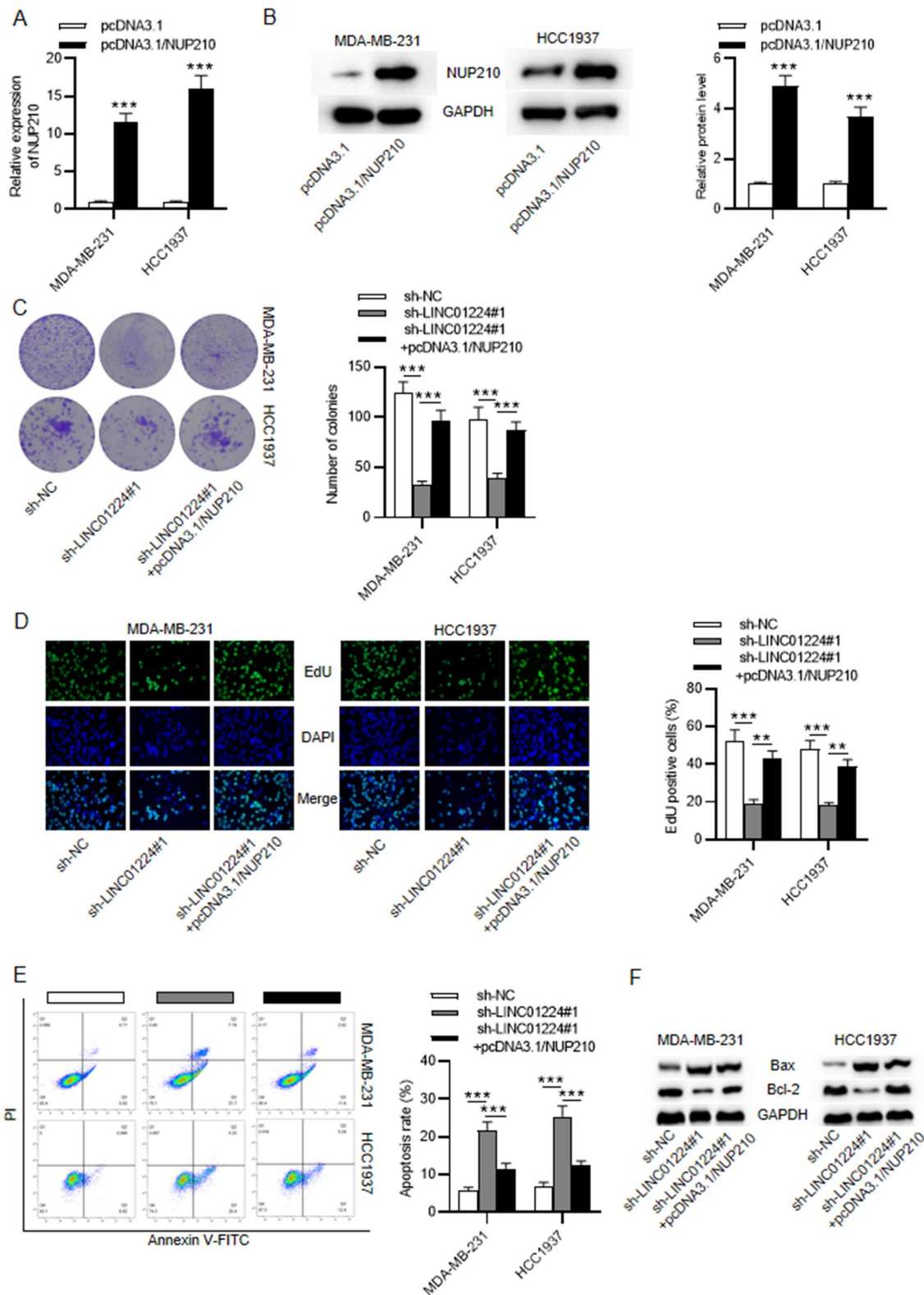


Fig. 4 LINC01224 facilitated the biological behaviors of TNBC cells via modulating the miR-193a-5p/NUP210 axis. **A, B** The qRT-PCR detected the overexpression efficiency of NUP210. **C, D** The change of proliferative ability in TNBC cells under the transfection of sh-LINC01224#1 and sh-LINC01224#1 + pcDNA3.1/NUP210 was evaluated through colony formation (**C**) and EdU assays (**D**). **E, F** Effects

of NUP210 overexpression on LINC01224 knockdown-repressed cell apoptosis was investigated through Annexin V-FITC/PI staining on a flow cytometry (**E**) and western blotting (**F**). **G–I** Wound healing and Transwell assays examined the rescue effects of upregulated NUP210 on LINC01224 depletion mediated cell migration and invasion. ** $P < 0.01$, *** $P < 0.001$

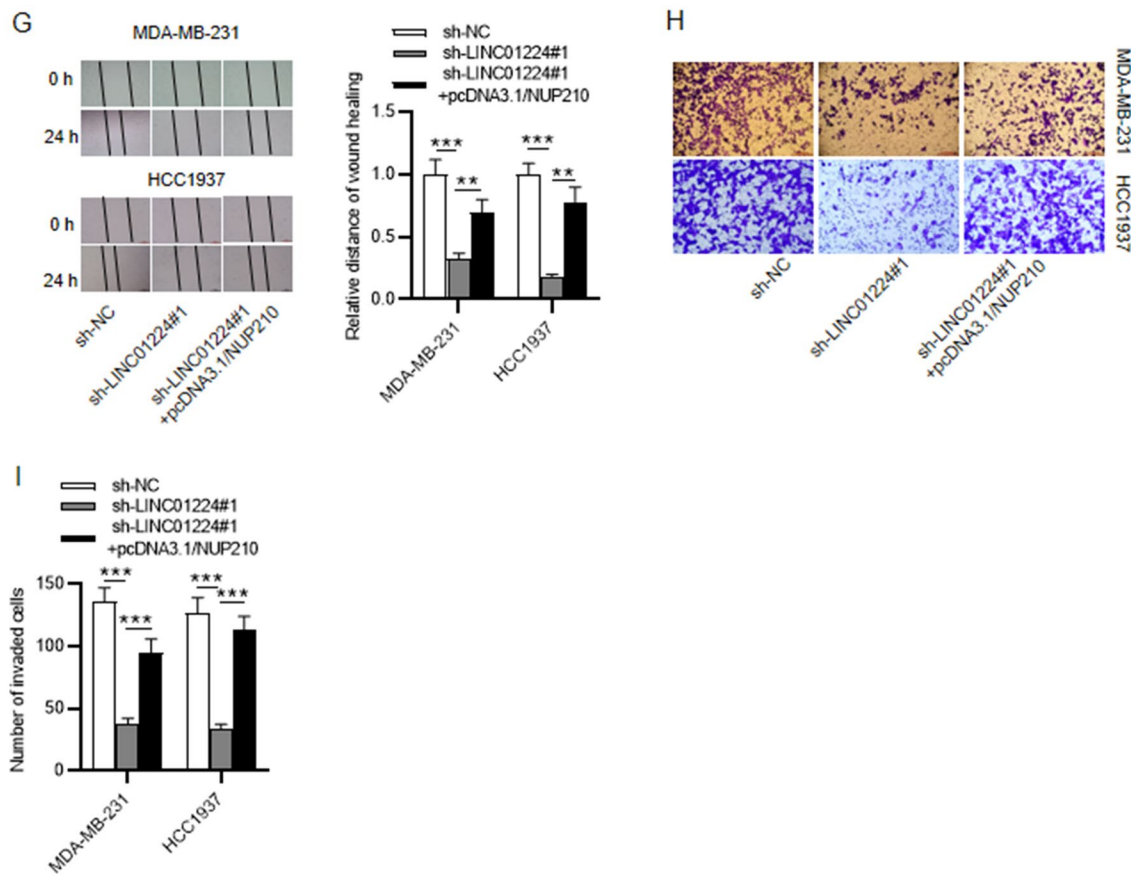


Fig. 4 (continued)

TNBC patients [52], indicating the potential role of NUP210 in TNBC progression. However, the specific role of NUP210 in TNBC has not been clarified. In the present study, we found that NUP210 was highly expressed in TNBC cells at both mRNA and protein levels. Furthermore, rescue experiments displayed that overexpression of NUP210 significantly reversed the suppressive effect of LINC01224 knockdown on TNBC cell proliferation, migration, and invasion. Additionally, NUP210 upregulation also attenuated LINC01224 silencing-mediated promotion in cell apoptosis. These results indicated that NUP210 might contribute to the aggressiveness of TNBC cells.

All in all, our research elucidated that LINC01224 was overexpressed in TNBC cells. Additionally, LINC01224 facilitated cell proliferation, migration, invasion while suppressed cell apoptosis in TNBC by sponging miR-193a-5p and upregulating NUP210. These results revealed the positive regulatory role of LINC01224/miR-193a-5p/NUP210 axis in TNBC cells, providing a further understanding for TNBC pathogenesis. Moreover, future studies are needed to further investigate the role of LINC01224/miR-193a-5p/NUP210 axis and the potential molecular mechanisms underlying the aggressiveness and high metastasis of TNBC.

Additionally, it is imperative to find more target genes of LINC01224/miR-193a-5p axis, which may help to provide novel agents that contribute to the malignant behaviors of TNBC cells.

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Declarations

Conflict of interest The authors declare that no competing interests was involved in this study.

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