Tropical Journal of Pharmaceutical Research April 2023; 22 (4): 713-722
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i4.2

Original Research Article

Expression of LINC00461 in breast cancer cells and its modulatory effect on miR-607/SLCLA3 axis

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Sent for review: 1 November 2022 *Revised accepted: 27 March 2023*

Abstract

Purpose: *To investigate the role and mechanisms of action of long non-coding RNA (lncRNA) (LINC00461) in breast cancer.*
Methods: *Human breast cancer cell lines and normal mammary epithelial cell lines as well as their corresponding negative controls (NC) were cultured and co-transfected with Lipofectamine 3000. Expressions of LINC00461 and miR-607 were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR), while the SLC1A3 level was evaluated by western blot. The role of LINC00461 in cell proliferation, apoptosis, cycle arrest, glycolysis, and chemoresistance was determined by MTT, colony formation, flow cytometry, and ELISA assays.*
Results: *The LINC00461 level was overexpressed in breast cancer cell lines ($p < 0.05$). Knockdown of LINC00461 in MCF-7 cells inhibited cell viability ($p < 0.01$) and the degree of colony formation ($p < 0.001$), induced cell apoptosis ($p < 0.001$) and cycle arrest ($p < 0.01$), and suppressed glucose consumption ($p < 0.001$), lactate production ($p < 0.001$), LDHA activity ($p < 0.05$) and cisplatin sensitivity ($p < 0.05$). Overexpression of LINC00461 in MDA-MB-468 cells resulted in reverse outcomes. LINC00461 positively regulated the expression of SLC1A3 via miR-607 in breast cancer cells. It was mechanistically established that LINC00461 is bound to miR-607 and miR-607 bound to SLC1A3, and this was confirmed by luciferase assay.*
Conclusion: *LINC00461 induces cell proliferation, cycle arrest, glycolysis, and chemoresistance by modulating miR-607/SLC1A3 axis in breast cancer. The results lay the theoretical basis for monitoring and therapy of breast cancer.*

Keywords: *Breast cancer, LINC00461, miR-607, SLC1A3, Glycolysis, Cell cycle, Chemoresistance*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Breast cancer is the most prevalent malignancy, with approximately one-third of morbidity and one-quarter of mortality in females around the world [1]. In medical practice, interventions such as surgery, radiation, chemotherapy, and immunotherapy are candidates for the management of breast cancer [2]. Nevertheless, the incidence of breast cancer is still reported to be on the increase. It has been demonstrated that progress in breast cancer has stagnated, due to limited screening and treatment [1]. Therefore, further investment in carrier-targeted

diagnosis and treatment will significantly reduce the morbidity and mortality rates associated with breast cancer.

Evidence has revealed that long non-coding RNAs (lncRNAs) regulate the development of cancers, including breast cancers (cell proliferation, invasion, and apoptosis) [3], as well as the overall survival of patients with breast cancer [4]. For instance, lncRNA A1BG antisense RNA 1 (A1BG-AS1) enhances cell growth, mobility, and invasion, and inhibits apoptosis in breast cancer. The downregulation of lncRNA small nucleolar RNA host gene 1 (SNHG1) contributes to growth retardation, cell cycle redistribution, and migration suppression in breast cancer.

LncRNA small nucleolar RNA host gene 5 (SNHG5) facilitates the proliferation of glycolysis in breast cancer cells. LncRNA LINC00461 is located at an intergenic region of the human chromosome 5, between myocyte enhancer factor 2C (MEF2C) and transmembrane protein 161B (TMEM161B) [5], which is a crucial regulator in a variety of cancers. It enhances cell proliferation and invasion of non-small cell lung cancer. The downregulation of LINC00461 inhibits cell growth and promotes apoptosis in gastric cancer. An interference by LINC00461 promotes cell radiosensitivity of lung adenocarcinoma.

LINC00461 is upregulated in breast cancer, which is related to its proliferation, invasion, mobility, and docetaxel resistance [6,7]. However, its detailed cancer-promoting mechanism in breast cancer is not well understood. Thus, in this study, the roles of LINC00461 in cell proliferation, cell cycle, glycolysis, and cisplatin sensitivity were investigated. Furthermore, its corresponding mechanisms were determined in order to lay a theoretical foundation for the development of the diagnosis and treatment of breast cancer.

EXPERIMENTAL

Cell culture

Human breast cancer cell lines T47D (CL-0228), MCF-7 (CL-0149), MDA-MB-231 (CL-0150), and MDA-MB-468 (CL-0290B), as well as human normal mammary epithelial cell line MCF 10A (CL-0525), were bought from Procell (Wuhan, China). All cells were maintained in DMEM/F12 medium (PM150312, Procell), supplied with 10 % fetal bovine serum (FBS, 164210-500, Procell) and 1 % streptomycin-penicillin (P/S, PB180120, Procell) at 37 °C with 5 % CO_2.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA from the cells was extracted with TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) and reverse-transcription was executed with RevertAid™ First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The qRT-PCR was conducted on the A PIKORed 96 (Thermo Fisher Scientific, Waltham, MA, USA) with SYBR Green PCR Kit (Takara, Dalian, China). The expression was calculated with $2^{-\Delta\Delta Ct}$ method. The sequences of primers used in this study were listed in Table 1.

Cell transfection and treatment

Two small interfering RNAs (siRNA) for LINC00461- si LINC00461#1 and si LINC00461#2, LINC00461 mimics, miR-607 mimics, miR-607 inhibitor, as well as their corresponding negative controls (NC) were synthesized using GenePharma (Shanghai, China). The MDA-MB-468 and MCF-7 cells were sowed into 6-well plates and cultured at 37 °C with 5 % CO_2. When the cells reached 80 % confluency, siRNAs, mimics, inhibitors, or their corresponding NC were co-transfected with Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the operating manual. To analyze the role of LINC00461 in chemoresistance, transfected MDA-MB-468, and MCF-7 cells were treated with 1 μg/mL cis-platinum for 48 h, and then collected for the determination of MTT and colony formation assays.

MTT analysis

Following the transfection for 48 h, MDA-MB-468, and MCF-7 cells were enriched and plated into 96-well plates with a density of 5×10^4 cells/well in 5 % CO_2 at 37 °C.

Table 1: The sequences of primers

Gene	Forward (5'-3')	Reverse (5'-3')
LINC00461	GACATTTACGCCACAACCCACG	AGACAGACCCTCAGATTCCCCA
GAPDH	CAGCTAGCCGCATCTTCTTTT	GTGACCAGGCGCCCAATAC
miR-607	GTTCAAATCCAGATCTATAAC	TGGTGTCGTGGAGTCG
U6	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

After being cultured for 24 h, the cells were treated with 10 μL MTT solution (Sigma-Aldrich, St. Louis, MO, USA) for 4 h. The culture supernatant was abandoned, and then each well was provided with 100 μL dimethyl sulfoxide (DMSO) to dissolve the crystals. The absorbance was read at 570 nm by the microplate reader (Thermo Fisher Scientific, Waltham, MA, USA).

Colony formation

MDA-MB-468 and MCF-7 cells with a density of 1×10^6 cells/well were sowed in 6-well plates after they were transfected for 48 h. The cells were hatched in 5 % CO_2 at 37 °C for two weeks with replacement of the media every week. The clones were immobilized with 4 % paraformaldehyde (Solarbio) for 15 min and stained with 0.1 % crystal violet (Sigma-Aldrich, St. Louis, MO, USA) for 10 min. The colonies numbers were manually counted and imaged with a digital camera (Canon, Japan).

Flow cytometry

The apoptosis and cell cycle of MDA-MB-468 and MCF-7 cells were analyzed by the flow cytometry assay. The transfected MDA-MB-468 and MCF-7 cells with a density of 2.5×10^5 cells/well were sowed into 24-well plates and cultured at 37 °C with 5 % CO_2. The cells were collected, washed with phosphate buffer saline (PBS) (Solarbio), resuspended by 0.5 mL of bind buffer, and stained with 5 μL propidium iodide (PI) and 5 μL Annexin V/FITC (Thermo Fisher Scientific, Waltham, MA, USA) for 15 min at room temperature.

In addition, the transfected MDA-MB-468 and MCF-7 cells with a density of 1×10^6 cells/well were inoculated into 6-well plates and incubated at 37 °C with 5 % CO_2. The cells were collected, rinsed, and resuspended with 250 μL ice-cold PBS. They were subsequently immobilized with 750 μL ice-cold ethanol (100 %, Solarbio) for 8 h, and washed with PBS. Subsequently, these cells were hatched with 50 μg/mL RNase (Thermo Fisher Scientific, Waltham, MA, USA) and PI for 30 min. The cell apoptosis and cycle were determined on a FACScan flow cytometry with CellQuest software (BD Biosciences, NJ, USA).

Western blot

Transfected MDA-MB-468 and MCF-7 cells were treated with RIPA lysis buffer (Beyotime, Shanghai, China) to obtain the total proteins. Following quantification with BCA kit (Thermo Fisher Scientific, Waltham, MA, USA), 20 μg protein samples were dissolved with 10 % SDS-PAGE, and then electrically transferred onto PVDF membranes.

The membranes were sealed with 5 % skim milk (Anchor, Switzerland) for 1 h at room temperature, hatched with primary antibodies at 4 °C overnight, administered with corresponding secondary antibodies for 2 h at room temperature, and visualized with an ECL kit (Beyotime, Shanghai, China). The primary antibodies were anti-CyclinD1 (1:5000, ab226977), anti-p21 (1:3000, ab227443), anti-CDK4 (1:3000, ab137675), anti-SLC1A3 (1:1000, ab95302) and anti-β-actin (1:5000, ab8227) from Abcam (Cambridge, UK).

Determination of glycolysis level

Culture supernatants were harvested after the cells were transfected for 48 h to examine the levels of glucose, lactate, and lactate dehydrogenase A (LDHA) with a glucose assay kit (Sigma-Aldrich, St. Louis, MO, USA), lactate assay kit (Biovision, Mountain View, CA, USA) and Human LDHA ELISA Kit (Sangon Biotech, Shanghai, China), respectively, according to manufacturer's instructions. Lactate generation and glucose consumption were assessed by the ratio of glucose/lactate concentration in the treatment group and the control group.

Luciferase assay

The binding sites between LINC00461 and miR-607, as well as miR-607 and SLC1A3, were forecasted with miRDB online sites (http://www.mirdb.org/mirdb/index.html). Wild-type (WT) and mutant (MUT) LINC00461 or SLC1A3 were built into a pGL3-Basic luciferase vector (Promega, Madison, WI, USA), and then transfected into MDA-MB-468 and MCF-7. Both cells were subsequently co-transfected with miR-607 mimics or NC mimics (GenePharma) and with Lipofectamine 3000 (Invitrogen). Following transfection for 48 h, the luciferase activity was examined by Promega kit (Promega, Madison, WI, USA) based on the operating instructions.

Statistical analysis

Data are presented as mean ± standard error (SE), and statistical differences were determined by SPSS 22.0 (IBM, Armonk, New York, USA). Differences were tested by unpaired Student's *t*-test (for two groups) or one-way analysis of variance (ANOVA) with Dunnett's post hoc test (for three or more groups). $P < 0.05$ was regarded as statistically significant.

RESULTS

Knockdown of LINC00461 reduces breast cancer cell proliferation

The expression of LINC00461 in four human breast cancer cell lines, including T47D, MCF-7, MDA-MB-231, and MDA-MB-468 was first determined so as to discuss the role of LINC00461 in the development of breast cancer. As shown in Figure 1 A, compared with that in MCF 10 A cells, the level of LINC00461 in all four breast cancer cell lines was significantly increased, with the highest LINC00461 expression in MCF-7 cells, and the lowest in MDA-MB-468 cells. Hence, these two cell lines were selected for subsequent assays. Since LINC00461 level was highly expressed in MCF-7 cells, two siRNAs targeted to LINC00461, si LINC00461#1 and si LINC00461#2 were designed to downregulate the expression of LINC00461 in MCF-7 cells. Results from Figure 1 B showed that both two siRNAs targeted to LINC00461 significantly decreased the expression of LINC00461 in MCF-7 cells, suggesting a viable interference efficiency. Moreover, silencing LINC00461 with two siRNAs observably reduced the cell viability and numbers of colony formation (Figure 1 C and D), which indicated that the knockdown of LINC00461 in MCF-7 cells inhibited breast cancer cell proliferation. On the other hand, LINC00461 mimics were transfected into MDA-MB-468 cells in order to upregulate the level of LINC00461, owing to it being the lowest LINC00461 expression in MDA-MB-468 cells (Figure 1 E).

Overexpression of LINC00461 significantly enhanced the cell viability and numbers of colony formation in MDA-MB-468 cells (Figure 1 F and G), which demonstrated that the upregulation of LINC00461 in MDA-MB-468 cells promoted breast cancer cell proliferation. Thus, these results collectively expounded that the knockdown of LINC00461 suppressed breast cancer cell proliferation.

Downregulation of LINC00461 induces cell cycle arrest

Interference of LINC00461 with two siRNAs significantly enhanced the cell apoptosis and G0/G1 phase, while significantly impeding G2/M phase in MCF-7 cells (Figure 2 A and B). Meanwhile, the knockdown of LINC00461 with two siRNAs significantly declined the relative protein level of CyclinD1 and CDK4 but elevated the p21 protein expression (Figure 2 C). Furthermore, inverse results were observed in the cell apoptosis and cycle, as well as the relative protein expression of CyclinD1, p21, and CDK4 after MDA-MB-468 cells were introduced with LINC00461 mimics (Figure 2 D - F). Therefore, the silencing of LINC00461 evoked cell apoptosis and cycle arrest in breast cancer.

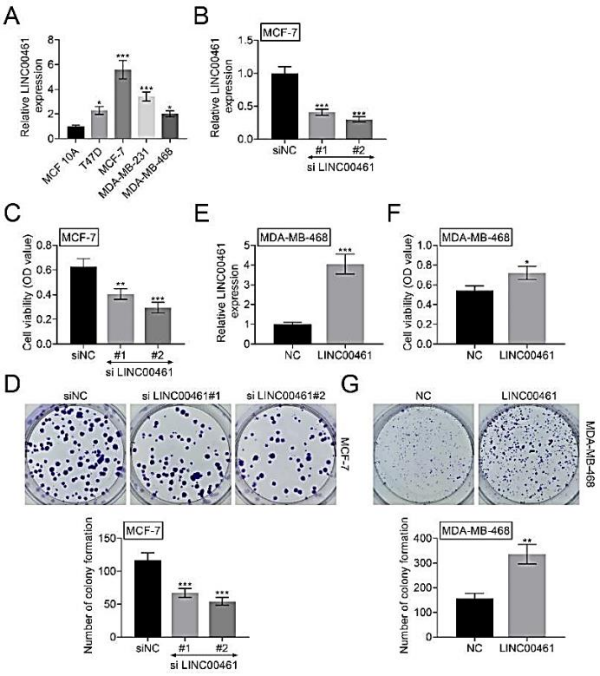

Figure 1: Downregulation of LINC00461 restrained breast cancer cell proliferation. (A): The expression of LINC00461 in four human breast cancer cell lines, including T47D, MCF-7, MDA-MB-231 and MDA-MB-468, as well as human normal mammary epithelial cell line MCF 10A. *P < 0.05 and ***p < 0.001 *vs.* MCF 10A; (B): The level of LINC00461 in MCF-7 cells transfected with si LINC00461#1 and si LINC00461#2. ***P < 0.001 *vs.* siNC; (C): The viability of MCF-7 cells transfected with si LINC00461#1 and si LINC00461#2. **P < 0.01 and ***p < 0.001 *vs.* siNC; (D): The numbers of colony formation counted after MCF-7 cells transfected with si LINC00461#1 and si LINC00461#2. ***P < 0.001 *vs.* siNC; (E): The level of LINC00461 in MDA-MB-468 cells transfected with LINC00461 mimics. ***P < 0.001 *vs.* NC; (F): The viability of MDA-MB-468 cells transfected with LINC00461 mimics. *P < 0.05 *vs.* NC; (G): The numbers of colony formation counted after MDA-MB-468 cells transfected with LINC00461 mimics. **P < 0.01 *vs.* NC

Interference of LINC00461 inhibits glycolysis and chemoresistance

Glucose consumption, lactate production, and LDHA activity were significantly diminished in MCF-7 cells transfected with two siRNAs, and targeted to LINC00461, while these were all significantly increased in MDA-MB-468 cells introduced with LINC00461 mimics (Figure 3 A - F). Meanwhile, the transfected MDA-MB-468 and

MCF-7 cells were treated with 1 μg/mL cisplatinum for 48 h to analyze the role of LINC00461 in chemoresistance. As shown in Figure 3 G - J, the knockdown of LINC00461 significantly reduced the cell viability and number of colony formations in transfected MCF-7 cells, while the overexpression of LINC00461 significantly increased the cell viability and number of colony formations in transfected MDA-MB-468 cells. Hence, the results suggested that the knockdown of LINC00461 repressed glycolysis and chemoresistance in breast cancer cells.

LINC00461 targets miR-607

One of the potential targets of LINC00461, miR-607 was predicted to bind with LINC00461 based on complementary base pairing through the miRDB online website (Figure 4 A). To further verify the direct binding between LINC00461 and miR-607, a luciferase assay was conducted in both MDA-MB-468 and MCF-7 cells. The relative fluorescence intensity was significantly reduced after both MDA-MB-468 and MCF-7 cells were co-transfected with LINC00461-WT and miR-607 mimics (Figure 4 B). The relative fluorescence intensity showed no statistical changes in both MDA-MB-468 and MCF-7 cells co-transfected with LINC00461-MUT and miR-607 mimics (Figure 4 B). Moreover, an increase in the expression of miR-607 was observed in both MDA-MB-468 and MCF-7 cells administrated with two siRNAs targeted to LINC00461, indicating a negative relation between LINC00461 and miR-607 (Figure 4 C). The results, therefore, clarified that LINC00461 directly targeted miR-607 breast cancer cells.

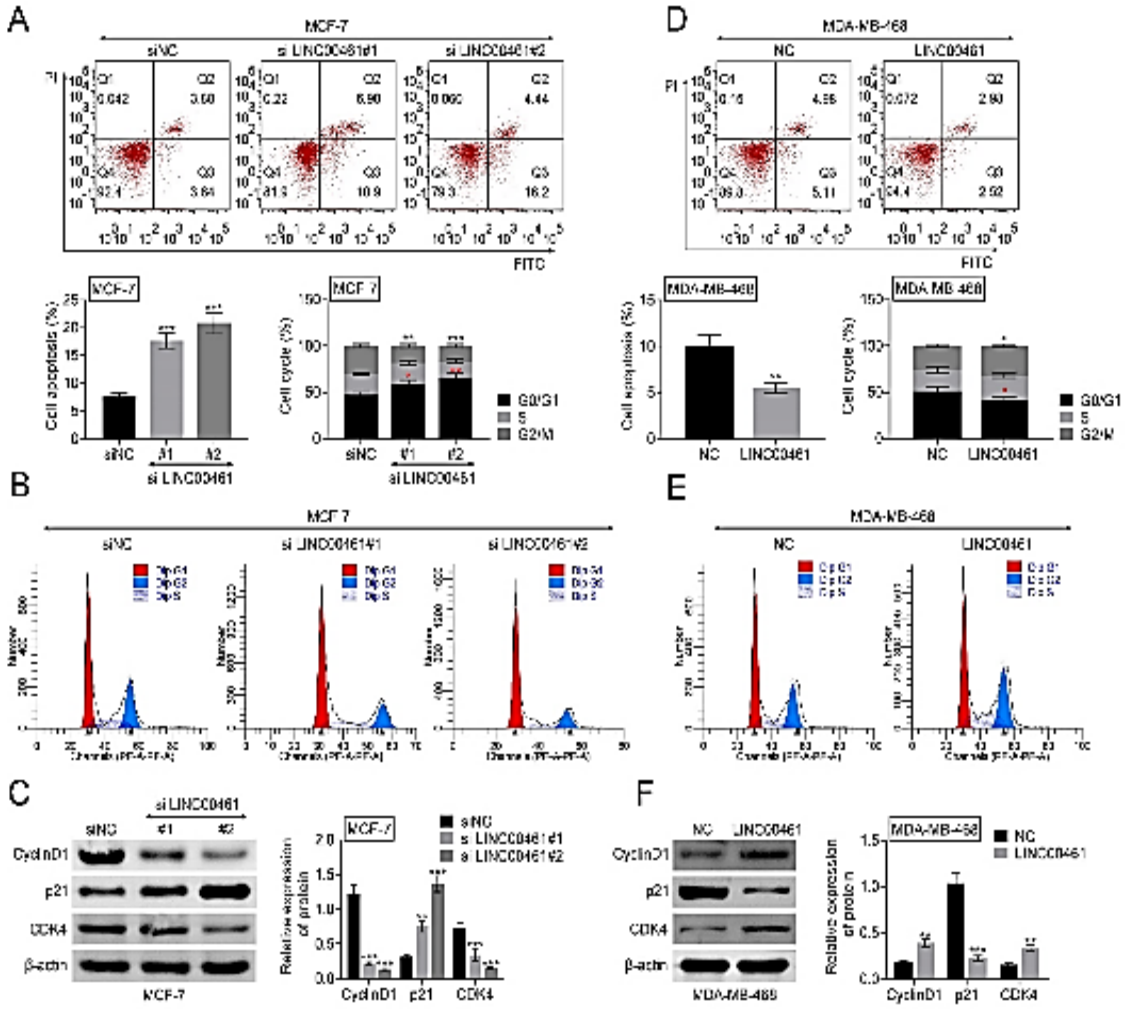

Figure 2: Knockdown of LINC00461 elicited cell apoptosis and cycle arrest in breast cancer. (A and B): Cell apoptosis and cycle after MCF-7 cells transfected with si LINC00461#1 and si LINC00461#2. *P < 0.05, **p < 0.01 and ***p < 0.001 *vs.* siNC; (C): Relative protein expression of CyclinD1, p21 and CDK4 of MCF-7 cells transfected with si LINC00461#1 and si LINC00461#2. **P < 0.01 and ***p < 0.001 *vs.* siNC; (D and E): Cell apoptosis and cycle after MDA-MB-468 cells treated with LINC00461 mimics. *P < 0.05 and **p < 0.01 *vs.* NC; (F): Relative protein expression of CyclinD1, p21 and CDK4 of MDA-MB-468 cells transfected with LINC00461 mimics. **P < 0.01 and ***p < 0.001 *vs.* NC

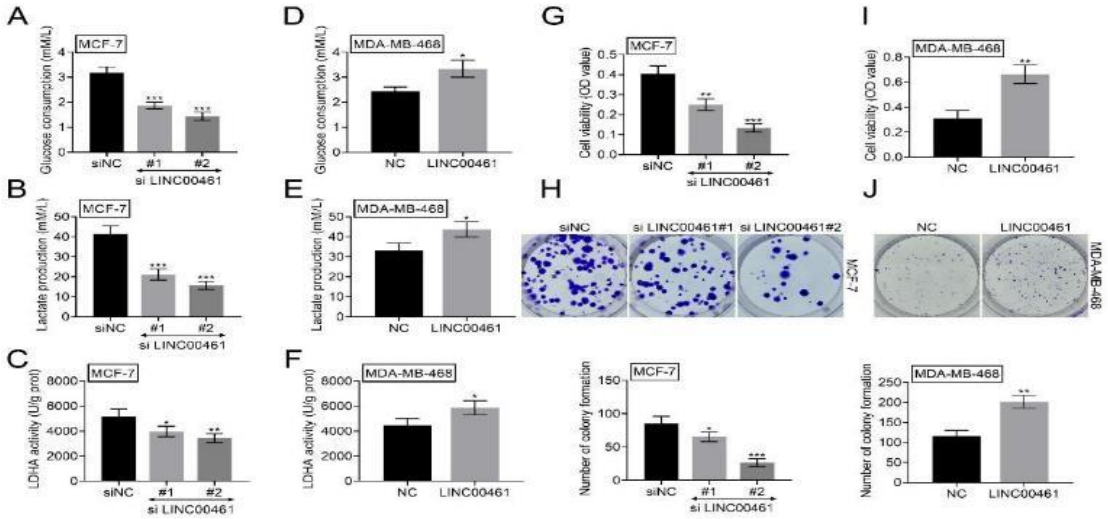

Figure 3: Silencing of LINC00461 suppressed glycolysis in breast cancer cells. (A-C): The glucose consumption, lactate production, and LDHA activity after MCF-7 cells were transfected with two siRNAs targeted to LINC00461. **P* < 0.05, ***p* < 0.01 and ****p* < 0.001 *vs.* siNC; (D-F): The glucose consumption, lactate production, and LDHA activity after MDA-MB-468 cells treated with LINC00461 mimics. **P* < 0.05 *vs.* NC; (G): The cell viability of transfected MCF-7 cells treated with cis-platinum. ***P* < 0.01 and ****p* < 0.001 *vs.* siNC; (H): The numbers of colony formation counted after the transfected MCF-7 cells treated with cis-platinum. **P* < 0.05 and ****p* < 0.001 *vs.* siNC; (I): The cell viability of transfected MDA-MB-468 cells treated with cis-platinum. ***P* < 0.01 *vs.* NC; (H): The numbers of colony formation counted after transfected MDA-MB-468 cells treated with cis-platinum. ***P* < 0.01 *vs.* NC

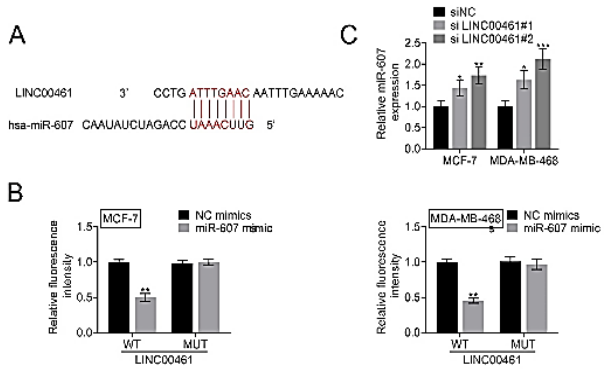

Figure 4: LINC00461 directly targeted miR-607 in breast cancer cells. (A): Binding between LINC00461 and miR-607 (B): Direct binding between LINC00461 and miR-607 in both MCF-7 and MDA-MB-468 cells. ***P* < 0.01 *vs.* NC mimics; (C): Expression of miR-607. **P* < 0.05, ***p* < 0.01 and ****p* < 0.001 *vs.* siNC

MiR-607 targets SLC1A3

Similarly, SLC1A3 was forecasted to bind with miR-607 based on complementary base pairing through the miRDB online website (Figure 5 A), which was confirmed in both MDA-MB-468 and MCF-7 cells by the luciferase assay (Figure 5 B). A positive relation between miR-607 and SLC1A3 was exhibited, as evidenced by the significantly decreased expression of SLC1A3 protein in both MDA-MB-468 and MCF-7 cells treated with miR-607 mimics, and the increased level of SLC1A3 protein in both MCF-7 and MDA-MB-468 cells treated with miR-607 inhibitors (Figure 5 C). Meanwhile, co-transfection of miR-607 inhibitors significantly rescued the si LINC00461-induced relative protein level of SLC1A3 in both MDA-MB-468 and MCF-7 cells (Figure 5 D), which suggested that LINC00461 positively regulated the expression of SLC1A3 via miR-607 in breast cancer cells. Thus, the data revealed that miR-607 targeted SLC1A3 in breast cancer cells.

LINC00461 affects breast cancer cell cycle, glycolysis, and sensitivity to cisplatin

To verify whether LINC00461 modulated breast cancer cell cycle, glycolysis, and sensitivity to cisplatin through miR-607/SLC1A3 axis, si LINC00461#2 and/or miR-607 inhibitor, as well as their corresponding NCs were transfected into MCF-7 cells. Results from Figure 6 A showed that the relative protein level of SLC1A3 was significantly decreased with the administration of si LINC00461#2, and enhanced with the administration of miR-607 inhibitor, which was reversed with the introduction of the combination of LINC00461#2 and miR-607 inhibitor. Co-transfection of si LINC00461#2 and miR-607 inhibitor inverted the si LINC00461#2- and induced the promotion of G0/G1 phase and the impediment of G2/M phase, as well as the miR-607 inhibitor induced the suppression of G0/G1 phase and the enhancement of G2/M phase (Figure 6 B and B').

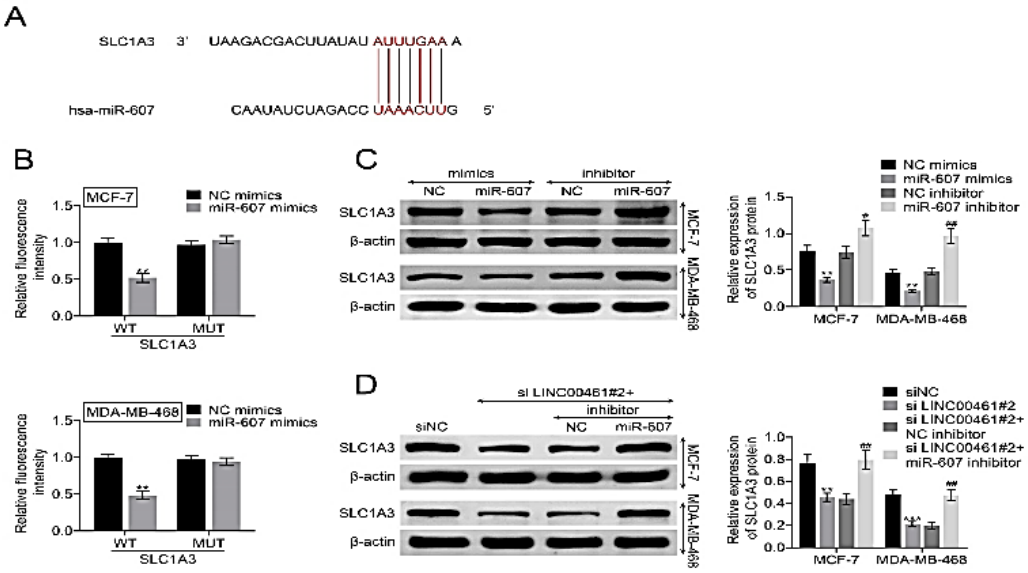

Figure 5: MiR-607 targeted SLC1A3 in breast cancer cells. (A); The bond between miR-607 and SLC1A3. (B): The direct bond between miR-607 and SLC1A3 in both MCF-7 and MDA-MB-468 cells. ***P* < 0.01 *vs.* NC mimics; (C): The relative protein expression of SLC1A3. ***P* < 0.01 *vs.* NC mimic; $^{\#}p$ < 0.05 and $^{\#\#}p$ < 0.01 *vs.* NC inhibitor. (D): The relative protein expression of SLC1A3. ***P* < 0.01 and ***p < 0.001 *vs.* siNC; $^{\#\#}p$ < 0.01 *vs.* si LINC00461#2 + NC inhibitor

The relative protein level of CyclinD1 and CDK4 was significantly diminished in MCF-7 cells transfected with si LINC00461#2 and enhanced in MCF-7 cells administrated with miR-607 inhibitor, which was both significantly antagonized with co-transfection of si LINC00461#2 and miR-607 inhibitor in MCF-7 cells (Figure 6 C). On the contrary, the opposite results were observed in the relative protein expression of p21 in MCF-7 cells (Figure 6 C). Moreover, the co-transfection of si LINC00461#2 and miR-607 inhibitor, neutralized the si LINC00461#2-induced reduction of the glucose consumption, lactate production, and LDHA activity, as well as the miR-607 inhibitor-induced elevation of the glucose consumption, lactate production, and LDHA activity in MCF-7 cells (Figure 6 D - F). Furthermore, similar results were observed in the cell viability in MCF-7 cells treated with cis-platinum (Figure 6 G). In total, LINC00461 induced cell proliferation, cycle arrest, glycolysis, and sensitivity to cisplatin by regulating miR-607/SLC1A3 axis in breast cancer.

DISCUSSION

Breast cancer is the most frequent malignant tumor in females around the world [1]. Thus, effective targets for the diagnosis and treatment are urgently needed for breast cancer. LncRNA LINC00461 has been identified as a candidate for the diagnosis and treatment of different cancers, such as gastric cancer and non-small cell lung cancer. Although LINC00461 involves the proliferation, invasion, migration, and docetaxel resistance of breast cancer [6,7], its detailed mechanisms need to be explored in breast cancer. Here, the data revealed that the LINC00461 level was upregulated in breast cancer cell lines. The knockdown of LINC00461 in MCF-7 cells inhibited the cell viability and numbers of colony formation, induced cell apoptosis and cycle arrest, and suppressed glucose consumption, lactate production, and LDHA activity. While the overexpression of LINC00461 in MDA-MB-468 cells resulted in the reverse outcomes. Furthermore, the knockdown of LINC00461 reduced the cell viability and number of colony formations in transfected MCF-7 cells treated with cis-platinum, while the overexpression of LINC00461 significantly increased these indices in transfected MDA-MB-468 cells treated with cis-platinum.

LINC00461 has been reported to be highly expressed in breast cancer, therefore, LINC00461 generally acts as a carcinogenic modulator through the regulation of the progress of breast cancer [8]. The high LINC00461 level involved in the TNM stage and differentiation enhances the migration and invasion of breast cancer, which is also verified from the findings by Zhang *et al* [6]. In addition, the level of LINC00461 is overexpressed in breast cancer cells and tissues, and this promotes the growth and migration, and docetaxel resistance of breast cancer [7].

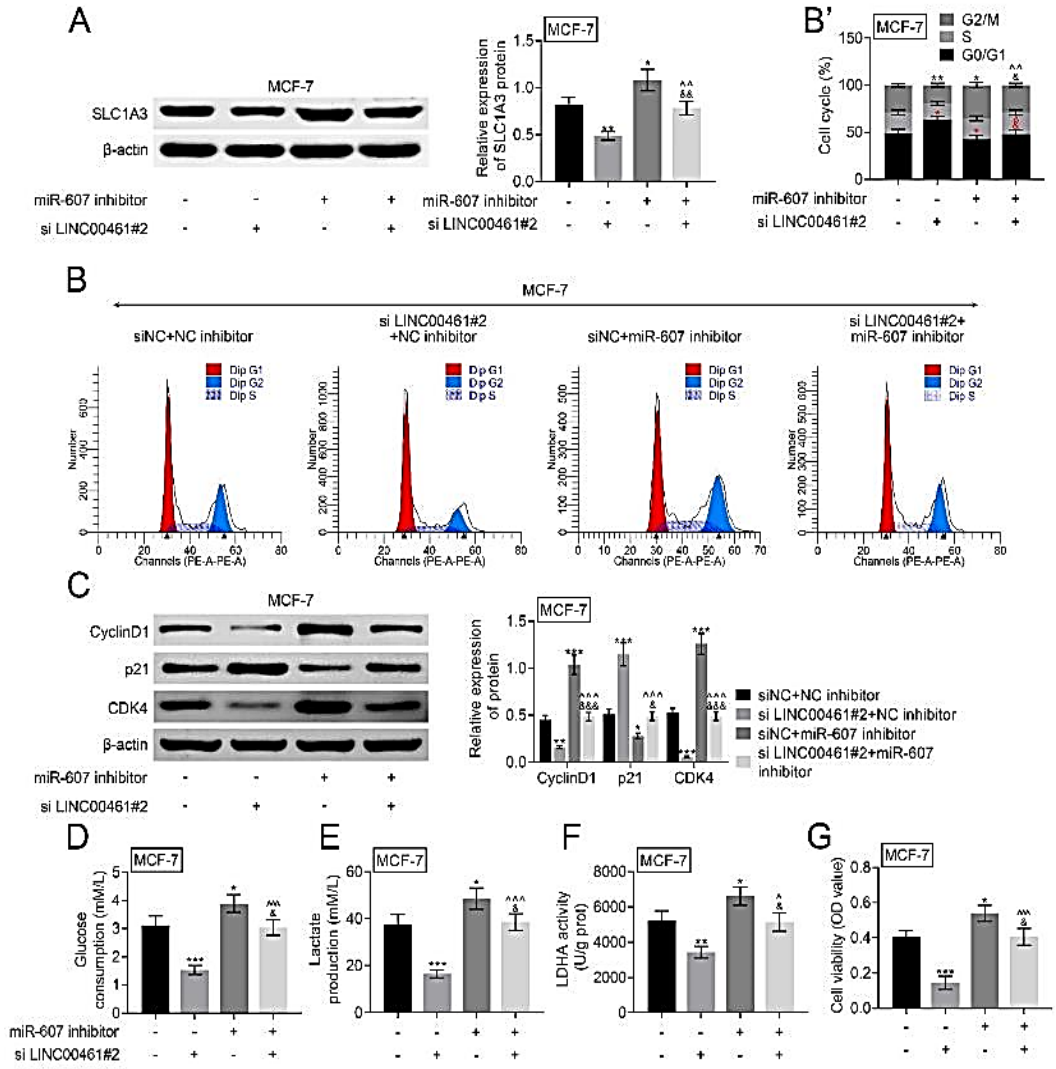

Figure 6: LINC00461 elicited cell cycle arrest and glycolysis by modulating miR-607/SLC1A3 axis in breast cancer. Si LINC00461#2 and/or miR-607 inhibitor, as well as their corresponding NCs, transfected into MCF-7 cells; (A): The relative protein expression of SLC1A3 (B): Cell cycle (C): The relative protein expression of CyclinD1, p21, and CDK4 (D-F): The glucose consumption, lactate production and LDHA activity (G): The cell viability. *P < 0.05, **p < 0.01 and ***p < 0.001 *vs.* siNC + NC inhibitor; $^{\&}p$ < 0.05, $^{\&\&}p$ < 0.01 and $^{\&\&\&}p$ < 0.001 *vs.* si LINC00461#2 + NC inhibitor; ^p < 0.05, ^^p < 0.01 and ^^^p < 0.001 *vs.* si NC+ miR-607 inhibitor

In the current study, the level of LINC00461 was consistently upregulated in four breast cancer cell lines. Also, both the knockdown and overexpression of LINC00461 confirmed that LINC00461 facilitated cell growth and declined cell apoptosis in breast cancer cells. Meanwhile, LINC00461 induced cell cycle arrest, accompanied by the enhanced expression of CyclinD1 and CDK4 protein, and the reduced level of p21 protein. Cyclin D1 that binds to CDK4 mainly contributes to the transformation of cells from G1 phase to S phase, as well as the process of S phase gene synthesis, thereby functioning on cell cycle progression. P21 is a cell cycle kinase inhibitory protein that can coordinate the relationship between cell cycle, DNA replication, and repair, by suppressing the activity of cyclin-dependent kinases (CDKs). Hence, both loss and gain-of-function assays illustrated that LINC00461 enhanced proliferation and cell cycle arrest, and suppressed apoptosis in breast cancer.

More evidence has demonstrated that abnormal glucose metabolism is a hallmark of tumors, in which superfluous glucose uptake for transformation into aerobic glycolysis leads to the generation of lactate, also designated as the Warburg effect. Significant number of the reports have identified the pivotal role of glycolysis in the development of breast cancer. The silencing of lncRNA SNHG7 reduces the expression of LDHA and glycolysis in breast cancer [9]. The

knockdown of lncRNA SNHG5 diminishes glucose consumption and lactate generation of breast cancer cells [10], and the stimulative role of lncRNA LINC00346 in glycolysis is also confirmed in breast cancer cells [11]. Thus, lncRNA has been summarized as a critical modulator of glycolysis in breast cancer. Here, the glucose consumption, lactate production, and LDHA activity were significantly decreased in MCF-7 cells introduced with two siRNAs targeted to LINC00461, while these were all significantly enhanced in MDA-MB-468 cells introduced with LINC00461 mimics, indicating that LINC00461 enhanced glycolysis in breast cancer cells. Additionally, the knockdown of LINC00461 reduced the cell viability and number of colony formations in transfected MCF-7 cells treated with cis-platinum, while the overexpression of LINC00461 significantly increased these indexes in transfected MDA-MB-468 cells treated with cis-platinum. Qu *et al* [12] reports that LINC00461 through the miR-593-5p/CCND1 axis regulates cisplatin resistance in rectal cancer. Guan *et al* [13] showed that LINC00461 decreases cisplatin chemosensitivity via the miR-195/HOXA10 axis in head and neck squamous cell carcinoma. Taken together, LINC00461 was essential for maintaining glycolysis and chemoresistance in breast cancer cells.

Mechanistically, LINC00461 enhanced the proliferation, cell cycle arrest, glycolysis and chemoresistance by regulating the miR-607/SLC1A3 axis in breast cancer. LncRNA can always sponge to miRNAs to competitively restrain their function, thus regulating the expression level of miRNA target genes. It was observed that LINC00461 bound to miR-607 and miR-607 bound to SLC1A3, and this was confirmed by the luciferase assay. MiRNAs has been shown to participate in the progression of breast cancer [14]. MiR-607 has been reported to be lowly expressed in various tumors, such as lung cancer, pancreatic ductal adenocarcinoma, and chronic lymphocytic leukemia. More importantly, it has been revealed that miR-607 can be sponged by different lncRNA to modulate the progression of tumors, including lncRNA LINC00115 sponging miR-607 in lung cancer [15], and lncRNA LINC01559 sponging miR-607 in pancreatic cancer [16]. SLC1A3 (solute carrier family 1 member 3) belongs to the high-affinity glutamate transporter family that associates with the progression and metabolism of tumors. In breast cancer, SLC1A3 is identified to be involved in drug resistance [17].

Here, it was shown that LINC00461 positively modulated the expression of SLC1A3 via miR-607 in breast cancer cells. Moreover, the promoted role of LINC00461 in the growth, cell cycle arrest, glycolysis and chemoresistance of breast cancer cells was verified to be through the modulation of the miR-607/SLC1A3 axis. Therefore, the data elaborated that LINC00461 enhanced the proliferation, cell cycle arrest, glycolysis and chemoresistance by regulating miR-607/SLC1A3 axis in breast cancer.

Limitations of this study

Multiple methods may be needed to confirm the conviction of the results, such as RNA pull down and RNA immunoprecipitation assay, in order to confirm the binding between LINC00461 and miR-607, as well as between miR-607 and SLC1A3. Secondly, the role of LINC00461 in chemoresistance will need to be further expounded in drugs sensitive breast cancer cells.

CONCLUSION

The LINC00461 level is overexpressed in breast cancer. The silencing of LINC00461 in MCF-7 cells suppresses proliferation, cell cycle arrest, glycolysis and chemoresistance, while the overexpression of LINC00461 in MDA-MB-468 cells results in reverse outcomes. Mechanistically, LINC00461 induces proliferation, cell cycle arrest, glycolysis and chemoresistance by regulating miR-607/SLC1A3 axis in breast cancer. Although *in vivo* verification will be an inevitable choice for subsequent studies, the results lay the theoretical basis for monitoring and therapy of breast cancer.

DECLARATIONS

Acknowledgements

None provided.

Funding

This work was supported by Natural Science Foundation of Xinjiang Uygur Autonomous Region (Grant no. 2022D01C531).

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the

current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Dan Li designed the experiments; Dan Li, Shuangjian Li, Qian Zhao, Dilixiati Jinsihan and Jinchun Feng carried them out, analyzed and interpreted the data, Dan Li prepared the manuscript. All authors read and approved the manuscript for publication.

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