Life Sciences 228 (2019) 135–144

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

LINC00511 knockdown enhances paclitaxel cytotoxicity in breast cancer via regulating miR-29c/CDK6 axis

Hualong Zhang[a], Bin Zhao[a,*], Xiuxia Wang[b], Fan Zhang[a], Wenlong Yu[a]

[a] Department of Breast and Thyroid Surgery, Shanxian Central Hospital, Heze 274300, China
[b] Department of Gynecology, Shanxian Central Hospital, Heze 274300, China

ARTICLE INFO

Keywords:
LINC00511
miR-29c
CDK6
Paclitaxel cytotoxicity
Breast cancer

ABSTRACT

Aims: Drug resistance is becoming a major clinical challenge to the success of breast cancer treatment. Compelling evidence has shown the association between the deregulated long non-coding RNAs (lncRNAs) and drug resistance in various malignancies. However, the effects of long intergenic noncoding RNA 00511 (LINC00511), a newly identified oncogenic lncRNA, on the drug resistance of breast cancer cells remain unknown.

Main methods: RT-qPCR was performed to detect the expressions of LINC00511, miR-29c, and cyclin dependent kinase 6 (CDK6) in breast cancer tissues and cells. Pearson correlation analysis was used to analyze the correlation between miR-29c, CDK6 and LINC00511 expression in breast cancer tissues. The interactions between LINC00511, CDK6 and miR-29c were explored by luciferase reporter assay, RT-qPCR and western blot. MTT assay and flow cytometry analysis were applied to evaluate paclitaxel cytotoxicity.

Key findings: LINC00511 and CDK6 were upregulated while miR-29c was downregulated in breast cancer tissues and cells. miR-29c was negatively correlated with LINC00511 and CDK6 expression while LINC00511 was positively correlated with CDK6 expression in breast cancer tissues. LINC0051 directly interacted with miR-29c to suppress its expression. LINC00511 knockdown enhanced paclitaxel cytotoxicity in breast cancer cells by upregulating miR-29c. CDK6 was identified as a target of miR-29c. CDK6 knockdown attenuated the effects of miR-29c inhibition on paclitaxel cytotoxicity in breast cancer cells. LINC00511 positively regulated CDK6 expression in breast cancer cells.

Significance: LINC00511 knockdown enhanced paclitaxel cytotoxicity in breast cancer cells via regulating miR-29c/CDK6 axis.

1. Introduction

Breast cancer remains one of the most frequently diagnosed gynecological malignancies, currently accounting for 29% of all new cancer cases, and is the second leading cause of cancer-associated mortality in females all over the world [1,2]. According to statistics in 2018, there were an estimated 266,120 new diagnosed cases of breast cancer and 40,920 patients eventually succumb to breast cancer in USA [3]. In addition to surgical and radiation therapies, conventional chemotherapy is currently the major anticancer therapy for breast cancer patients [4]. Paclitaxel, a microtubule-stabilizing agent, is an effective first-line chemotherapeutic agent commonly used in the treatment of breast cancer [5]. However, its efficacy is severely limited due to the emergence of intrinsic or acquired drug resistance [6]. Drug resistance is becoming a major clinical challenge to the success of breast cancer treatment [7]. Therefore, elucidating the underlying mechanism of drug resistance in breast cancer is desperately needed to develop new therapeutic interventions for reversing the resistance to the existing drugs.

Recently, high-throughput transcriptome analysis shows that protein-coding genes make up only about 2% of the human genome, while the vast majority of the human genome encodes large amounts of non-coding RNAs (ncRNAs), including microRNAs (miRNAs) and long ncRNAs (lncRNAs) [8]. miRNAs are a class of small endogenous ncRNAs that consist of typically 18–22 nucleotides in length. The crucial roles of miRNAs in drug resistance of human malignancies including breast cancer have been extensively studied [9]. LncRNAs are a set of newly identified ncRNAs longer than 200 nucleotides and control various physiological or pathological processes, including cell proliferation, differentiation, apoptosis, and migration [10]. Increasing

* Corresponding author at: Department of Breast and Thyroid Surgery, Shanxian Central Hospital, No.1 Wenhua Road, Heze 274300, China.
E-mail address: zhaobin_doctor@126.com (B. Zhao).

https://doi.org/10.1016/j.lfs.2019.04.063
Received 15 February 2019; Received in revised form 12 April 2019; Accepted 28 April 2019
Available online 07 May 2019
0024-3205/

Table 1
Clinicopathologic characteristics of patients.

Characteristics		Case no.	%
Age (years)	≥50	12	57.1%
	< 50	9	42.9%
Tumor size (cm)	≥2	13	61.9%
	< 2	8	38.1%
TNM stage	I–II	13	61.9%
	III–IV	8	38.1%
Tumor grade	I	7	33.3%
	II	9	42.9%
	III	5	23.8%
Subtype	Luminal A	6	28.6%
	Luminal B	10	47.6%
	HER-2 positive	2	9.5%
	Triple negative	3	14.3%

evidence has indicated that lncRNAs are abnormally expressed in almost all malignant human tumors and serve as oncogenes or tumor suppressors [11]. Moreover, compelling evidence has shown the association between the aberrantly expressed lncRNAs and drug resistance in various malignancies [12]. Long intergenic noncoding RNA 00511 (LINC00511), located onto chromosome 17q24.3, is a newly identified cancerogenic lncRNA that is highly expressed in non-small-cell lung cancer [9], tongue squamous cell carcinoma [13], and breast cancer [14]. However, the effects of LINC00511 on the drug resistance of breast cancer cells remain unknown.

Recently, a novel lncRNA-miRNA-mRNA regulatory mechanism has been proposed that lncRNAs behave as competing endogenous RNAs (ceRNAs) to suppress the expression and biological functions of miRNAs, thereby resulting in the derepression of miRNA targets [15]. According to our bioinformatics analysis data, we found that

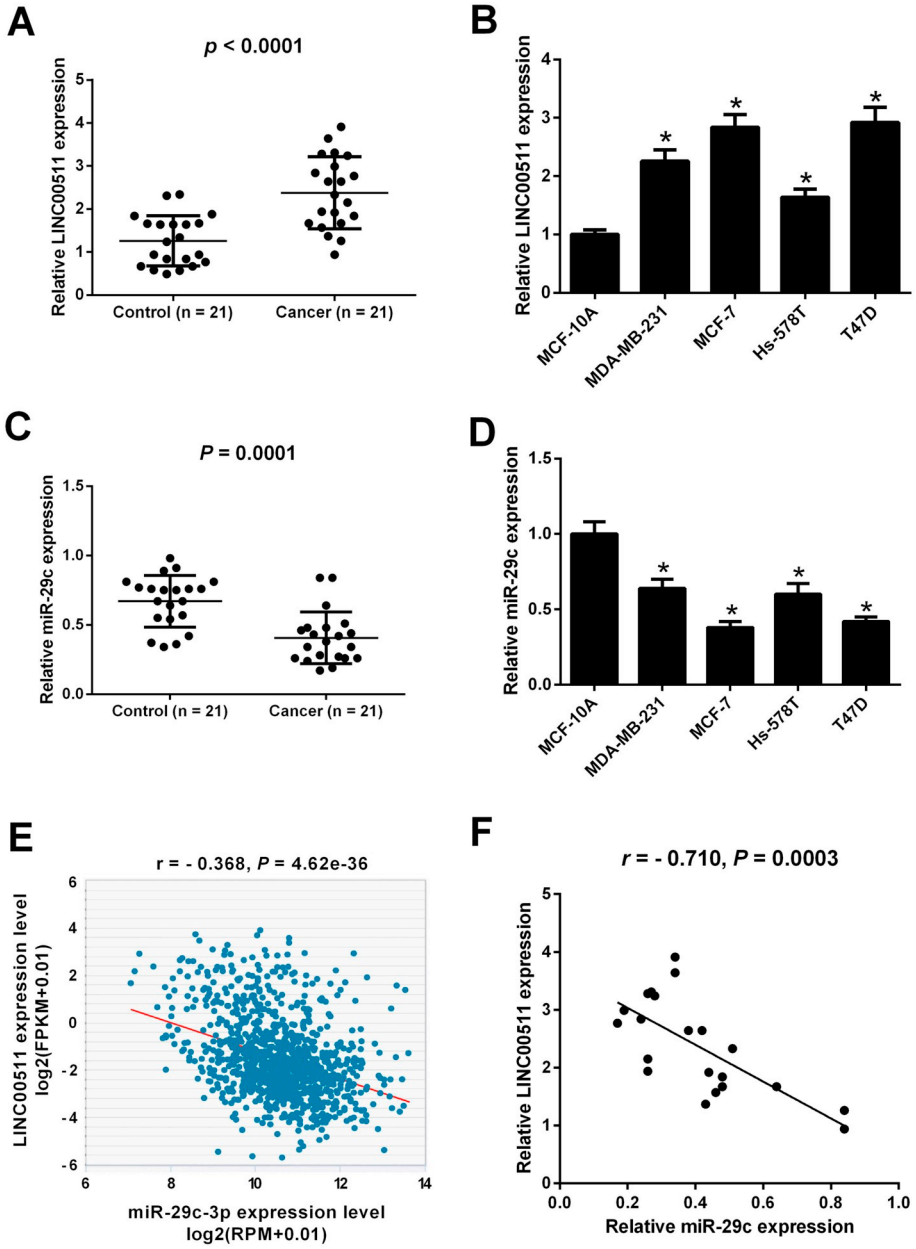

Fig. 1. The expression profiles of LINC00511 and miR-29c in breast cancer tissues and cells. RT-qPCR analysis of LINC00511 (A and B) and miR-29c (C and D) in 21 paired breast cancer tissues and adjacent normal tissues, as well as breast cancer cells (MDA-MB-231, MCF-7, Hs-578T, and T47D) and the immortalized breast epithelial cell line MCF-10A. (E) Analysis of The Cancer Genome Atlas (TCGA) dataset from starBase of the correlation between LINC00511 and miR-29c expression in 1085 breast cancer samples. (F) Pearson correlation analysis of the correlation between LINC00511 and miR-29c expression in 21 breast cancer tissues. *$P < 0.05$.

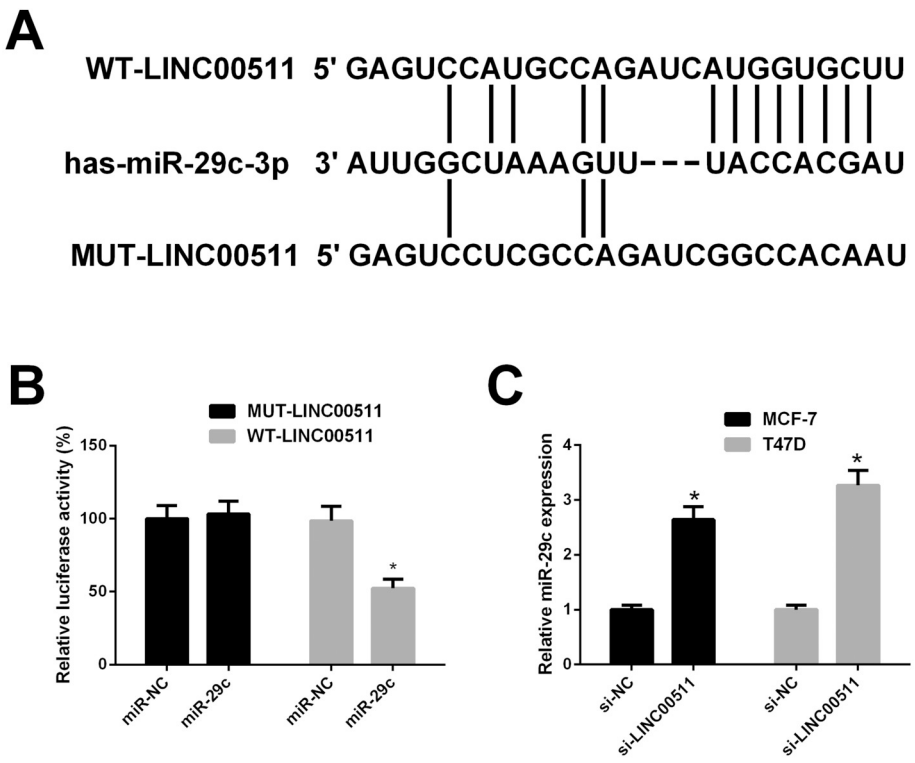

Fig. 2. The interaction between LINC00511 and miR-29c in breast cancer cells. (A) The predicted potential binding sites between LINC00511 and miR-29c. (B) MCF-7 cells were cotransfected with WT-LINC00511 or MUT-LINC00511 and miR-29c or miR-NC and luciferase reporter assay was applied to measure the luciferase activity at 48 h after transfection. (C) miR-29c expression in si-LINC00511- or si-NC-transfected MCF-7 and T47D cells was detected by RT-qPCR. $^{*}P < 0.05$.

LINC00511 contained the binding sites pairing with the seed region of miR-29c. miR-29c, a member of the miR-29 family, has been reported to be downregulated in multiple types of tumors, such as hepatocellular carcinoma [16] and colorectal cancer [17]. Notably, it was previously reported that miR-29c was downregulated in breast cancer tissues and played a tumor suppressive role in the progression of breast cancer [18]. Therefore, we supposed that LINC00511 and miR-29c were involved in the ceRNA network in breast cancer.

In the present study, we aimed to determine the role of LINC00511 in paclitaxel cytotoxicity in breast cancer and the underlying molecular mechanism.

2. Materials and methods

2.1. Patients and tissue samples

Breast cancer tissues and their corresponding adjacent non-tumor specimens were collected from 21 patients who were histopathologically diagnosed as breast cancer and underwent radical surgery at Shanxian Central Hospital. The clinicopathologic characteristics of patients are presented in Table 1. These surgical tissue samples were snap-frozen in liquid nitrogen immediately and store in a −80 °C refrigerator before total RNA extraction. The clinical research was carried out with the approval of the Ethics Committee of Shanxian Central Hospital, and the written informed consent was obtained from all participants before the start of the study.

2.2. Cell culture and transfection

The breast cancer cell lines (MDA-MB-231, MCF-7, Hs-578T, and T47D) and the immortalized breast epithelial cell line MCF-10A were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). All cells were maintained in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; GE Healthcare, Logan, UT, USA), and 100 U/ml penicillin/streptomycin (Sigma-Aldrich, St. Louis, MO, USA) in a humidified incubator with 5% CO_2/95% air at 37 °C. miR-29c mimics (miR-29c), mimic negative control (miR-NC), miR-29c inhibitor (anti-miR-29c), inhibitor negative control (anti-miR-NC), small interfering RNA specifically targeting LINC00511 (si-LINC00511), siRNA specifically targeting cyclin dependent kinase 6 (CDK6) (si-CDK6) and the scramble control (si-NC) were purchased from RiboBio (Guangzhou, China). Transient transfection with these oligonucleotides into MCF-7 and T47D cells were conducted using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

2.3. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

Cell viability was evaluated using MTT colorimetric kit (Dojindo, Tokyo, Japan). In brief, MCF-7 and T47D cells in the logarithmic phase of growth were plated into 96-well plates at 3×10^4 cells/well and cotransfected with si-LINC00511, si-CDK6 or si-NC and anti-miR-NC or anti-miR-29c, followed by treatment with 50 nM paclitaxel (Sigma-Aldrich) for 48 h. Then, 20 μl of MTT reagent (5 mg/ml; Sigma-Aldrich) was added to each well, followed by cultivation for another 4 h at 37 °C. After the removal of supernatant medium, 150 μl dimethyl sulfoxide (DMSO) was supplemented to dissolve the generated formazan. Finally, the absorbance at a wavelength of 490 nm was recorded using a microplate reader (Molecular Devices Corp., Sunnyvale, CA, USA). The experiments were independently repeated three times in triplicate.

2.4. Apoptosis assay by flow cytometry analysis

Cell apoptosis was assessed by Annexin V-FITC Apoptosis Detection Kit (BD Biosciences, Franklin Lakes, NJ, US) according to the manufacturer's protocols. After treatment as above, MCF-7 and T47D cells were collected by centrifugation and washed twice with ice-cold PBS. The cells were then resuspended in 200 μl binding buffer containing Annexin V-FITC and propidium iodide (PI) and incubated for 15 min in the dark at room temperature. The stained cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences) equipped with CellQuest software (BD Biosciences). The annexin V and PI double-negative cells were identified as live cells, whereas the annexin V and PI double-

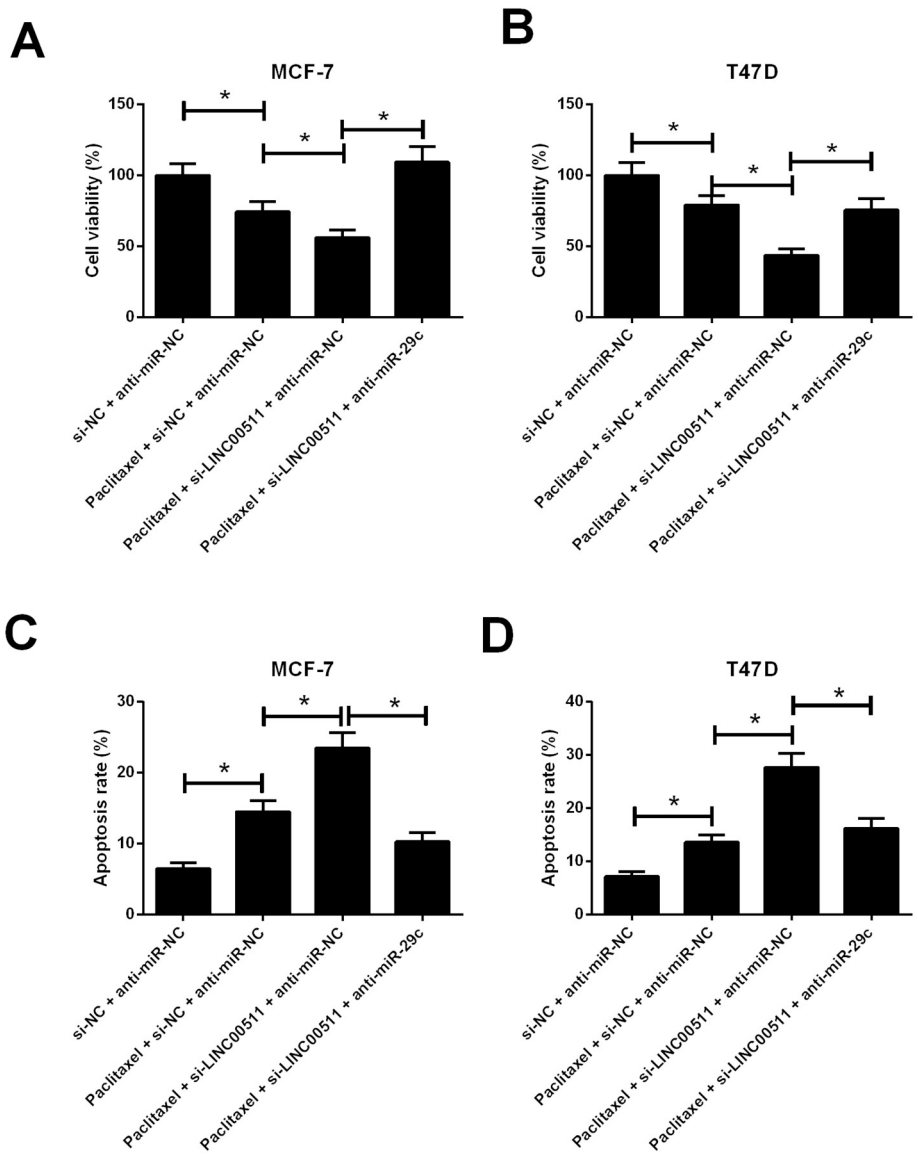

Fig. 3. Effects of LINC00511 or combined with miR-29c on the paclitaxel cytotoxicity of breast cancer cells. MCF-7 and T47D cells were cotransfected with si-LINC00511 or si-NC and anti-miR-NC or anti-miR-29c, followed by treatment with 50 nM paclitaxel for 48 h. (A and B) MTT assay was performed to determine cell viability in the treated MCF-7 and T47D cells. (C and D) Flow cytometry analysis was conducted to evaluate apoptosis of the treated MCF-7 and T47D cells. $^{*}P < 0.05$.

positive cells were recognized as late apoptotic cells. The annexin V-positive and PI-negative cells were recognized as early apoptotic cells, and the annexin V-negative and PI-positive cells were recognized as necrotic cells. The experiments were independently repeated three times.

2.5. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis

Total RNA from collected tissues and cells was isolated using RNAiso Plus (Takara, Tokyo, Japan) and RNA concentration was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). For the detection of LINC00511 and CDK6 mRNA expressions, 1 μg of total RNA was reversely transcribed into first-strand cDNA using a PrimeScript RT Reagent Kit (Takara) and qPCR were then performed using the SYBR Green PCR kits (Roche, Mannheim, Germany), with GAPDH as an internal control. For the detection of miR-29c expression, cDNA was synthesized from 1 μg of total RNA using miRNA cDNA synthesis kit (CWBIO, Beijing, China) and TaqMan miRNA assays (Applied Biosystems, Carlsbad, CA, USA) was used to examine miR-29c expression, with U6 small nuclear RNA (snRNA) as an endogenous control. All PCR reactions were conducted on a CFX96 real-time PCR System (Bio-Rad, Hercules, CA, USA). Relative gene expression was calculated by $2^{-\Delta\Delta Ct}$ method. The primers used are as follows: LINC00511, 5′-AAA GGA AGA AAT GAC CGA GGG-3′ (forward), 5′-GAG TCC TCA TGC CTA TAA TCA CG-3′ (reverse); CDK6 mRNA, 5′-TGG AGA CCT TCG AGC ACC-3′ (forward), 5’-CAC TCC AGG CTC TGG AAC TT-3′ (reverse); GAPDH, 5′-GAA GGT GAA GGT CGG AGT C-3′ (forward), 5′-GAA GAT GGT GAT GGG ATT TC-3′ (reverse); miR-29c, 5′-TGA CCG ATT TCT CCT GG-3′ (forward), 5′-GTG CAG GGT CCG AGG T-3′ (reverse); U6, 5′-TGC GGG TGC TCG CTT CGG CAG C-3′ (forward), 5′-CCA GTG CAG GGT CCG AGG T-3′ (reverse). The experiments were independently repeated three times.

2.6. Western blot analysis

Protein was extracted from cultured cells using ice-cold RIPA lysate

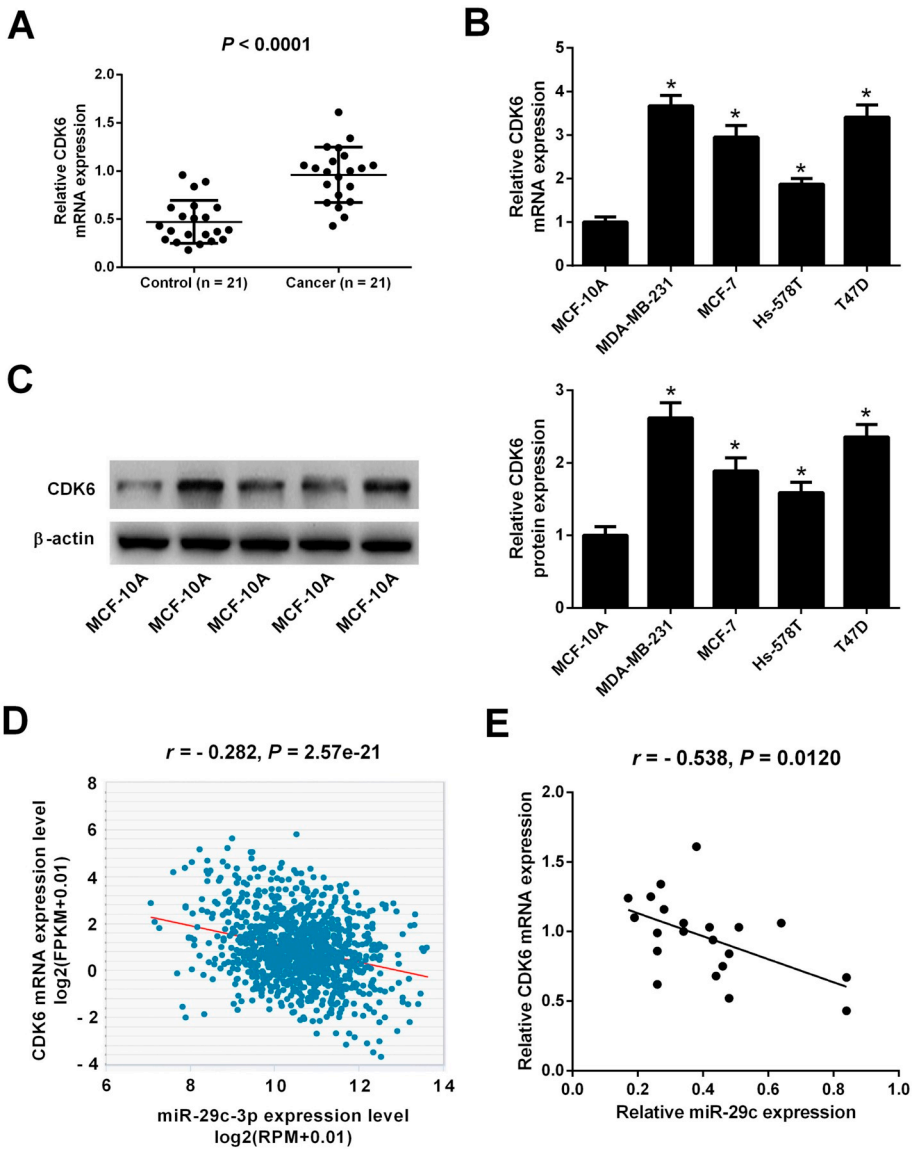

Fig. 4. The expression of CDK6 in breast cancer tissues and cells. (A) RT-qPCR analysis of CDK6 mRNA expression in 21 paired breast cancer tissues and adjacent normal tissues. (B and C) RT-qPCR and western blot analysis of CDK6 expression at mRNA and protein levels in breast cancer cells (MDA-MB-231, MCF-7, Hs-578T, and T47D) and the immortalized breast epithelial cell line MCF-10A. (D) The correlation between miR-29c and CDK6 mRNA expression in 1085 breast cancer samples from TCGA dataset from starBase. (E) Pearson correlation analysis of the correlation between miR-29c and CDK6 mRNA expressions in 21 breast cancer tissues. *$P < 0.05$.

buffer (Beyotime, Shanghai, China) supplemented with protease inhibitor cocktail (Sigma-Aldrich). The protein concentrations were then determined by the BCA protein assay kit (Beyotime). Equal amounts of protein lysates were subjected to 12% SDS-PAGE gels, and then electrophoretically transferred onto nitrocellulose membranes (Millipore, Billerica, MA, USA). After that, the membranes were blocked with 5% non-fat dried milk in Tris-buffered saline with 0.1% Tween (TBST) for 1 h at room temperature, followed by incubation with primary antibodies against CDK6 (Abcam, Cambridge, UK) and β-actin (Abcam) at 4 °C overnight. After being washed with TBST for three times, the membranes were incubated with horseradish peroxidase (HRP)-tagged secondary antibody (Abcam) for 2 h. Finally, the protein bands were detected using a chemiluminescence detection kit (Amersham Pharmacia Biotech, Piscataway, NJ). The experiments were independently repeated three times.

2.7. Luciferase reporter assay

The fragments of LINC00511 containing the binding sites of miR-29c were prepared by PCR amplification and subcloned into a pGL3 basic vector (Promega, Madison, WI, USA) to produce WT-LINC00511. The mutations of the miR-29c binding sites within the LINC00511 luciferase reporter vector (MUT-LINC00511) were generated using the QuikChange site-directed mutagenesis kit (Agilent Technologies, Inc., Santa Clara, CA, USA). Similarly, the luciferase reporter constructs incorporating the wild-type (WT) or mutated (MUT) CDK6 3′UTR fragments within the binding sites of miR-29c were constructed, namely WT-CDK6 and MUT-CDK6, respectively. MCF-7 cells were grown in triplicate in 24-well plates and cotransfected with 100 ng constructed luciferase reporter plasmids and 200 ng miR-29c or miR-NC using Lipofectamine™ 2000 (Invitrogen). At 48 h post-transfection, Renilla and firefly luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega). The experiments were independently repeated three times.

2.8. Statistical analysis

All experimental data are displayed as mean ± standard deviation (SD). Statistical analysis was carried out using GraphPad Prism 5 (GraphPad Software, San Diego, CA, USA). Student's *t*-test and one-way analysis of variance (ANOVA) were used to determine the differences between two or multiple groups, and a P value < 0.05 was considered to be statistically significant.

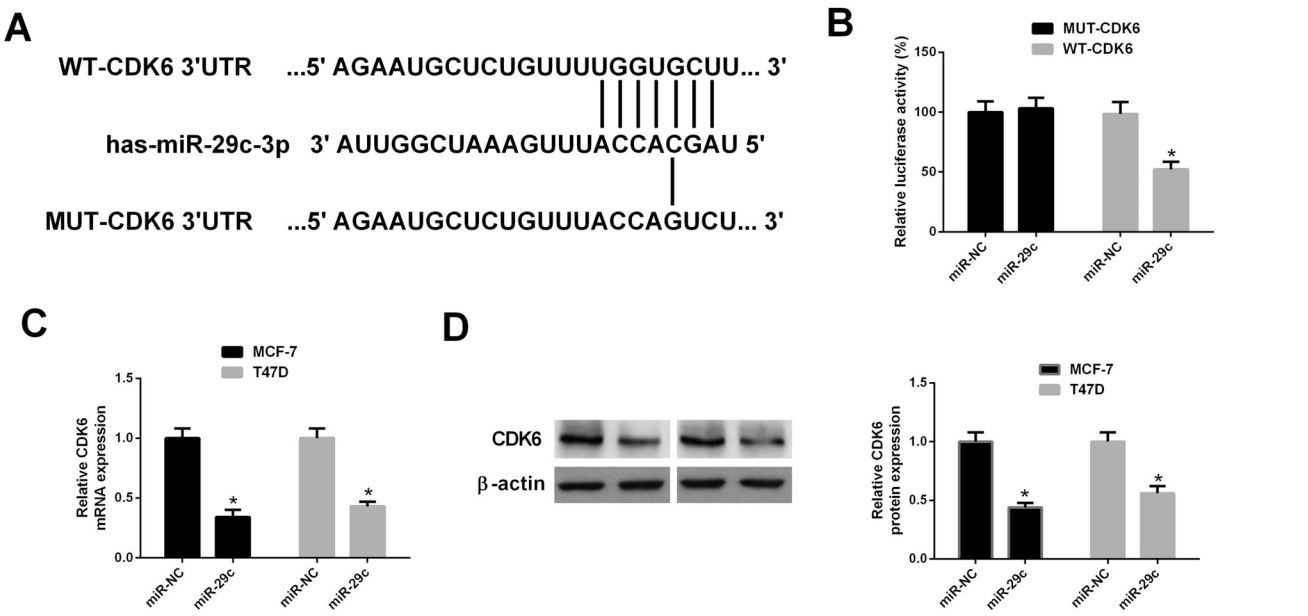

Fig. 5. The interaction between CDK6 and miR-29c in breast cancer cells. (A) Schematic of the predicted miR-29c binding sites in the 3′UTR of CDK6. (B) MCF-7 cells were cotransfected with WT-CDK6 or MUT-CDK6 and miR-29c or miR-NC. At 48 h post-transfection, luciferase activity was measured using luciferase reporter assay. (C and D) The mRNA and protein expressions of CDK6 were examined by RT-qPCR and western blot, respectively, in MCF-7 and T47D cells transfected with miR-29c or miR-NC for 48 h. *$P < 0.05$.

3. Results

3.1. LINC00511 was upregulated while miR-29c was downregulated in breast cancer tissues and cells

To explore the significance of LINC00511 and miR-29c in breast cancer, we initially detected the expressions of LINC00511 and miR-29c in 21 paired breast cancer tissues and adjacent normal tissues by RT-qPCR. As displayed in Fig. 1A and C, LINC00511 expression was upregulated while miR-29c expression was downregulated in breast cancer tissues as compared with that in corresponding adjacent normal tissues. We also examined the expressions of LINC00511 and miR-29c in breast cancer cell lines and the RT-qPCR results demonstrated that LINC00511 expression was higher (Fig. 1B) and miR-29c expression was lower (Fig. 1D) in MDA-MB-231, MCF-7, Hs-578T, and T47D breast cancer cells than the immortalized breast epithelial cell line MCF-10A, in line with the results from breast cancer tissues. The Cancer Genome Atlas (TCGA) dataset from starBase showed an inverse correlation between LINC00511 and miR-29c expression in 1085 breast cancer samples (Fig. 1E). Besides, Pearson correlation analysis suggested that LINC00511 was also negatively correlated with miR-29c expression in the 21 breast cancer tissues (Fig. 1F).

3.2. miR-29c was a target of LINC00511 in breast cancer cells

To determine the relationship between LINC00511 and miR-29c, the bioinformatic database starBase was used to predict the potential miRNAs that can be regulated by LINC00511 and we found that LINC00511 contained binding sequences complementary to miR-29c seed regions, as shown in Fig. 2A. To further validate the direct binding between LINC00511 and miR-29c, luciferase reporter assay was performed and the results manifested that luciferase activity was decreased in MCF-7 cells after cotransfection with WT-LINC00511 and miR-29c relative to the control group (Fig. 2B). However, no significant difference was observed in MCF-7 cells when cotransfected with MUT-LINC00511 and miR-29c compared to the control group (Fig. 2B). Moreover, RT-qPCR analysis demonstrated that miR-29c expression was elevated in si-LINC00511-transfected MCF-7 and T47D cells with respect to si-NC-introduced group (Fig. 2C). These data suggested that LINC00511 acted as a molecular sponge of miR-29c in breast cancer cells.

3.3. LINC00511 knockdown enhanced paclitaxel cytotoxicity in breast cancer cells by upregulating miR-29c

To clarify the effects of LINC00511 on the paclitaxel cytotoxicity of breast cancer cells as well as the underlying mechanism, MCF-7 and T47D cells were cotransfected with si-LINC00511 or si-NC and anti-miR-NC or anti-miR-29c, followed by treatment with 50 nM paclitaxel for 48 h. MTT assay implicated that LINC00511 knockdown exacerbated paclitaxel-induced inhibition of cell viability in MCF-7 (Fig. 3A) and T47D cells (Fig. 3B), suggesting that LINC00511 knockdown improved paclitaxel cytotoxicity in breast cancer cells. However, this effect was substantially relieved following cotreatment with LINC00511 depletion and miR-29c inhibition. Meanwhile, flow cytometry analysis demonstrated that paclitaxel exposure resulted in a remarkable increase of apoptosis in MCF-7 (Fig. 3C) and T47D cells (Fig. 3D), which was enhanced by LINC00511 silencing, suggesting that LINC00511 knockdown improved paclitaxel cytotoxicity by prompting paclitaxel-induced apoptosis in breast cancer cells. Nevertheless, suppression of miR-29c attenuated the effect of LINC00511 silencing on paclitaxel-induced apoptosis in MCF-7 (Fig. 3C) and T47D cells (Fig. 3D). Collectively, these findings indicated that LINC00511 knockdown enhanced paclitaxel cytotoxicity in breast cancer cells by upregulating miR-29c.

3.4. CDK6 was upregulated in breast cancer cells

CDK6, a member of CDK family, was previously demonstrated to be a direct target of miR-29c in bladder cancer [19] and gastric cancer [20]. Moreover, it has been proposed that new drugs targeting CDK4/6 provided new directions for drug-resistant breast cancer [21]. Therefore, we supposed whether miR-29c could directly target CDK6 in breast cancer. We firstly detected the expression of CDK6 in breast cancer tissues by RT-qPCR. As shown in Fig. 4A, CDK6 mRNA expression was increased in breast cancer tissues versus that in adjacent

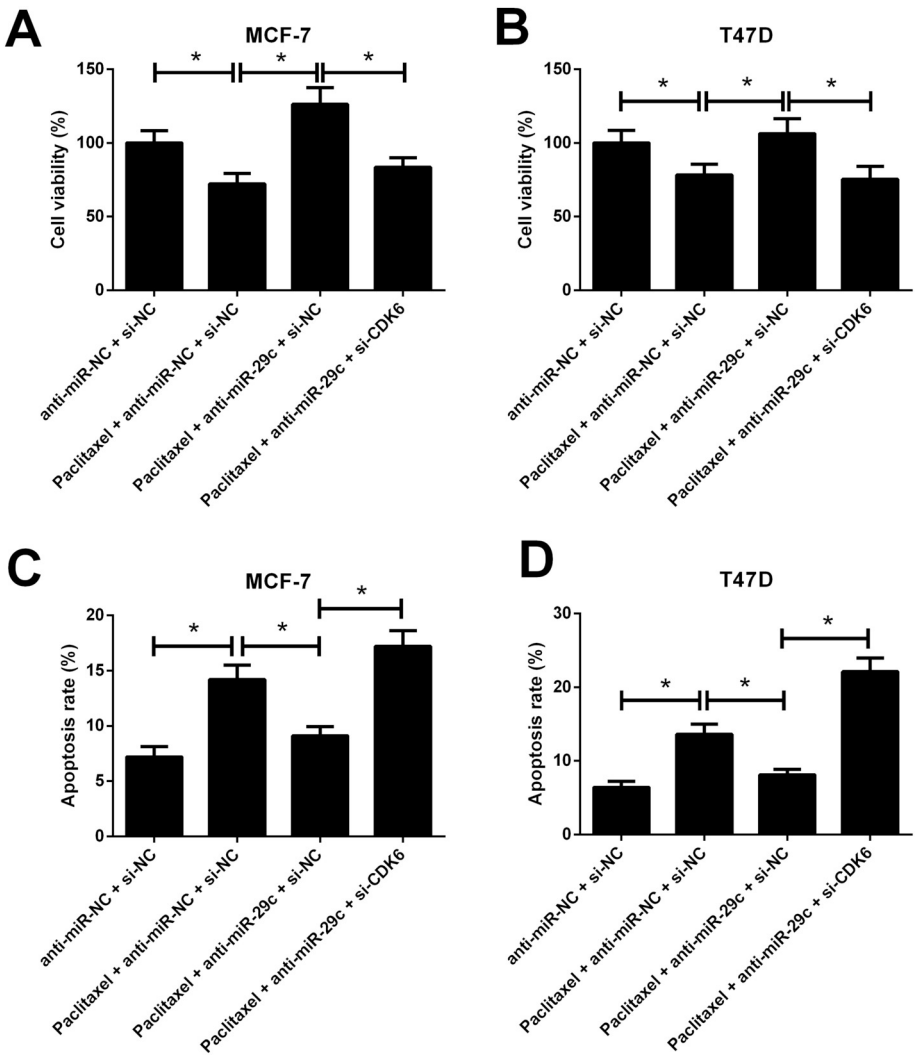

Fig. 6. Effects of miR-29c or along with CDK6 on the paclitaxel cytotoxicity in breast cancer cells. MCF-7 and T47D cells were cotransfected with anti-miR-NC or anti-miR-29c and si-CDK6 or si-NC, followed by exposure to 50 nM paclitaxel for 48 h. (A and B) MTT assay was carried out to assess cell viability in the treated MCF-7 and T47D cells. (C and D) Flow cytometry analysis was performed to analyze the apoptosis of treated MCF-7 and T47D cells. $^{*}P < 0.05$.

normal tissues. Meanwhile, we discovered that CDK6 expression at both mRNA (Fig. 4B) and protein (Fig. 4C) levels was highly expressed in breast cancer cell lines including MDA-MB-231, MCF-7, Hs-578T, and T47D relative to the immortalized breast epithelial cell line MCF-10A. Interestingly, TCGA dataset and Pearson correlation analysis both exhibited a negative correlation between miR-29c and CDK6 expressions in breast cancer tissues (Fig. 4D and E).

3.5. CDK6 was a target of miR-29c in breast cancer cells

The bioinformatic database TargetScan showed that CDK6 was predicted to exhibit miR-29c-binding sequences in its 3′UTR regions, as presented in Fig. 5A. To confirm the direct interaction between CDK6 and miR-29c, luciferase reporter vectors containing the WT or MUT CDK6 3′UTR fragments within the binding sites of miR-29c were constructed. The subsequent luciferase reporter assay demonstrated that MCF-7 cells cotransfected with miR-29c and WT-CDK6 showed a reduced luciferase activity compared with that cotransfected with miR-NC and WT-CDK6 (Fig. 5B). Furthermore, RT-qPCR and western blot analysis revealed that the mRNA and protein expressions of CDK6 were suppressed following transfection with miR-29c in MCF-7 and T47D cells (Fig. 5C and D). Therefore, we concluded that CDK6 was a target of miR-29c in breast cancer cells.

3.6. CDK6 knockdown attenuated the effects of miR-29c inhibition on paclitaxel cytotoxicity in breast cancer cells

To explore whether miR-29c regulated paclitaxel cytotoxicity in breast cancer cells via a CDK6-dependent manner, MCF-7 and T47D cells were cotransfected with anti-miR-NC or anti-miR-29c and si-CDK6 or si-NC, followed by exposure to 50 nM paclitaxel for 48 h. MTT assay revealed that paclitaxel treatment led to a significant repression of cell viability in MCF-7 and T47D cells, while miR-29c inhibition attenuated paclitaxel-induced reduction of cell viability (Fig. 6A and B). Nevertheless, CDK6 knockdown effectively abolished the effect of anti-miR-29c on cell viability in MCF-7 and T47D cells in the presence of paclitaxel (Fig. 6A and B). Flow cytometry analysis further implicated that transfection with anti-miR-29c distinctly weakened paclitaxel-induced apoptosis in MCF-7 and T47D cells, which was reversed following reintroduction with si-CDK6 (Fig. 6C and D). Collectively, these data suggested that miR-29c inhibition decreased paclitaxel cytotoxicity in breast cancer cells by upregulating CDK6.

3.7. LINC00511 positively regulated CDK6 expression in breast cancer cells

We further investigated the effects of LINC00511 on CDK6 expression in breast cancer cells. RT-qPCR and western blot analysis proved

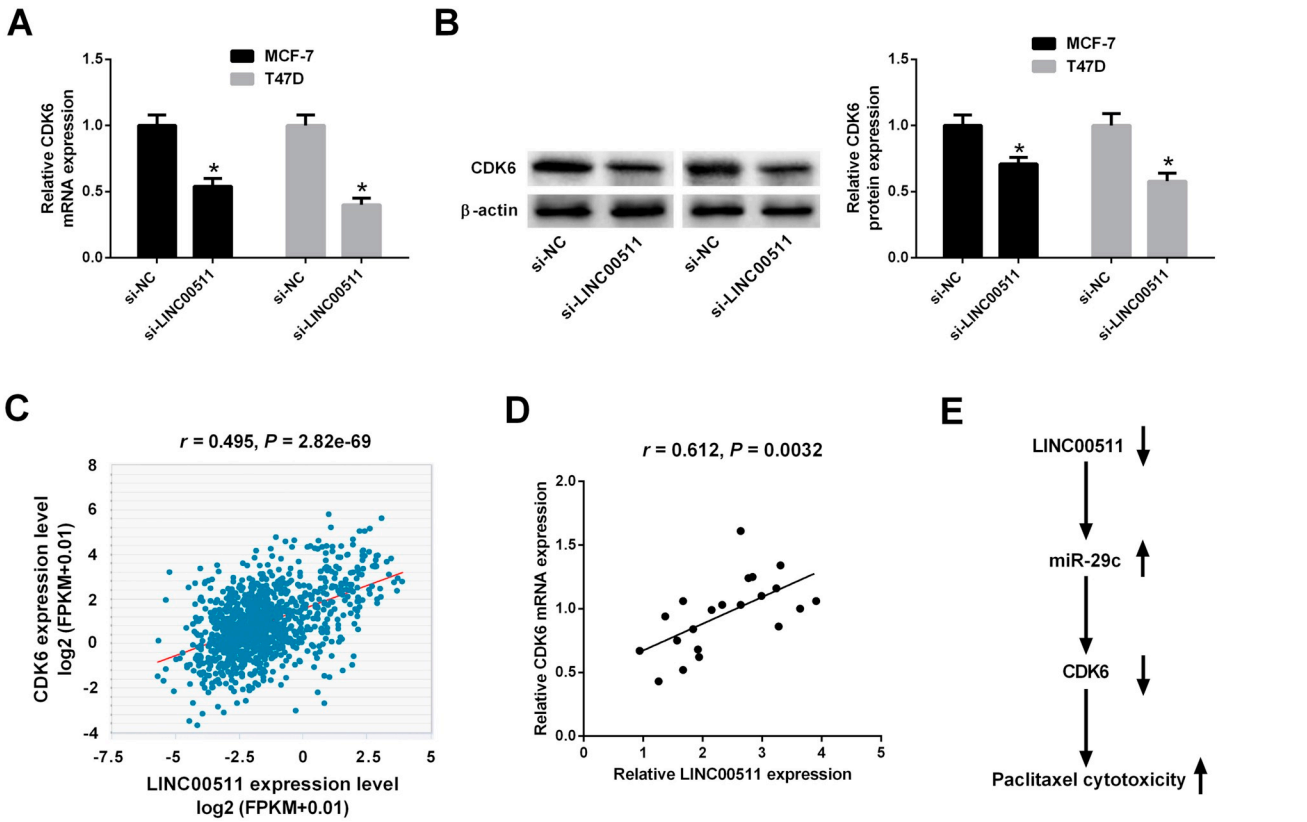

Fig. 7. Effects of LINC00511 on CDK6 expression in breast cancer cells. (A) RT-qPCR analysis of CDK6 mRNA expression in MCF-7 and T47D cells transfected with si-LINC00511 or si-NC for 48 h. (B) Western blot analysis of CDK6 protein level in si-LINC00511 or si-NC-introduced MCF-7 and T47D cells. (C) The correlation between LINC00511 and CDK6 mRNA expression in 1104 breast cancer samples from TCGA dataset from starBase. (D) Pearson correlation analysis of the correlation between LINC00511 and CDK6 mRNA expressions in 21 breast cancer tissues. (E) Schematic illustration showing that LINC00511 knockdown enhances paclitaxel cytotoxicity by miR-29c/CDK6 axis in breast cancer cells. $^*P < 0.05$.

that CDK6 expression at mRNA and protein levels was decreased in si-LINC00511-introduced MCF-7 (Fig. 7A) and T47D cells (Fig. 7B). By analysis of TCGA dataset from starBase, we found that LINC00511 expression was positively correlated with CDK6 expression in 1104 breast cancer samples (Fig. 7C). Also, Pearson correlation analysis implied that LINC00511 was positively correlated with CDK6 expression in 21 breast cancer tissues (Fig. 7D). These results demonstrated that LINC00511 positively regulated CDK6 expression in breast cancer cells.

4. Discussion

Currently, chemotherapy is widely applied in the treatment of various types of human malignancies including breast cancer. Nevertheless, the emergence of drug resistance in breast cancer severely limits the efficacy of conventional chemotherapies in clinical setting and remains a considerable hurdle contributing to treatment failure [22]. In recent decades, increasing studies have suggested that dysregulated lncRNAs are associated with drug resistance/sensitivity in breast cancer via a variety of intrinsic mechanisms [23]. For example, knockdown of lncRNA H19 elevated tamoxifen sensitivity in tamoxifen-resistant breast cancer cells by inhibiting Wnt pathway and epithelia-mesenchymal transition process [24]. LncRNA GAS5 overexpression enhanced cell sensitivity to tamoxifen in breast cancer cells by serving as a molecular sponge for miR-222 to modulate phosphatase and tensin homologs (PTEN) expression [25]. LncRNA UCA1 enhanced tamoxifen resistance in breast cancer cells through a miR-18a-hypoxia inducible factor-1α (HIF-1α) feedback regulatory loop [26]. LINC00511, a newly identified cancerogenic lncRNA, was found to be highly expressed and contribute to tumorigenesis and stemness in breast cancer [27]. Consistently, the present study demonstrated that LINC00511 was upregulated in breast cancer tissues and cells. It should be noted that Qu et al. raised a question as to whether the MCF-10A cells served as a suitable model for normal mammary epithelial cells, and this question warranted further investigation [28]. To the best of our knowledge, we provided the first evidence that LINC00511 knockdown enhanced paclitaxel cytotoxicity in breast cancer cells by prompting paclitaxel-induced apoptosis. A recent study suggested that LINC00511 depletion decreased proliferative ability, endothelial tube formation, and metastasis by regulating vascular endothelial growth factor A/miR-29b-3p axis in pancreatic ductal adenocarcinoma [29]. LINC00511 interacted with miR-765 and modulated the progression of tongue squamous cell carcinoma via targeting laminin subunit gamma 2 [30]. LINC00511 was also reported to promote osteosarcoma cell proliferation, colony formation, and migration by targeting miR-765 [31].

miR-29c has been widely recognized as an anticancer miRNA in many tumors [18,32]. Li et al. reported that miR-29c played a suppressive role in breast cancer by regulating the tissue inhibitor of metalloproteinases 3 (TIMP3)/signal transducer and activator of transcription 1 (STAT1)/forkhead box protein O1 (FOXO1) pathway [18]. miR-29c suppressed breast cancer progression via down-regulating its target gene, a disintegrin and metalloproteinase 12 (ADAM12) [33]. It is reportedly involved in the modulation of drug resistance in several types of human tumors. For example, it was reported that miR-29c was downregulated in cisplatin-resistant liver cancer cells and overexpression of miR-29c restored cisplatin chemosensitivity in liver cancer by direct targeting sirtuin 1 [34]. In pancreatic cancer, miR-29c enhanced the chemosensitivity to gemcitabine in vitro and in vivo by inhibiting ubiquitin specific peptidase (USP)-22-mediated autophagy [35]. However, the role of miR-29c in drug resistance of breast cancer cells remains unclear. Herein, our study demonstrated that miR-29c

was downregulated in breast cancer tissues and cells. Notably, TCGA dataset and Pearson correlation analysis both revealed a negative correlation between LINC0051 and miR-29c expression in breast cancer samples. Bioinformatics analysis and luciferase reporter assay further suggested that miR-29c was a target of LINC0051 and LINC0051 suppressed miR-29c expression via acting as a ceRNA. Rescue experiments revealed that miR-29c inhibition significantly abolished the effects of LINC0051 silencing on paclitaxel cytotoxicity in breast cancer cells, suggesting that LINC00511 knockdown enhanced paclitaxel cytotoxicity in breast cancer cells by sponging miR-29c.

CDK6, a cyclin-D1-dependent kinase, has attracted increasing research interest due to its involvement in the control of G1 progression and G1/S phase transition of the cell cycle, and inhibition of CDK6 can induce cell cycle arrest and uncontrolled tumor cell proliferation [36–38]. Recent studies have revealed that CDK6 was aberrantly amplified or overexpressed in various cancers such as bladder cancer [39], glioblastoma [40], osteosarcoma [41], and breast cancer [42], and exerted a cancerogenic activity in experimental models [43]. In our study, we demonstrated that CDK6 expression at mRNA and protein levels was higher in breast cancer tissues and cells. In addition, CDK6 was discovered to be inversely correlated with miR-29c expression and positively correlated with LINC00511 expression in breast cancer tissues, as demonstrated by TCGA dataset and Pearson correlation analysis. CDK6 was further identified as a target of miR-29c in breast cancer cells, which was similar with the previous reports in bladder cancer [19] and gastric cancer [20]. Moreover, we found that LINC00511 served as a molecular sponge of miR-29c to harbor its expression, thus leading to the release of CDK6 from miR-29c. Mechanistically, CDK6 knockdown attenuated the effects of miR-29c on paclitaxel cytotoxicity in breast cancer cells. Collectively, these results demonstrated that LINC00511 knockdown enhanced paclitaxel cytotoxicity in breast cancer cells by regulating the miR-29c/CDK6 axis.

5. Conclusion

In summary, we demonstrated for the first time that LINC00511 knockdown enhanced paclitaxel cytotoxicity in breast cancer cells by working as a molecular sponge of miR-29c and in turn decreasing CDK6 expression (Fig. 7E), contributing to better understanding the molecular mechanism underlying the drug resistance in breast cancer. Therefore, LINC00511 may be a promising therapeutic target for enhancing the efficacy of paclitaxel-based chemotherapy during the treatment of breast cancer. However, an in vivo experiment must be conducted to confirm our results.

Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] C.C. O'Sullivan, C.L. Loprinzi, T.C. Haddad, Updates in the evaluation and management of breast cancer, Mayo Clin. Proc. 93 (2018) 794–807.
[2] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2016, CA Cancer J. Clin. 66 (2016) 7–30.
[3] R.L. Siegel, K.D. Miller, A. Jemal, Cancer statistics, 2018, CA Cancer J. Clin. 68 (2018) 7–30.
[4] X. Chen, P. Lu, D.D. Wang, S.J. Yang, Y. Wu, H.Y. Shen, S.L. Zhong, J.H. Zhao, J.H. Tang, The role of miRNAs in drug resistance and prognosis of breast cancer formalin-fixed paraffin-embedded tissues, Gene 595 (2016) 221–226.
[5] E.T. Quispe-Soto, G.M. Calaf, Effect of curcumin and paclitaxel on breast carcinogenesis, Int. J. Oncol. 49 (2016) 2569–2577.
[6] L. O'Driscoll, M. Clynes, Biomarkers and multiple drug resistance in breast cancer, Curr. Cancer Drug Targets 6 (2006) 365–384.
[7] V. Hlavac, V. Brynychova, R. Vaclavikova, M. Ehrlichova, D. Vrana, V. Pecha, R. Kozevnikovova, M. Trnkova, J. Gatek, D. Kopperova, I. Gut, P. Soucek, The expression profile of ATP-binding cassette transporter genes in breast carcinoma, Pharmacogenomics 14 (2013) 515–529.
[8] C.A. Thomas Jr., The genetic organization of chromosomes, Annu. Rev. Genet. 5 (1971) 237–256.
[9] W. Hu, C. Tan, Y. He, G. Zhang, Y. Xu, J. Tang, Functional miRNAs in breast cancer drug resistance, Onco Targets Ther. 11 (2018) 1529–1541.
[10] M. Haemmerle, T. Gutschner, Long non-coding RNAs in cancer and development: where do we go from here? Int. J. Mol. Sci. 16 (2015) 1395–1405.
[11] R. Spizzo, M.I. Almeida, A. Colombatti, G.A. Calin, Long non-coding RNAs and cancer: a new frontier of translational research? Oncogene 31 (2012) 4577–4587.
[12] R. Heery, S.P. Finn, S. Cuffe, S.G. Gray, Long non-coding RNAs: key regulators of epithelial-mesenchymal transition, tumour drug resistance and cancer stem cells, Cancer 9 (2017) 38.
[13] J. Ding, C. Yang, S. Yang, LINC00511 interacts with miR-765 and modulates tongue squamous cell carcinoma progression by targeting LAMC2, J. Oral Pathol. Med. 47 (2018) 468–476.
[14] S. Xu, D. Kong, Q. Chen, Y. Ping, D. Pang, Oncogenic long noncoding RNA landscape in breast cancer, Mol. Cancer 16 (2017) 129.
[15] S. Jalali, D. Bhartiya, M.K. Lalwani, S. Sivasubbu, V. Scaria, Systematic transcriptome wide analysis of lncRNA-miRNA interactions, PLoS One 8 (2013) e53823.
[16] H.J. Bae, J.H. Noh, J.K. Kim, J.W. Eun, K.H. Jung, M.G. Kim, Y.G. Chang, Q. Shen, S.J. Kim, W.S. Park, J.Y. Lee, S.W. Nam, MicroRNA-29c functions as a tumor suppressor by direct targeting oncogenic SIRT1 in hepatocellular carcinoma, Oncogene 33 (2014) 2557–2567.
[17] I. Cristobal, J. Madoz-Gurpide, R. Manso, F. Rojo, J. Garcia-Foncillas, MiR-29c downregulation contributes to metastatic progression in colorectal cancer, Ann. Oncol. 26 (2015) 2199–2200.
[18] W. Li, J. Yi, X. Zheng, S. Liu, W. Fu, L. Ren, L. Li, D.S.B. Hoon, J. Wang, G. Du, miR-29c plays a suppressive role in breast cancer by targeting the TIMP3/STAT1/FOXO1 pathway, Clin. Epigenetics 10 (2018) 64.
[19] X. Zhao, J. Li, S. Huang, X. Wan, H. Luo, D. Wu, MiRNA-29c regulates cell growth and invasion by targeting CDK6 in bladder cancer, Am. J. Transl. Res. 7 (2015) 1382–1389.
[20] M. Matsuo, C. Nakada, Y. Tsukamoto, T. Noguchi, T. Uchida, N. Hijiya, K. Matsuura, M. Moriyama, MiR-29c is downregulated in gastric carcinomas and regulates cell proliferation by targeting RCC2, Mol. Cancer 12 (2013) 15.
[21] M. Nichols, New directions for drug-resistant breast cancer: the CDK4/6 inhibitors, Future Med. Chem. 7 (2015) 1473–1481.
[22] A.M. Gonzalez-Angulo, F. Morales-Vasquez, G.N. Hortobagyi, Overview of resistance to systemic therapy in patients with breast cancer, Adv. Exp. Med. Biol. 608 (2007) 1–22.
[23] A. Malhotra, M. Jain, H. Prakash, K.M. Vasquez, A. Jain, The regulatory roles of long non-coding RNAs in the development of chemoresistance in breast cancer, Oncotarget 8 (2017) 110671–110684.
[24] H. Gao, G. Hao, Y. Sun, L. Li, Y. Wang, Long noncoding RNA H19 mediated the chemosensitivity of breast cancer cells via Wnt pathway and EMT process, Onco Targets Ther. 11 (2018) 8001–8012.
[25] J. Gu, Y. Wang, X. Wang, D. Zhou, C. Shao, M. Zhou, Z. He, Downregulation of lncRNA GAS5 confers tamoxifen resistance by activating miR-222 in breast cancer, Cancer Lett. 434 (2018) 1–10.
[26] X. Li, Y. Wu, A. Liu, X. Tang, Long non-coding RNA UCA1 enhances tamoxifen resistance in breast cancer cells through a miR-18a-HIF1α feedback regulatory loop, Tumour Biol. 37 (2016) 14733–14743.
[27] G. Lu, Y. Li, Y. Ma, J. Lu, Y. Chen, Q. Jiang, Q. Qin, L. Zhao, Q. Huang, Z. Luo, S. Huang, Z. Wei, Long noncoding RNA LINC00511 contributes to breast cancer tumourigenesis and stemness by inducing the miR-185-3p/E2F1/Nanog axis, J. Exp. Clin. Cancer Res. 37 (2018) 289.
[28] Y. Qu, B. Han, Y. Yu, W. Yao, S. Bose, B.Y. Karlan, A.E. Giuliano, X. Cui, Evaluation of MCF10A as a reliable model for normal human mammary epithelial cells, PLoS One 10 (2015) e0131285.
[29] X. Zhao, Y. Liu, Z. Li, S. Zheng, Z. Wang, W. Li, Z. Bi, L. Li, Y. Jiang, Y. Luo, Q. Lin, Z. Fu, C. Rufu, Linc00511 acts as a competing endogenous RNA to regulate VEGFA expression through sponging hsa-miR-29b-3p in pancreatic ductal adenocarcinoma, J. Cell. Mol. Med. 22 (2018) 655–667.
[30] J. Ding, C. Yang, S. Yang, LINC00511 interacts with miR-765 and modulates tongue squamous cell carcinoma progression by targeting LAMC2, J. Oral Pathol. Med. 47 (2018) 468–476.
[31] L. Yan, X. Wu, Y. Liu, W. Xian, LncRNA Linc00511 promotes osteosarcoma cell proliferation and migration through sponging miR-765, J. Cell. Biochem. 120 (2019) 7248–7256.
[32] J. Li, F. Fu, X. Wan, S. Huang, D. Wu, Y. Li, Up-regulated miR-29c inhibits cell proliferation and glycolysis by inhibiting SLC2A3 expression in prostate cancer, Gene 665 (2018) 26–34.
[33] S.Y. Wu, M.D. Yan, A.T. Wu, K.S. Yuan, S.H. Liu, Brown seaweed fucoidan inhibits cancer progression by dual regulation of mir-29c/ADAM12 and miR-17-5p/PTEN axes in human breast cancer cells, J. Cancer 7 (2016) 2408–2419.
[34] W. Zhang, P. Luo, MicroRNA-29c restores cisplatin sensitivity in liver cancer through direct inhibition of sirtuin 1 expression, Oncol. Lett. 16 (2018) 1543–1550.
[35] L. Huang, C. Hu, H. Cao, X. Wu, R. Wang, H. Lu, H. Li, H. Chen, MicroRNA-29c increases the chemosensitivity of pancreatic cancer cells by inhibiting USP22 mediated autophagy, Cell. Physiol. Biochem. 47 (2018) 747–758.
[36] S.A. Wander, E.L. Mayer, H.J. Burstein, Blocking the cycle: cyclin-dependent kinase 4/6 inhibitors in metastatic, hormone receptor-positive breast cancer, J. Clin. Oncol. 35 (2017) 2866–2870.
[37] J. Rader, M.R. Russell, L.S. Hart, M.S. Nakazawa, L.T. Belcastro, D. Martinez, Y. Li, E.L. Carpenter, E.F. Attiyeh, S.J. Diskin, S. Kim, S. Parasuraman, G. Caponigro, R.W. Schnepp, A.C. Wood, B. Pawel, K.A. Cole, J.M. Maris, Dual CDK4/CDK6 inhibition induces cell-cycle arrest and senescence in neuroblastoma, Clin. Cancer Res. 19 (2013) 6173–6182.
[38] K. Handschick, K. Beuerlein, L. Jurida, M. Bartkuhn, H. Muller, J. Soelch, A. Weber,

O. Dittrich-Breiholz, H. Schneider, M. Scharfe, M. Jarek, J. Stellzig, M.L. Schmitz, M. Kracht, Cyclin-dependent kinase 6 is a chromatin-bound cofactor for NF-kappaB-dependent gene expression, Mol. Cell 53 (2014) 193–208.
[39] G. Wang, L. Zheng, Z. Yu, G. Liao, L. Lu, R. Xu, Z. Zhao, G. Chen, Increased cyclin-dependent kinase 6 expression in bladder cancer, Oncol. Lett. 4 (2012) 43–46.
[40] W.R. Wiedemeyer, I.F. Dunn, S.N. Quayle, J. Zhang, M.G. Chheda, G.P. Dunn, L. Zhuang, J. Rosenbluh, S. Chen, Y. Xiao, G.I. Shapiro, W.C. Hahn, L. Chin, Pattern of retinoblastoma pathway inactivation dictates response to CDK4/6 inhibition in GBM, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 11501–11506.
[41] W. Yuan, D. Wang, Y. Liu, D. Tian, Y. Wang, R. Zhang, L. Yin, Z. Deng, miR494 inhibits cell proliferation and metastasis via targeting of CDK6 in osteosarcoma, Mol. Med. Rep. 16 (2017) 8627–8634.
[42] Y. Zhang, H. Zhang, H. Kang, W. Huo, Y. Zhou, Y. Zhang, Knockdown of long non-coding RNA HOST2 inhibits the proliferation of triple negative breast cancer via regulation of the let-7b/CDK6 axis, Int. J. Mol. Med. 43 (2019) 1049–1057.
[43] M. Malumbres, M. Barbacid, Cell cycle, CDKs and cancer: a changing paradigm, Nat. Rev. Cancer 9 (2009) 153–166.