LINC00680 Modulates Docetaxel Resistance in Breast Cancer via miR-320b/CDKL5 Axis

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Abstract

Background

The purpose of this study was to explore regulatory mechanism of the long non-coding RNA LINC00680 underlying the breast cancer chemoresistance via miR-205/CDKL5 axis.

Methods

Bioinformatics methods were applied to screen lncRNAs, miRNAs and mRNAs for construction of a competing endogenous RNA network. Dual-luciferase reporter assay were used to explore the relationship between LINC00680, miR-320b and CDKL5. CCK-8 assay and Transwell assay were utilized to evaluate the proliferation, migration and invasion in docetaxel-resistant cells.

Results

LINC00680 and CDKL5 protein levels were both up-regulated when induced by different concentration of docetaxel. LINC00680 knockdown decreased the expression level of drug resistance related genes (MDR1, MRP5, LRP1), proliferation, invasion of breast cancer cells. Bioinformatics prediction and dual-luciferase assay reveled that miR-320b both targeted the 3'-UTR of LINC00680 and CDKL5, suggesting that the modulation of LINC00680 on CDKL5 through sponging miR-320b.

Conclusions

Overall, the results indicate the important role of LINC00680 in the docetaxel resistance through miR-320b/CDKL5 pathway, providing a novel therapy strategy for breast cancer drug resistance.

Background

Breast cancer (BC) is the most common malignancy tumor in women worldwide, and it is the leading cause of cancer-related mortality among women[1]. Despite remarkable progress in the treatments of breast cancer over the past decade, women dying from this cancer type were gradually increasing due to the frequent chemotherapeutic resistance and tumor metastasis [2, 3]. So in depth understanding of the molecular mechanisms regulating the drug resistance is of great importance, which would provide more treatment options for those with end-stage disease.

Taxanes, including paclitaxel and docetaxel, are commonly accepted as the common chemotherapeutic agents for breast cancer in clinical practice[4], while the resistance to docetaxel remains a persistent problem. In recent years, several genes have been found to be associated with docetaxel resistance, for example, the differential expression of the ATP-binding cassette subfamily B member 1 (*ABCB1*) gene is the putative biomarkers in docetaxel-resistance cancers[5, 6]; Permeability glycoprotein (Pgp) acts as an ATP-dependent efflux pump and reduced docetaxel concentration by expelling the drug[7]. Also cyclin-dependent kinase-like 5 (CDKL5), a kind of widely expressed serine/threonine kinase, is an X-linked gene.

CDKL5 deficiency or mutations cause a distinct disorder, especially in neuro system because it is expressed throughout the brain starting at late embryonic stages. However, Jiang *et., al* [8] found that CDKL5 could promote progression and β-lapachone resistance of glioma, Robin *et., al*[9] indicated CDKL5 presented as prognostic marker for glioblastoma, and Masahiro *et., al*[10] suggested CDKL5 peptide can be used for cytotoxic T-lymphocyte-mediated immunotherapy, so it's of great interest for us to find the role of CDKL5 in the progression of breast cancer.Long non-coding RNAs (lncRNAs) is a kind of RNAs with length over 200 nucleotides, and usually described to influence mRNA generation and expression[11]. Previous studied have shown that lncRNAs are also implicated in chemotherapy resistance. Long non-coding RNA cancer susceptibility candidate 2 (CASC2), lncRNA ferritin heavy chain 1 pseudogene 3 (FTH1P3) are both reported to contribute to paclitaxel resistance of breast cancer[12, 13]. Two lncRNAs, lncRNA SNHG14 and H19, both contributes to the trastuzumab resistance of breast cancer[14, 15]. Thus, lncRNAs are found to regulate gene expression at post-transcription level via sponging microRNAs[15] and modulate transcriptional gene[16, 17]. So the investigation of the role of lncRNAs in breast cancer could help with the understanding of chemotherapy resistance and provide the novel therapeutic strategy. LINC00680 is firstly reported as a protective biomarker and an independent prognostic indicator of soft-tissue sarcoma[18], then it was reported to promote the progression of non-small cell lung cancer and glioblastoma cells[19, 20]. Nevertheless, the expression pattern, biological function and underlying mechanism of LINC00680 in breast cancer progression and docetaxel resistance are scarcely unresearched. In this study, we found that LINC00680 regulated breast cancer progression and docetaxel resistance via regulating CDKL5 via sponging miR-320b. To verify this hypothesis, we determined the expression of LINC00680 in breast cancer cell lines, and performing *in vitro* experiments to investigate the functional relevance of LINC00680 with chemotherapy resistance.

Methods

Cell lines and culture

Human breast cancer cell lines MCF-7 was obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China). The docetaxel-resistant variant of MCF-7 cells (MCF-7/Doc) was generated in a stepwise manner by exposing drug-sensitive MCF-7 cells to increasing doses of docetaxel as recently described[21]. MCF-7/Doc was maintained in drug-free medium for 2 weeks before subsequent experiments to avoid the influence of toxic insult, and parental MCF-7 was cultured unexposed to docetaxel, as a control for all experiments.

All cell lines were grown in Dulbecco's modified Eagle's medium (DMEM) high glucose (Hyclone, Logan, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Grand Island, NY, UT, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin (both purchased from Beyotime Ltd, Shanghai, China) under a humidified incubator at 37℃ in an atmosphere of 5% CO_2.

Drug-resistance and proliferation ability analysis

Drug cytotoxicity and proliferation ability were measured by Cell Counting Kit-8 (CCK-8) assays *in vitro*. Approximately 5,000 cells suspended in 100 μl culture medium were plated in 96-well plates and incubated with increasing concentration of docetaxel. To calculate the drug lethal to 50% of the cells (IC_{50}), the proliferative ability of no-docetaxel treated cells was seated as 100% standard. After incubation for 48 h, 10 μl Cell Counting Kit-8 reagents were added and the absorbance of 450 nm were detected according to the manufacturer's instructions using the Multiskan FC (ThermoFisher Scientific, Waltham, MA, USA).

RNA interference and transfection

RNA interference sequence for LINC00680, miR-320b and CDKL5 were designed and synthesized by Gene pharma company (Shanghai, China). siRNA sequence targeting was as follows: si-LINC00680, forward oligo, 5'- UAUAGUUCAAGUCAUAAACUG -3', reverse oligo, 5'-GUUUAUGACUUGAACUAUAGG-3'; si-CDKL5, forward oligo, 5'-UCACAUAUUUGUUCUAAUCAU-3'; reverse oligo, 5'-GAUUAGAACAAAUAUGUGAAA-3'. Interfering RNAs were transfected into cells in 6-well plates using Lipofectamine^{TM} 2000 reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions.

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

RNA from cells was isolated using the TRIZol reagent (ThermoFisher Scientific) and its quantifications were assessed by Nanodrop 2000 spectrophotometer (ThermoFisher Scientific). Total 2 μg RNA was reversed to cDNA using SuperScript^{TM} IV One-Step RT-PCR System (Thermo Fisher Scientific). qRT-PCR reaction was performed using an ABI7500 system (Applied Biosystems, Foster City, CA, USA) and SYBR Green PCR Master Mix (Thermo Fisher Scientific). The primers were designed and shown in Table 1. Relative levels of gene expression were expressed relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or SncU6 using $2^{-\Delta\Delta Ct}$ method.

Table 1 Primers for qRT-PCR assay

Gene Name	Primers (5’ to 3’)	
LINC00680	Forward	AGATGGTGAGAACTGGTCTGA
	Reverse	GGGCTCTGGGTCTGAATCTT
miR-320b	Forward	GATGCTGAAAAGCTGGGTTG
	Reverse	TATGGTTGTTCTGCTCTCTGTCTC
CDKL5	Forward	CTGGGGAAGGTAAAGCGGC
	Reverse	TTAAATGACTCCCCCGCCGA
MDR1	Forward	GGCTACATGAGAGCGGAGGA
	Reverse	GGAATGTTCTGGCTTCCGTTG
MRP5	Forward	TTGCTACGTGAGTGTACGCC
	Reverse	GATGTGAGGACTGGCTGGTT
LRP5	Forward	GTTACACTGGAGAGAGCAGCAT
	Reverse	CCTCTGTCCTCTCATCCTTCA
GAPDH	Forward	ACAGTCAGCCGCATCTTCTT
	Reverse	GACTCCGACCTTCACCTTCC
SncU6	Forward	CTCGCTTCGGCAGCACA
	Reverse	AACGCTTCACGAATTTGCGT

Western blot analysis

Cells were lysed by RIPA lysis buffer (Beyotime) added within protease inhibitors cocktail (Roche Applied Science, Indian polis, IN, USA), and then were incubated at 4°C for 30 min, the samples were centrifuged at 12,000 × g for 15 min at 4°C. Then, the suspending protein was transferred onto sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and then blotted onto PVDF membrane (Millipore, Billerica, MA, USA). PVDF membrane was incubated with primary antibodies against CDKL5 and β-actin (both purchased from Abcam, Cambridge, CA, USA) at 4°C overnight. The membrane was incubated with second antibody (horseradish peroxidase-conjugated goat anti-rabbit or horseradish peroxidase-conjugated goat anti-mouse) at room temperature for 2 h. Lastly, blots were detected using ECL chemiluminescence detection kit (Thermo Fisher Scientific).

Transwell invasion assay

Briefly, cells (1×10^4 per well) were seeded and suspended in medium containing 1% FBS, then the cells were plated into the upper chamber of Transwell plates which was pre-coated with Matrigel, the lower chamber was filled with DMEM medium with 10% FBS. After 24 h of incubation, the non-invaded cells on

upper surface of membrane were removed using cotton. The cells invaded through the membrane were fixed with methanol for 20 min at room temperature, after washing with PBS, the cells were stained with 0.1% crystal violet. The number of invaded cells in five randomly selected fields of each group was counted, all experiments were performed in triplicate.

Dual-luciferase reporter assay

The 3’-UTR of LINC 00680 and CDKL5 mRNA containing miR-320b binding sites were amplified and cloned into the pGL3-Basic luciferase vector (Promega, Madison, WI, USA). Then the LINC00680 and CDKL5 mRNA, wild type or mutant type sequences were synthesized and transfected into HEK-293T cells with miR-320b mimics or negative controls using Lipofectamine 2000 (ThermoFisher Scientific). After 24 h of transfection, the luciferase activity was measured using Dual-Luciferase Reported System comparing to Rinella luciferase (Promega).

Statistical analysis

Data were presented as mean ± SD. The statistical analyses were performed using SPSS version 19.0 software (IBM, Chicago, IL, USA) ad charted using GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA, USA). The statistical analysis was performed using one-way ANOVA for difference among groups and student’s t-test for comparing within groups. The P value less than 0.05 were considered statistical significance.

Results

LINC00680 and CDKL5 protein were up-regulated in docetaxel resistance of breast cancer cells *in vitro*

To investigate the expression levels of LINC00680, qRT-PCR was performed in docetaxel resistance breast cancer cells. Results showed that LINC00680 expression was significantly upregulated in MCF-7/Doc cells compared with its parental MCF-7 cells. Besides, IC50 value of docetaxel for MCF-7/Doc cells was significantly higher than that in parental MCF-7 cells. Furthermore, the LINC00680 expression presented time- and dose-dependent characteristic in MCF-7/Doc cells when treated with different dose of docetaxel or different processed time (0, 6, 12, 24 h). Meanwhile, we found that CDKL5 protein was up-regulated with the docetaxel treatment. Therefore, above data suggested that LINC00680 and CDKL5 protein might be the potential key molecular in chemotherapy of breast cancer.

LINC00680 modulated CDKL5 expression through targeting miR-320b

In present experiments, we had verified that LINC00680 and CDKL5 protein were both up-regulated in MCF-7/Doc cells when treated with docetaxel, so bioinformatics tools were performed to investigate the interaction within LINC00680 and CDKL5 in the deep study. Results showed that miR-320b shared with LINC00680 3'-UTR, and miR-320b was upregulated in MCF-/Doc cells transfected with si-LINC00680. Moreover, miR-320b was predicted to bind with the position of 931- 937 in CDKL5 3'-UTR and validated by dual-luciferase reporter assay. The data indicated LINC00680 and CDKL5 mRNA were both up-regulated

when transfected with miR-320b inhibitor. Furthermore, Western blot analysis showed that CDKL5 protein expression was decreased when transfected with si-LINC00680 and increased when transfected with miR-320b inhibitor. In summary, we hold the hypothesis that LINC00680 might modulate the expression of CDKL5 protein through targeting miR-320b.

The associated role of LINC00680, miR-320b and CDKL5 on breast cancer proliferation, invasion and docetaxel resistance

In present research, we reported the LINC00680/miR-320b/CDKL5 pathway in breast cancer tumorigenesis, and then functional experiments were performed to confirm it. The results showed that the levels of multidrug resistance related genes (MDR1, MRP5, LRP1) decreased in CDKL5 knockdown or LINC00680 silencing cells, while miR-320b inhibitor transfection reversed the inhibition of LINC00680 knockdown. CCK-8 assay also indicated that CDKL5 knockdown or LINC00680 knockdown suppressed the proliferation vitality, and miR-320b inhibitor reversed the inhibition of LINC00680 knockdown. Transwell invasion assay showed that the invaded cells reduced in CDKL5 siRNA transfection cells and miR-320b inhibitor reversed this inhibition function of LINC00680 silencing. Therefore, the above data suggested the role of LINC00680/miR-320b/CDKL5 signaling on breast cancer proliferation, invasion and docetaxel resistance.

Discussion

Several studies revealed the function of LINC00680 in cancer, including promoting malignancy in glioblastoma[19], predicting the clinical outcome and survival of patients with sarcoma[18] and promoting the progression of non-small cell cancer[22]. Our work confirmed a new role of LINC00680 in breast cancer, breast cancer cells with docetaxel resistance shown increasing expression of both LINC00680 and CDKL5 (Fig. 1). Docetaxel resistance of breast cancer cells was inhibited by the knockdown of LINC00680 (Fig. 2). These results indicated that LINC00680 associated with chemo-resistance of breast cancer cells.

Both the role of lncRNA in miRNA generation and miRNA negative regulation were identified[23, 24]. In present study, it was shown that both LINC00680 and 3'-UTR of CDKL5 were targeted by miR-320b (Fig. 3). The expression level of CDKL5 was regulated by LINC00680 (Fig. 3) through competing the miR-320b target sites of CDKL5 3'-UTR, which is known as “competing endogenous RNA (ceRNA)” hypothesis[25].

The function of CDKL5 is not well understood. It was reported that CDKL5 localizes at multiple subcellular structures[26, 27], and involved in cell division[26]. The association between CDKL5 and drug resistance was identified[28, 29]. In vitro experiments suggested that knockdown of CDKL5 and LINC00680 decreased the expression of MDR1, MRP5 and LRP1 (Fig. 4), which are known as drug-resistance genes in various cancers[30-33]. The role of MDR1 and MRP7 in docetaxel resistance were demonstrated in different types of cancer[34-37]. As shown by Jiang *et al.*[29], CDKL5 promotes chemotherapeutic drug resistance of glioma may through PI3K/AKT pathway. Our study hinted that the expression of CDKL5 and LINC00680 together with chemo-resistance genes MDR1, MRP5 and LRP1 play

an important role in docetaxel resistance of breast cancer. The interaction between CDKL5 and MDR1, MRP5 and LRP1 needs further study.

Conclusion

In consistent with previous findings in other cancers, our results have shown that LINC00680 promote cell proliferation, migration, and invasion in breast cancer. Taking together, the results shown in present study implied that LINC00680 may act as miR-320b competitor, which regulates the promote cell proliferation, migration, invasion and docetaxel resistance of breast cancer cells through CDKL5.

Abbreviations

BC, breast cancer; CDKL5, cyclin dependent kinase like 5; ABCB1, ATP-binding cassette subfamily B member 1; Pgp, permeability glycoprotein; CAS2, cancer susceptibility candidate 2; FTH1P3, ferritin heavy chain 1 pseudogene; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MDR1, multidrug resistance 1; MRP5, multidrug resistance associated protein 5; LRP1, LDL receptor related protein 1; UTR, untranslated regions; PI3K, phosphatidylinositol 3 kinase; AKT, protein kinase B; DMEM, Dulbecco's modification of Eagle's medium; FBS, fetal bovine; CCK-8, cell counting kit-8; SDS-PAGE, sodium salt-polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; HRP, horseradish peroxidase; qRT-PCR, quantitative reverse transcription polymerase chain reaction;

Declarations

Acknowledgments

Not applicable.

Authors' contributions

L and J. K: writing, literature research, data analysis and statistical analysis. J. L, J. K and CL. Q: literature search, clinical research and data analysis; X. Z: manuscript writing, literature research, study design and manuscript review. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D: **Global cancer statistics**. *CA Cancer J Clin* 2011, **61**(2):69-90.
2. Li T, Mello-Thoms C, Brennan PC: **Descriptive epidemiology of breast cancer in China: incidence, mortality, survival and prevalence**. *Breast Cancer Res Treat* 2016, **159**(3):395-406.
3. Fan L, Strasser-Weippl K, Li JJ, St Louis J, Finkelstein DM, Yu KD, Chen WQ, Shao ZM, Goss PE: **Breast cancer in China**. *Lancet Oncol* 2014, **15**(7):e279-289.
4. King KM, Lupichuk S, Baig L, Webster M, Basi S, Whyte D, Rix S: **Optimal use of taxanes in metastatic breast cancer**. *Curr Oncol* 2009, **16**(3):8-20.
5. Kim HJ, Im SA, Keam B, Ham HS, Lee KH, Kim TY, Kim YJ, Oh DY, Kim JH, Han W: **ABCB1 polymorphism as prognostic factor in breast cancer patients treated with docetaxel and doxorubicin neoadjuvant chemotherapy**. *Cancer ence* 2015, **106**(1):86-93.
6. **Acquisition of docetaxel resistance in breast cancer cells reveals upregulation of ABCB1 expression as a key mediator of resistance accompanied by discrete upregulation of other specific genes and pathways**. *Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine* 2015.
7. Chen CJ: **Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells**. *Cell* 1986, **47**(3):381-389.
8. Jiang Z, Gong T, Wei H: **CDKL5 promotes proliferation, migration, and chemotherapeutic drug resistance of glioma cells via activation of the PI3K/AKT signaling pathway**. *FEBS Open Bio* 2019, **10**(2).
9. Varghese RT, Liang Y, Guan T, Franck CT, Sheng Z: **Survival kinase genes present prognostic significance in glioblastoma**. *Oncotarget* 2016, **7**(15):20140-20151.
10. Kawahara M, Hori T, Matsubara Y, Okawa K, Uchiyama T: **Cyclin-dependent kinaselike 5 is a novel target of immunotherapy in adult T-cell leukemia**. *Journal of Immunotherapy* 2007, **30**(5):499-505.

11. Geisler S, Coller J: **RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts**. *Nature Reviews Molecular Cell Biology* 2013, **14**(11):699-712.
12. Zheng P, Dong L, Zhang B, Dai J, Qin S: **Long noncoding RNA CASC2 promotes paclitaxel resistance in breast cancer through regulation of miR-18a-5p/CDK19**. *Histochemistry and Cell Biology* 2019, **152**(8).
13. Ruoming W, Tengteng Z, Zhen Y, Chunxia J, Jingjing S: **Long non-coding RNA FTH1P3 activates paclitaxel resistance in breast cancer through miR-206/ABCB1**. *Journal of Cellular & Molecular Medicine* 2018.
14. Huaying D, Wei W, Shaowei M, Qiang L, Xin C, Ru C, Yu Z, Kejian Z, Mulin Y, Xionghui H: **Long non-coding RNA SNHG14 induces trastuzumab resistance of breast cancer via regulating PABPC1 expression through H3K27 acetylation**. *Journal of cellular and molecular medicine* 2018, **22**.
15. Sun Z, Zhang C, Wang T, Shi P, Guo Y: **Correlation between long non-coding RNAs (lncRNAs) H19 expression and trastuzumab resistance in breast cancer**. *Journal of cancer research and therapeutics* 2019, **15**(4):933.
16. Polyak K, Weinberg RA: **Transitions between epithelial and mesenchymal states: acquisition of malignant and stem cell traits**. *Nature Reviews Cancer* 2009, **9**(4):265-273.
17. Shi SJ, Wang LJ, Yu B, Li YH, Jin Y, Bai XZ: **LncRNA-ATB promotes trastuzumab resistance and invasion-metastasis cascade in breast cancer**. *Oncotarget* 2015, **6**(13).
18. He RQ, Wei QJ, Tang RX, Chen WJ, Yang X, Peng ZG, Hu XH, Ma J, Chen G: **Prediction of clinical outcome and survival in soft-tissue sarcoma using a ten-lncRNA signature**. *Oncotarget* 2017, **8**(46):80336-80347.
19. Tang W, Wang D, Shao L, Liu X, Zheng J, Xue Y, Ruan X, Yang C, Liu L, Ma J *et al*: **LINC00680 and TTN-AS1 Stabilized by EIF4A3 Promoted Malignant Biological Behaviors of Glioblastoma Cells**. *Molecular therapy Nucleic acids* 2020, **19**:905-921.
20. Wang H, Feng L, Zheng Y, Li W, Liu L, Xie S, Zhou Y, Chen C, Cheng D: **LINC00680 Promotes the Progression of Non-Small Cell Lung Cancer and Functions as a Sponge of miR-410-3p to Enhance HMGB1 Expression**. *OncoTargets and therapy* 2020, **13**:8183-8196.
21. Li W, Zhong S, Wu Y, Xu W, Xu J, Tang J, Zhao J: **Systematic expression analysis of genes related to multidrug-resistance in isogenic docetaxel- and adriamycin-resistant breast cancer cell lines**. *Molecular Biology Reports* 2013, **40**(11):6143-6150.
22. Wang H, Feng L, Zheng Y, Li W, Liu L, Xie S, Zhou Y, Chen C, Cheng D: **LINC00680 Promotes the Progression of Non-Small Cell Lung Cancer and Functions as a Sponge of miR-410-3p to Enhance HMGB1 Expression**. *OncoTargets and Therapy* 2020, **13**:8183-8196.
23. Cao MX, Jiang YP, Tang YL, Liang XH: **The crosstalk between lncRNA and microRNA in cancer metastasis: orchestrating the epithelial-mesenchymal plasticity**. *Oncotarget* 2017, **8**(7):12472-12483.
24. Dykes IM, Emanueli C: **Transcriptional and Post-transcriptional Gene Regulation by Long Non-coding RNA**. *Genomics Proteomics Bioinformatics* 2017, **15**(3):177-186.

25. Salmena L, Poliseno L, Tay Y, Kats L, Pandolfi PP: **A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language?** *Cell* 2011, **146**(3):353-358.
26. Barbiero I, Valente D, Chandola C, Magi F, Bergo A, Monteonofrio L, Tramarin M, Fazzari M, Soddu S, Landsberger N *et al*: **CDKL5 localizes at the centrosome and midbody and is required for faithful cell division**. *Sci Rep* 2017, **7**(1):6228.
27. Oi A, Katayama S, Hatano N, Sugiyama Y, Kameshita I, Sueyoshi N: **Subcellular distribution of cyclin-dependent kinase-like 5 (CDKL5) is regulated through phosphorylation by dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A)**. *Biochem Biophys Res Commun* 2017, **482**(2):239-245.
28. Parrini E, Marini C, Mei D, Galuppi A, Cellini E, Pucatti D, Chiti L, Rutigliano D, Bianchini C, Virdo S *et al*: **Diagnostic Targeted Resequencing in 349 Patients with Drug-Resistant Pediatric Epilepsies Identifies Causative Mutations in 30 Different Genes**. *Hum Mutat* 2017, **38**(2):216-225.
29. Jiang Z, Gong T, Wei H: **CDKL5 promotes proliferation, migration, and chemotherapeutic drug resistance of glioma cells via activation of the PI3K/AKT signaling pathway**. *FEBS Open Bio* 2019.
30. Tanaka K, Kiguchi K, Mikami M, Aoki D, Iwamori M: **Involvement of the MDR1 gene and glycolipids in anticancer drug-resistance of human ovarian carcinoma-derived cells**. *Hum Cell* 2019, **32**(4):447-452.
31. Zhou SF, Wang LL, Di YM, Xue CC, Duan W, Li CG, Li Y: **Substrates and inhibitors of human multidrug resistance associated proteins and the implications in drug development**. *Curr Med Chem* 2008, **15**(20):1981-2039.
32. Wielinga P, Hooijberg JH, Gunnarsdottir S, Kathmann I, Reid G, Zelcer N, van der Born K, de Haas M, van der Heijden I, Kaspers G *et al*: **The human multidrug resistance protein MRP5 transports folates and can mediate cellular resistance against antifolates**. *Cancer Res* 2005, **65**(10):4425-4430.
33. Wang B, Shen C, Li Y, Zhang T, Huang H, Ren J, Hu Z, Xu J, Xu B: **Oridonin overcomes the gemcitabine resistant PANC-1/Gem cells by regulating GST pi and LRP/1 ERK/JNK signalling**. *Onco Targets Ther* 2019, **12**:5751-5765.
34. Hour TC, Chung SD, Kang WY, Lin YC, Chuang SJ, Huang AM, Wu WJ, Huang SP, Huang CY, Pu YS: **EGFR mediates docetaxel resistance in human castration-resistant prostate cancer through the Akt-dependent expression of ABCB1 (MDR1)**. *Arch Toxicol* 2015, **89**(4):591-605.
35. Kruh GD, Guo Y, Hopper-Borge E, Belinsky MG, Chen ZS: **ABCC10, ABCC11, and ABCC12**. *Pflugers Arch* 2007, **453**(5):675-684.
36. Chen H, Shien K, Suzawa K, Tsukuda K, Tomida S, Sato H, Torigoe H, Watanabe M, Namba K, Yamamoto H *et al*: **Elacridar, a third-generation ABCB1 inhibitor, overcomes resistance to docetaxel in non-small cell lung cancer**. *Oncol Lett* 2017, **14**(4):4349-4354.
37. Kato T, Mizutani K, Kameyama K, Kawakami K, Fujita Y, Nakane K, Kanimoto Y, Ehara H, Ito H, Seishima M *et al*: **Serum exosomal P-glycoprotein is a potential marker to diagnose docetaxel resistance and select a taxoid for patients with prostate cancer**. *Urol Oncol* 2015, **33**(9):385 e315-320.

Figures

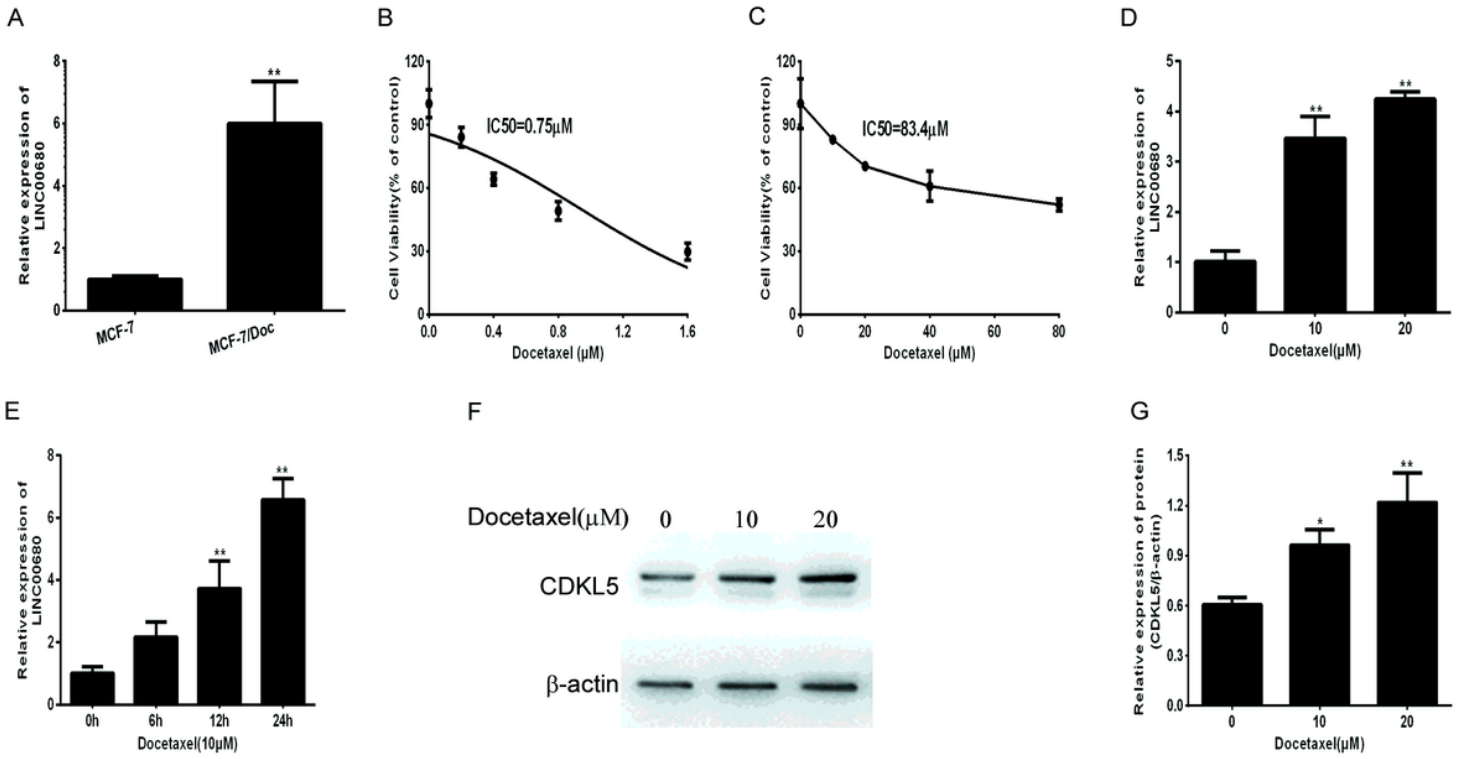

Figure 1

LINC00680 and CDKL5 were both up-regulated in docetaxel resistance BC cells. (A) qRT-PCR assay indicated the levels of LINC00680 in docetaxel resistance BC cells (MCF-7/Doc) compared with its parental cells. (B-C) The survival percentage of MCF-7/Doc and MCF-7 cells when treated with increasing concentration of docetaxel. (D-E) Expression of LINC00680 in MCF-7/Doc cells incubated with different dose of docetaxel or different processed time. (F-G) Western blot showed the expression level of CDKL5 in MCF-7/Doc cells treated with different dose of docetaxel. Data was expressed as mean ± SD, *P<0.05, **P<0.01, presents statistically difference.

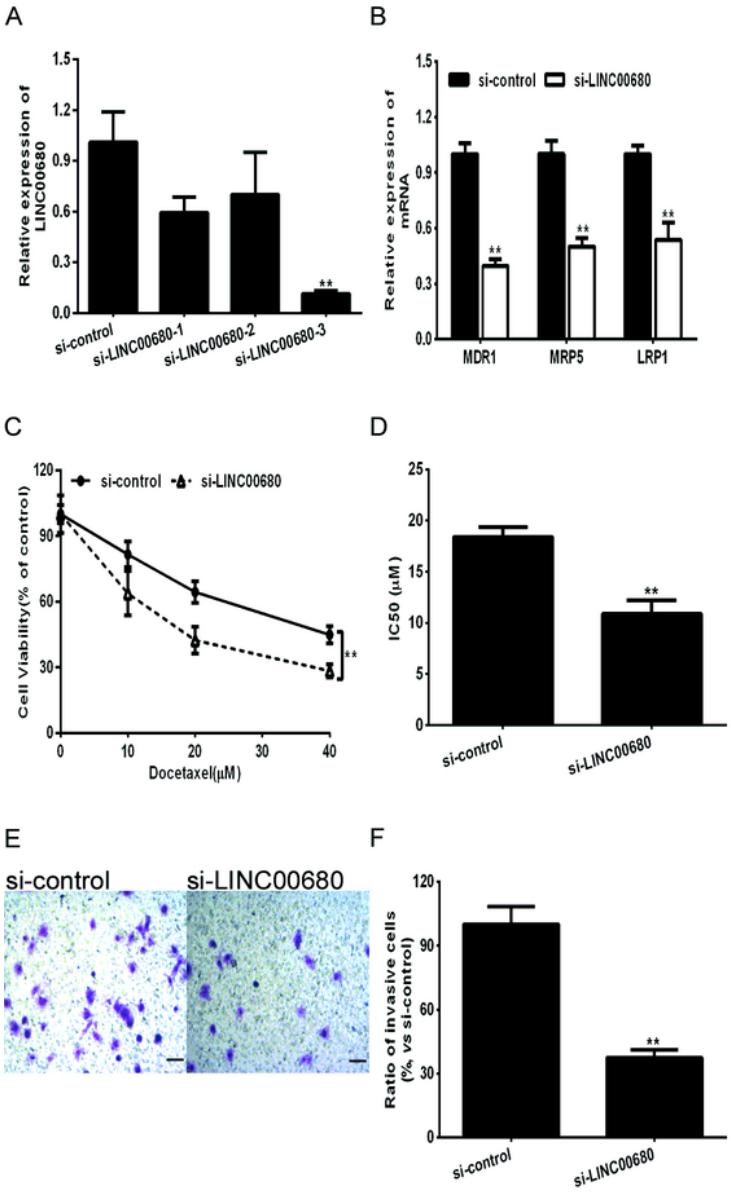

Figure 2

LINC00680 silencing suppressed the docetaxel resistance of BC cells in vitro. (A) qRT-PCR showed LINC00680 expression in MCF-7/Doc cells transfected with si-RNAs. (B) qRT-PCR showed the mRNA levels of drug resistance related genes in MCF-7/Doc cells transfected with si-RNAs. (C) Cell viability measured by CCK-8 assay and IC50 value for docetaxel in MCF-7/Doc cells transfected with si-LINC00680 or empty vector (si-control). (D) Transwell cell invasion assay indicated the invasive cell

number decreased in MCF-7/Doc cells transfected with si-LINC00680 compared with that transfected with empty vector. Data was expressed as mean ± SD, *P<0.05, **P<0.01, presents statistically difference.

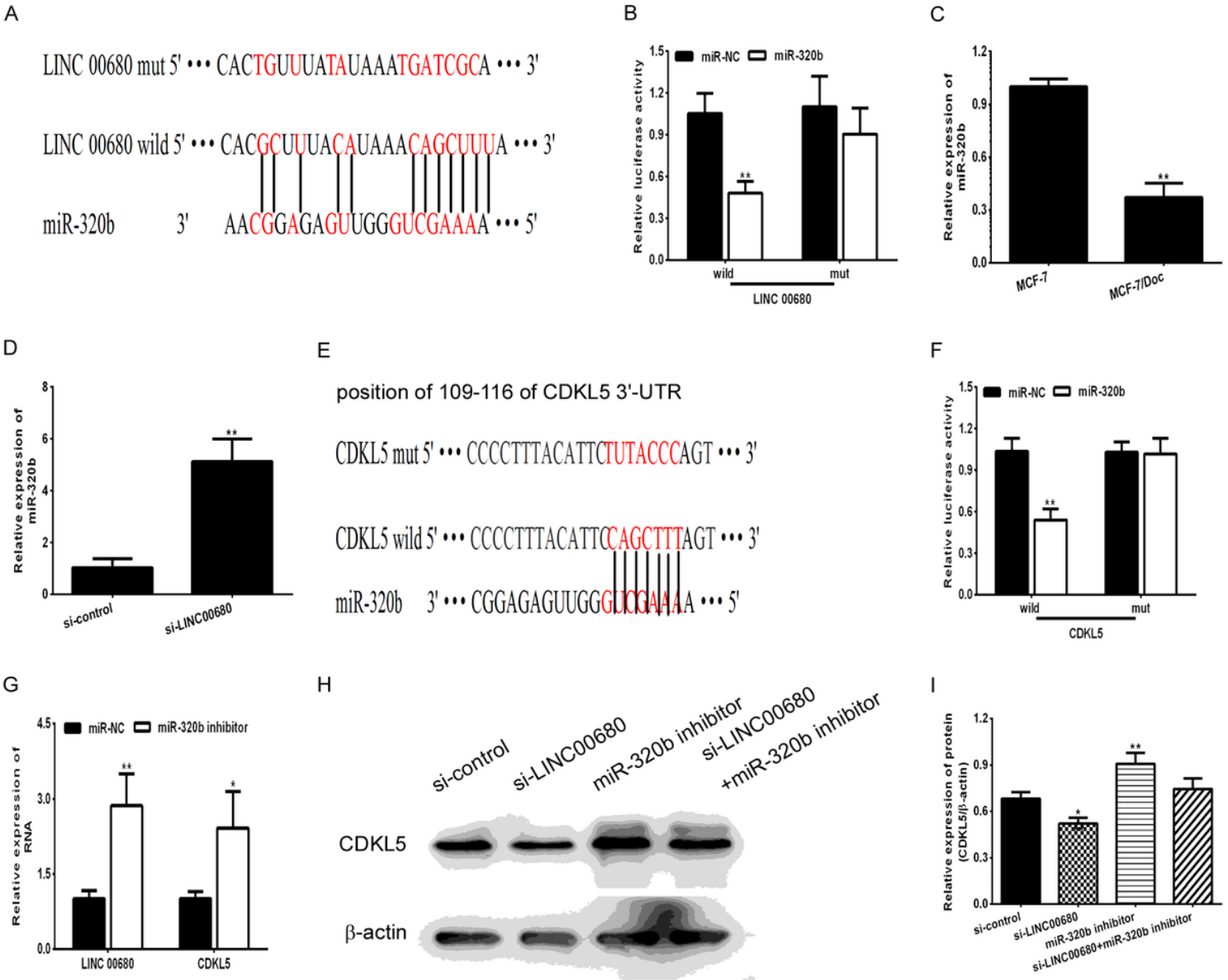

Figure 3

LINC00680 modulated CDKL5 expression via sponging the miR-320b. (A) Bioinformatics tools showed the complementary binding sequence of miR-320b and 3'-UTR of LINC00680 wild type and mutant type. (B) Dual-luciferase reporter assay showed the luciferase vitality within miR-320b/vector and 3'-UTR of LINC00680 mutant or wild type. (C) The levels of miR-320b in MCF-7/Doc cells transfected with si-LINC00680 detected by qRT-PCR assay. (D) Bioinformatics tools showed the complementary binding sequence of miR-320b and CDKL5 3'-UTR. (E) Dual-luciferase reporter assay showed the luciferase vitality within miR-320b/vector and 3'-UTR of CDKL5 mutant or wild type. (F, G) Western blot analysis showed the CDKL5 expression in MCF-7/Doc cells transfected with si-LINC00680 or/ and miR-320b inhibitor. Data was expressed as mean ± SD, *P<0.05, **P<0.01, presents statistically difference.

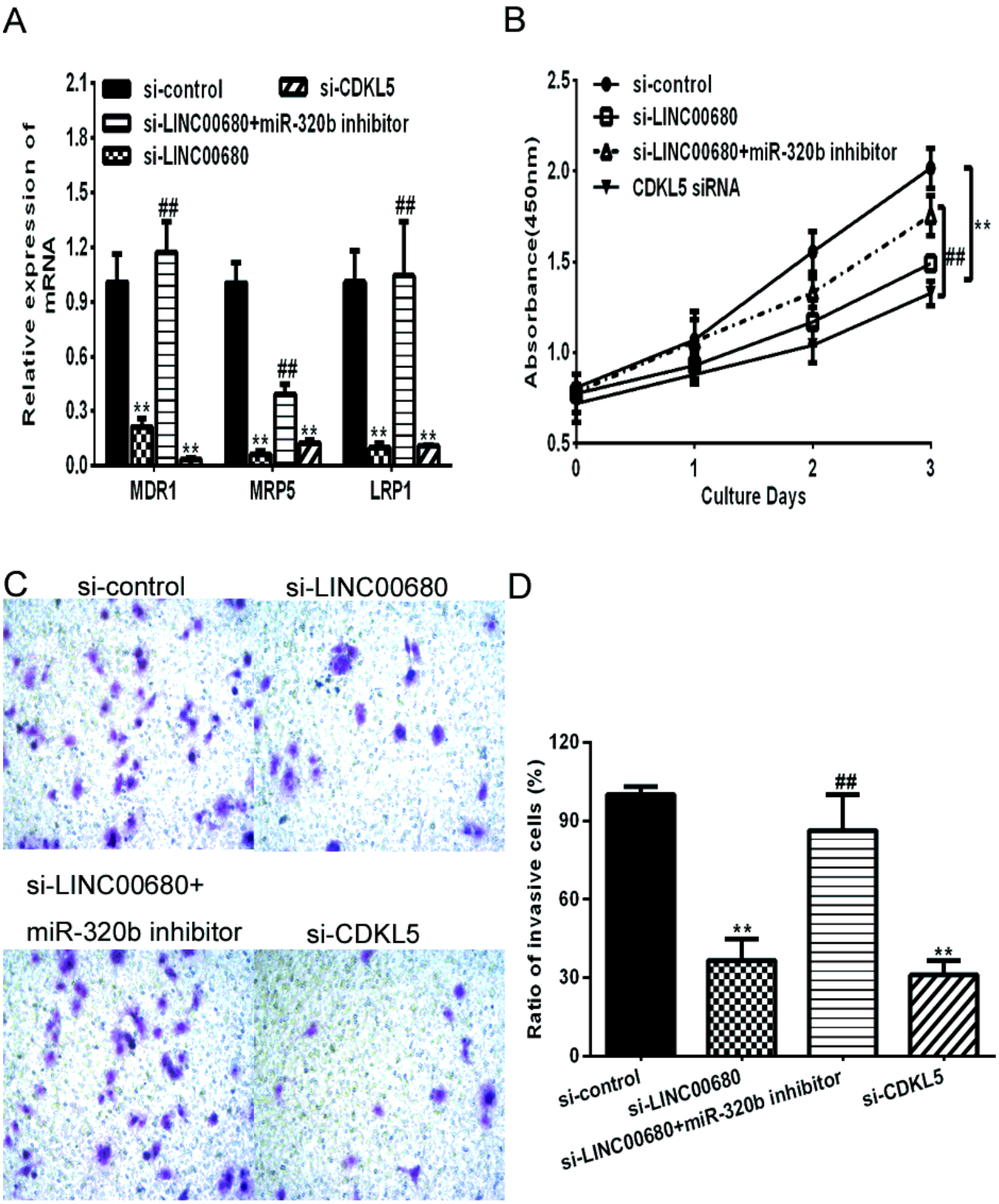

Figure 4

The associated role of LINC00680, miR-320b and CDKL5 on breast cancer proliferation, invasion and docetaxel resistance. (A) qRT-PCR showed the mRNA expression of drug resistance related gene in MCF-7/Doc cells transfected with si-LINC00680, miR-320b inhibitor or CDKL5 siRNA. (B) CCK-8 assay indicated the proliferation vitality of MCF-7/Doc cells. (C) Transwell invasion assay showed the invasive ability of MCF-7/Doc cells transfected with si-LINC00680 and/or miR-320b inhibitor and CDKL5 siRNA.

Data was expressed as mean ± SD, *P<0.05, **P<0.01, presents statistically difference comparing with si-control group. #P<0.05, ##P<0.01, presents statistically difference comparing with si-LINC00680 group.