


Long Non-Coding RNA PVT1 Regulates the Resistance of the Breast Cancer Cell Line MDA-MB-231 to Doxorubicin via Nrf2

Technology in Cancer Research & Treatment
 Volume 19: 1-9
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 DOI: 10.1177/1533033820980763
journals.sagepub.com/home/tct


Ying Luo^{1,2} , Wei Zhang³, Liang Xu², Yajun Chen³, Yao Xu⁴, and Lin Yuan¹

Abstract

Triple-negative breast cancer (TNBC) is one of the most common malignant tumor types in females and its drug resistance is a major clinical issue. An increasing number of long non-coding RNAs (lncRNAs) have been reported as key regulators of drug resistance in TNBC. Plasmacytoma variant translocation 1 (PVT1) has been proved to promote the development of various cancer types. The present study suggested that PVT1 enhances the resistance of the TNBC cell line MDA-MB-231 to doxorubicin and uncovered the molecular mechanism. PVT1 function assays and its target gene analyses were performed. We revealed that PVT1 promoted the protein stability of nuclear factor erythroid 2 like 2 (Nrf2) by inhibiting the binding of kelch-like ECH-associated protein 1 (Keap1) to Nrf2, which is beneficial to the resistance of MDA-MB-231 cells to doxorubicin. These novel results enhance the current knowledge regarding the versatile roles of PVT1 and lay a foundation for future developments of clinical applications.

Keywords

plasmacytoma variant translocation 1, Nrf2, triple-negative breast cancer, doxorubicin, drug-resistant

Abbreviations

TNBC, Triple-negative breast cancer; lncRNAs, long non-coding RNAs; PVT1, Plasmacytoma variant translocation 1; Nrf2, nuclear factor erythroid 2 like 2; Keap1, kelch-like ECH-associated protein 1; NAC, neoadjuvant chemotherapy; RIP RNA, immunoprecipitation; HO-1, heme oxygenase (HO)-1; NQO1, NAD(P)H quinone dehydrogenase 1 (NQO1)

Received: August 05, 2020; Revised: October 07, 2020; Accepted: October 28, 2020.

Introduction

With the advancement of treatment methods, breast cancer has become one of the cancers with high survival rate. As long as most patients can be detected early and receive standard treatment, the 5-year survival rate is as high as 80%. Nevertheless, breast cancer is still one of the most deadly cancers for women.^{1,2} Especially for triple-negative breast cancer (TNBC), which is called “the most difficult breast cancer to treat,” its high rate of metastasis and recurrence leads to the lack of effective treatment.

The standard treatment for TNBC is resection after neoadjuvant chemotherapy (NAC). Chemotherapy is very effective for nearly half of triple-negative breast cancer patients, but the rest of the patients will not respond to neoadjuvant chemotherapy at all. There is no approved new therapy to replace

¹ Hubei Provincial Key Laboratory of Occurrence and Intervention of Rheumatic Diseases, Hubei Minzu University, Enshi, Hubei, China

² College of Biological Science and Technology, Hubei Minzu University, Enshi, Hubei, China

³ School of Medicine, Wuhan University of Science and Technology, Wuhan, Hubei, China

⁴ Institute of Biology and Medicine, Wuhan University of Science and Technology, Wuhan, Hubei, China

Corresponding Authors:

Lin Yuan, Hubei Provincial Key Laboratory of Occurrence and Intervention of Rheumatic diseases, Hubei Minzu University, No.39 Xueyuan Road, Enshi, Hubei, China.

Email: yuanlines@163.com

Yao Xu, Institute of Biology and Medicine, Wuhan University of Science and Technology, No.2 Huangjiahu West Road, Wuhan, Hubei, China.

Email: xuyaowh@wust.edu.cn



Table 1. The Primers for the Realtime PCR.

Primer name		Sequence(5'→3')
β-actin	F	CATGTACGTTGCTATCCAGGC
	R	CTCCTTAATGTCACGCACGAT
Nrf2	F	CGACGGAAAGAGTATGAG
	R	GGCAACCTGGGAGTAG
Keap1	F	GAGGTATGAGCCAGAGCG
	R	CGGCATAAAGGAGACGAT
HO-1	F	GCTGGCAGGAGGTCAT
	R	TTTCTGGGCAATCTTTT
NQO1	F	AAAGGACATCACAGGTAAA
	R	CAGAATGGCAGGGACT
PVT1	F	TGGACGGACTTGAGAAC
	R	CATGGGCAGGGTAGAT

chemotherapy at present. At the same time, 30-50% of the patients who responded to neoadjuvant chemotherapy showed resistance to NAC, resulting in a lower survival rate. Therefore, to explore the resistance mechanism of TNBC resistance contributes to new drug research.

Long-chain non-coding RNA (lncRNA), one of regulatory ncRNA (non-coding RNA, ncRNA), whose length is more than 200 nt and has no protein coding function. It has a specific secondary structure and highly conserved local sequence, variable splicing form and specific subcellular localization. It has been confirmed that lncRNA is involved in many reactions in the body, even in the occurrence, development, metastasis, and drug resistance of tumors.³⁻⁷ The expression of lncRNA BORG correlated positively with chemoresistance in breast cancer.⁸ Overexpression of HCP5 contributed to cisplatin resistance in TNBC cells.⁹ Knocking down of lncRNA H19 could restore chemo-sensitivity in paclitaxel-resistant triple-negative breast cancer cells.¹⁰ Therefore, lncRNAs are a potential targets for TNBC treatment. To explore the relationship and mechanism between lncRNA and drug resistance in TNBC cells is of great significance to prevent the failure of TNBC treatment caused by drug resistance.

Long non-coding RNA plasmacytoma variant translocation 1 (PVT1) is a newly discovered oncogenic factor, and its abnormal expression has been confirmed to be closely related to the occurrence and development of cancer, including cervical cancer, colorectal cancer,¹¹ and lung cancer.¹² However, the molecular mechanisms underlying the role of PVT1 in triple-negative breast cancer, especially in drug resistance, remain unclear. Here, we try to explore the effect and molecular mechanism of lncRNA PVT1 in 3 negative breast cancer cell line MDA-MB-231 cells' drug resistance.

Materials and Methods

Cell Culture

Human breast cancer cells MDA-MB-231 (ATCC® HTB-26) were seeded in Leibovitz's L-15 medium (Gibco.) supplemented with 10% fetal bovine serum (FBS, Gibco) at 37°C in humidified air with 5% CO₂.

Cell Transfection

The full length of the synthesized PVT1 (Sangon Biotech, gene ID: 5820) was inserted into the pcDNA3.1 vector to construct the PVT1 expression plasmid (pcDNA-PVT1). Specific small interfering RNAs targeted PVT1 or Nrf2 and scramble small interfering RNA were purchased from Ribobio Co. (Guangzhou). For transfection experiments, the MDA-MB-231 cells were cultured in growth medium without antibiotics at 60% confluence for 2 days, and then transfected with transfection reagent (Lipofectamine™ 2000, Thermo Scientific, 11668019) according to manufacturer's instructions. After incubation for 6 h, the medium was removed and replaced with normal culture medium for 24 h.

Quantitative Realtime PCR (qRT-PCR)

Total RNA was isolated from cells using Trizol reagent (Invitrogen), and 2 microgram of the sample were reverse-transcribed using M-MLV reverse transcriptase (Invitrogen, 28025021). β-actin was used as an internal control to show equal loading of the cDNA samples. Realtime PCR was performed in an Applied Biosystems Step One™ Real-Time PCR System. Fast SYBR® Green Master Mix was obtained from Applied Biosystems. Data were shown as relative expression level after being normalized by β-actin. The primers for the PCR analysis are listed in Table 1.

Western Blot

MDA-MB-231 cells after treatment were lysed in RIPA lysis (Thermo Scientific). Protein concentrations were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories). Then, 30mg of protein was analyzed by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Sigma) and transferred to polyvinylidene fluoride (PVDF) membranes (Millipore). Transferred membranes were blocked with 5% skim milk (BD) and the membranes were immunoblotted with anti-Keap1 (Abclonal, A17061), anti-β-actin (Abclonal, AC026), anti-Nrf2 (Abclonal, A1244), anti-HO-1 (Abclonal, A11919) and anti-NQO1 (Abclonal, A1518) antibodies overnight at 4°C, and then incubated with secondary antibodies (Abclonal, AS014 or AS003) for 60 min at RT. The specific proteins were visualized by Odyssey Infrared Imaging System (Gene Company Limited). β-actin expression was used as an internal control to show equal loading of the protein samples.

MTT Assay

In the 96 well plate, 10000 MDA-MB-231 cells were inoculated into each hole for 24 h. The cells were then treated with either 1 μg/mL doxorubicin (Sigma). Following 0, 24, 48, 72 h incubation at 37°C, 20 μL MTT solution (Sigma, 5 mg/mL) was added to each well and the plates were re-incubated for 4 h at 37°C. The formazan precipitate was dissolved in DMSO

(Sigma) the absorbance was determined using a microtiter plate reader at 570 and 630 nm for background (Bio-Rad).

CO-IP

Lysates of MDA-MB-231 cells or Cos-7 cells in different groups were incubated with anti-Nrf2 (Abclonal, A1244) and agarose beads (Santa Cruz, sc-2003). Then, the protein complex was eluted from the agarose beads and separated by sodium dodecyl sulfate-poly acrylamide gel electrophoresis (SDS-PAGE), Keap1 antibody was used to visualize Keap1.

Colony Formation Assay

MDA-MB-231 cells or endogenous Nrf2-silenced MDA-MB-231 cells were transfected with PVT1-expressing plasmid or with si-PVT1 interfering RNAs or their corresponding controls, as indicated. Twenty-four hours later, transfected cells were trypsinized, counted and replated at a density of 200 cells per 6 cm dish. Ten days later, colonies resulting from the surviving cells were fixed with 3.7% methanol, and stained with 0.1% crystal violet. Following capturing photos, the crystal violet stain was washed with 33% acetic acid and the absorbance was measured at 570 nm. Each assay was performed in triplicate.

Protein Stability Analysis

MDA-MB-231 cells transfected with PVT1 for 48 h were treated with 100 μ M cycloheximide (CHX) (Sigma) for 30 min. Endogenous Nrf2 was detected by western blot assay.

RNA Pull Down

PVT1 was in vitro transcribed with T7 RNA polymerase (Sigma, 18033019), biotin-labeled with the Biotin RNA Labeling Mix (Thermo Scientific, 89818), treated with RNase-free DNase I (Invitrogen, AM2224), and purified with an RNeasy Mini Kit (Qiagen, 74104). The following RNA-pulldown experiment was carried out according to the instructions (Thermo Scientific, 20164).

RNA Immunoprecipitation (RIP)

For anti-Keap1 RNA immunoprecipitation (RIP), 2×10^7 MDA-MB-231 cells were used with RIP kit (Millipore, 17-700). Normal rabbit IgG antibody was used as the negative control. The final results were detected by realtime PCR.

Statistical Analysis

Data were expressed as mean \pm SEM, accompanied by the number of experiments performed independently. The data of the 2 groups were analyzed by T-test. The data of the 3 groups and above were analyzed by a one-way ANOVA and Tukey test. Differences at $P < 0.05$ were considered statistically significant.

Results

PVT1 Can Improve the Resistance of MDA-MB-231 Cells to Doxorubicin

To illustrate the possible effect of PVT1 in MDA-MB-231, we made PVT1 overexpress or knock down in MDA-MB-231 cells. Realtime PCR was used to evaluate the effect of overexpression or silence of PVT1 (Figure 1A-B). The MTT assay showed the survival rate of PVT1 overexpressed MDA-MB-231 cells increased at 48 and 72 h after 20 μ M doxorubicin treatment (Figure 1C). When PVT1 was silenced, the resistance of MDA-MB-231 cells to doxorubicin decreased (Figure 1D). Further, the colony formation was used to confirm the survival rate of MDA-MB-231 cells with PVT1 overexpression. As shown in Figure 1E, proliferation rate of MDA-MB-231 cells with PVT1 overexpression increased significantly compared with control, after 20 μ M doxorubicin treatment. Instead, the result of colony formation in PVT1 silenced MDA-MB-231 cells decreased (Figure 1F). These data showed that PVT1 can improve the resistance of MDA-MB-231 cells to doxorubicin.

PVT1 Can Activate the Expression of Drug Resistance-Associated Genes

As shown in Figure S1, the expression of PVT1 and Keap1-Nrf2 pathway could be activated in doxorubicin-treated MDA-MB-231 cells. Further, the results of realtime PCR and western blot confirmed that PVT1 could activate the expression of HO-1 and NQO1, 2 genes downstream of Nrf2 related to drug resistance (Figure 2A-B). On the contrary, the expression of HO-1 and NQO-1 were decreased in MDA-MB-231 cells in the condition of PVT1 knock down (Figure 2C-D). Interestingly enough, PVT1 could increase the protein abundance of Nrf2 without affecting the level of Nrf2 mRNA (Figure 2A-D). These suggested that PVT1 might affect MDA-MB-231 cells' resistance through Nrf2. Notably, PVT1 could not effect on the expression of Keap1, which was a key regulatory factor of Nrf2 (Figure 2A-D).

PVT1 Enhances the Resistance of MDA-MB-231 Cells to Doxorubicin via Nrf2

To explore the potential molecular mechanism of PVT1 in MDA-MB-231 cells, siRNAs of Nrf2 were synthesized to silence endogenous Nrf2 (Figure 3A). Subsequently, after PVT1 overexpressed or knocked down in Nrf2 silenced MDA-MB-231 cells, realtime PCR was used to detect the expression of PVT1, Nrf2, HO-1 and NQO1. As a result, PVT1 had no effect on the expression of HO-1 and NQO1 after endogenous Nrf2 silence (Figure 3B-E). Then, as shown in Figure 3F, the resistance of MDA-MB-231 cells with Nrf2 silence to doxorubicin decreased compared with normal cells. Meanwhile, overexpression or silence of PVT1 could not change the toxicity of doxorubicin to MDA-MB-231 cells

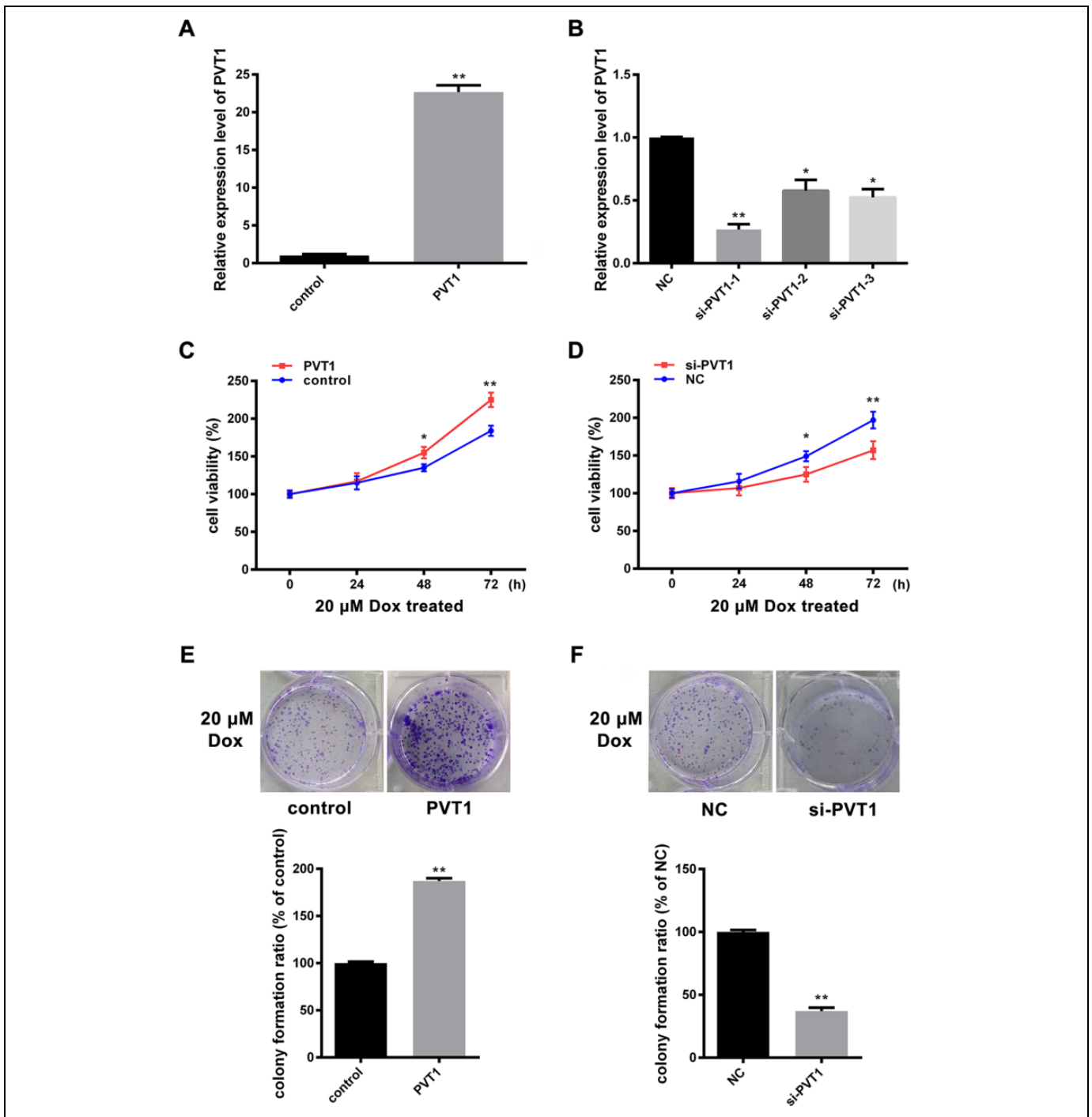


Figure 1. PVT1 can improve the resistance of MDA-MB-231 cells to doxorubicin. A and B, Realtime PCR was used to detect PVT1 overexpression or silencing (relative to β -actin) ($n = 4$, * $P < 0.05$, ** $P < 0.01$). C and D, When PVT1 was overexpressed or knocked down in MDA-MB-231 cells, MTT assay was used to detect the toxicity of doxorubicin to cells ($n = 4$, * $P < 0.05$, ** $P < 0.01$). E and F, When PVT1 was overexpressed or knocked down in MDA-MB-231 cells, the colony formation assay was used to detect the toxicity of doxorubicin to cells ($n = 4$, **, $P < 0.01$).

under the condition of Nrf2 silence. Finally, the results of colony formation assay further confirmed that PVT could not effect on the resistance of Nrf2 silenced MDA-MB-231 cells to doxorubicin (Figure 3G). The above data showed that PVT1 could affect MDA-MB-231 cells' resistance via Nrf2.

PVT1 Can Prevent Nrf2 Protein From Being Degraded by Competitive Binding With Keap1 for Nrf2 Protein

Based on the above results, the remaining puzzle is how PVT1 regulates the protein level of Nrf2. As predicted with

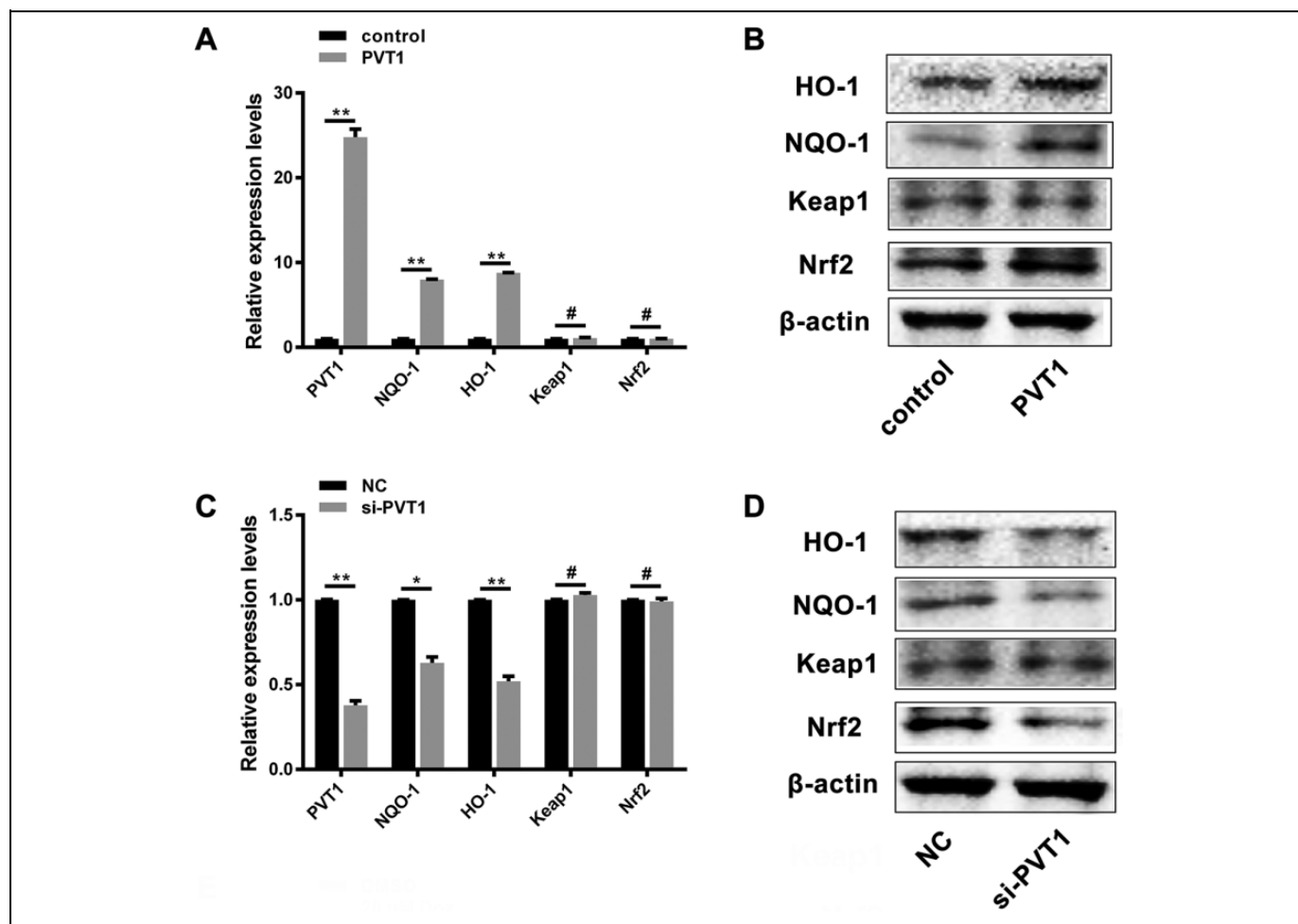


Figure 2. PVT1 can activate the expression of drug resistance-associated genes. A and C, Realtime PCR was used to determine the mRNA levels of PVT1, Keap1, Nrf2, HO-1 and NQO1 after overexpressing or knocking down PVT1 in MDA-MB-231 cells ($n = 3$, *, $P < 0.05$, **, $P < 0.01$, #, $P > 0.05$). B and D, Western blot was used to determine the protein levels of Keap1, Nrf2, HO-1 and NQO1 after overexpressing or knocking down PVT1 in MDA-MB-231 cells.

bioinformatics tool (<http://priddb.gdcb.iastate.edu/RPISeq/index.html>), there might exist RNA-protein interaction between PVT1 and Keap1. However, no potential interaction between PVT1 and Nrf2 was predicted. As known, Keap1 could directly induce the degradation of Nrf2 protein. These indicated that PVT1 might competitively inhibit the binding of Keap1 and Nrf2. As shown in Figure 4A and B, RIP and RNA-pulldown assay confirmed PVT1 could bind to Keap1. Further, we confirmed that the overexpression of PVT1 could inhibit the endogenous protein binding of Keap1 and Nrf2 by CO-IP experiment in MDA-MB-231 cells (Figure 4C). Finally, Keap1 and Nrf2 were co-transfected into Cos-7 cells with or without PVT1 existed. As shown in Figure 4D, the protein-protein interaction of Keap1 and Nrf2 was inhibited with PVT1 existed. These results showed PVT1 could prevent Nrf2 protein from being degraded by competitively inhibiting the binding of Keap1 and Nrf2. Moreover, the PVT1 overexpressed MDA-MB-231 cells were treated with cycloheximide (100 μ M) for 30 min. Then, the western blot results showed overexpression

of PVT1 in MDA-MB-231 cells significantly inhibited the degradation of Nrf2 protein with cycloheximide treated (Figure S2). So, these results confirmed PVT1 could prevent Nrf2 protein from being degraded by competitive binding with Keap1 for Nrf2 protein.

Discussion

Tri-negative breast cancer predominates in young women under 40 years of age, accounting for 15-25% of all breast cancer types. Both endocrine therapy and targeted therapy for HER-2 are ineffective for this type of cancer.¹³⁻¹⁵ Drug resistance is a very difficult problem in basic research and clinical management of breast cancer. In the process of chemotherapy, endocrine therapy and molecular targeted therapy, the occurrence of drug resistance will seriously affect the efficacy of treatment, leading to recurrence, metastasis and even death of TNBC patients.¹⁶⁻¹⁹ Therefore, studies on the mechanism of drug resistance in TNBC are expected to break the current

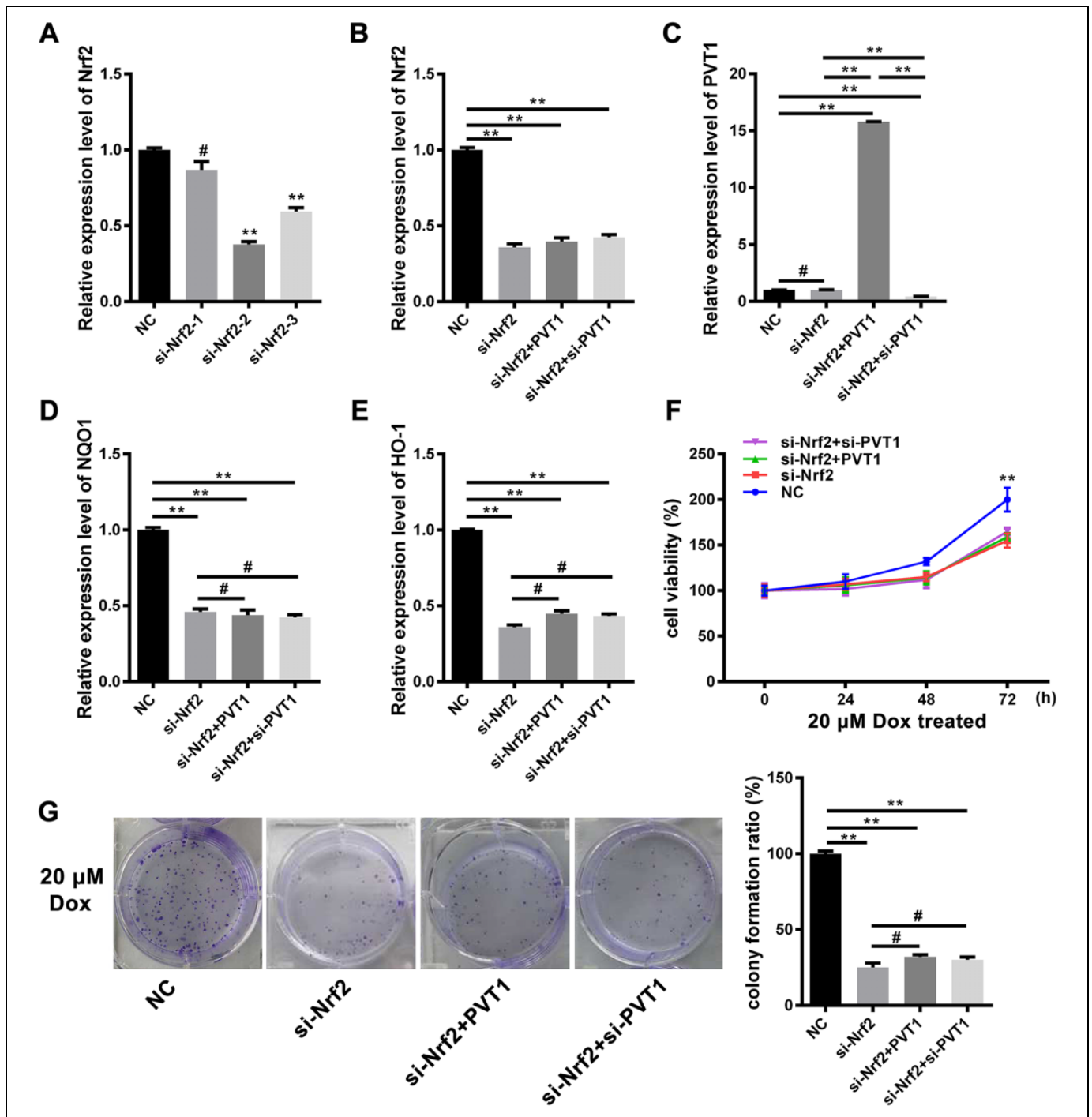


Figure 3. PVT1 enhances the resistance of MDA-MB-231 cells to doxorubicin via Nrf2. A, The detection of related siRNA interference effect for Nrf2 (n = 4, ** P < 0.01, # P > 0.05). B-E, After endogenous Nrf2 knocking down, the mRNA levels of PVT1, Nrf2, HO-1 and NQO1 were measured by realtime PCR following PVT1 overexpression or knock down (n = 4, **, P < 0.01, #, P > 0.05). F, After transfected with PVT1 or si-PVT1, MTT was used to detect the toxicity of doxorubicin to MDA-MB-231 cells with Nrf2 silence (n = 4, **, P < 0.01). G, Overexpression or knockdown of PVT1 in MD-MB-231 cells silencing endogenous Nrf2, the colony formation assay was used to detect the toxicity of doxorubicin to cells (n = 4, **, P < 0.01, #, P > 0.05).

situation of treatment failure caused by drug resistance in breast cancer treatment, and provide a strong guarantee for the therapeutic efficacy, prognosis and long-term survival of breast cancer patients.

Not only transcription factors lead to the development and drug resistance of breast cancer, but also non-coding RNAs, such as microRNAs and lncRNAs, are also closely related to the development and drug resistance of breast cancer.²⁰⁻²⁴ In

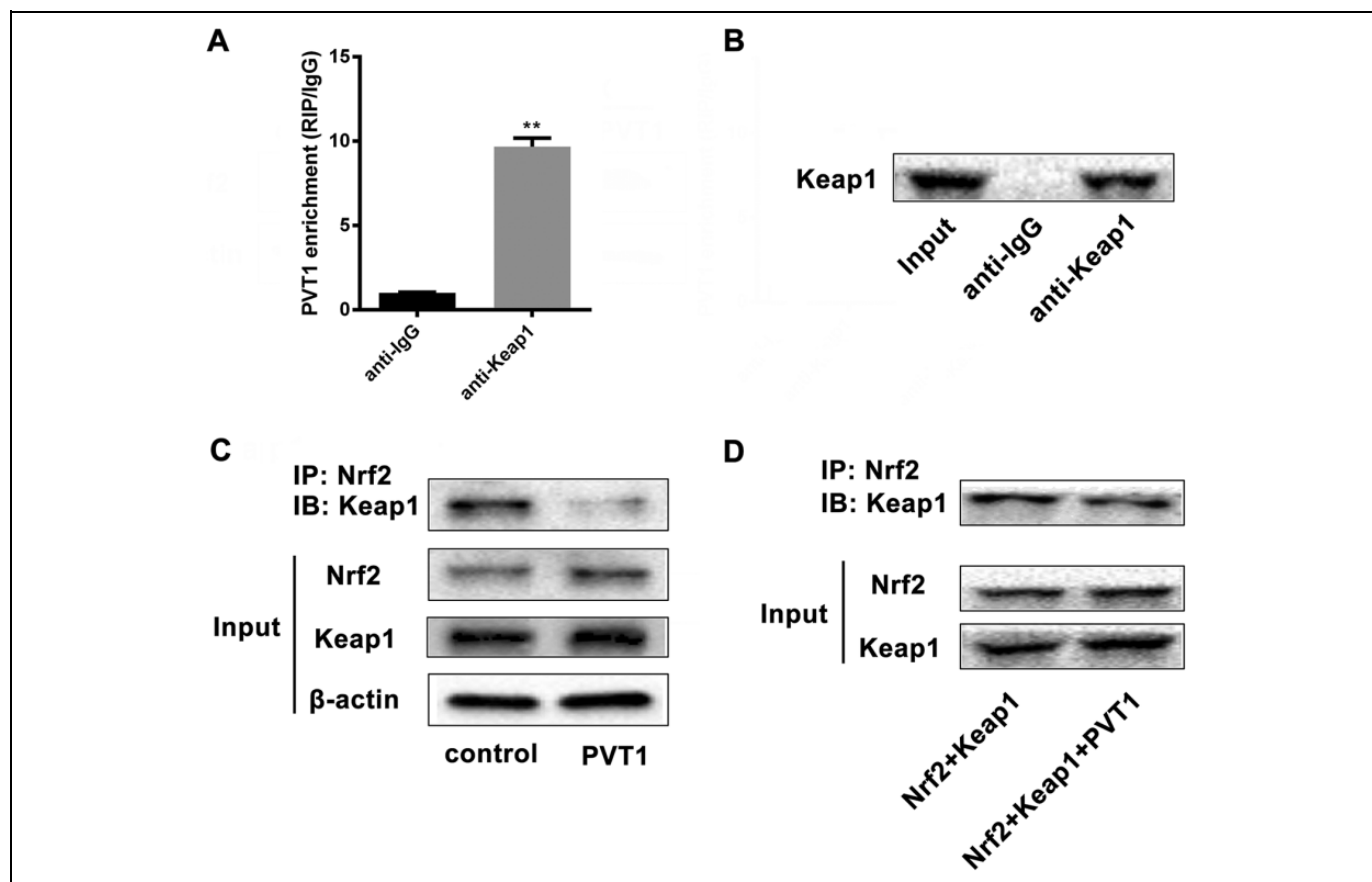


Figure 4. PVT1 can prevent Nrf2 protein from being degraded by competitive binding with Keap1 for Nrf2 protein. A, RIP assay was used to detect the combination of Keap1 and PVT1 ($n = 4$, **, $P < 0.01$). B, RNA-pulldown experiment using cell lysate of MDA-MB-231 cells followed by western blot of indicated proteins. C, CO-IP was used to detect protein-protein interactions between Keap1 and Nrf2 in MDA-MB-231 cells with or without PVT1 overexpression. D, After Keap1 and Nrf2 were transfected into Cos-7 cells with or without PVT1 overexpression, CO-IP was used to detect protein-protein interactions between Keap1 and Nrf2.

recent years, more and more evidences have shown that lncRNAs play an important role in mediating and regulating drug resistance in breast cancer.²⁵ LncRNA HOTAIR could transmit tamoxifen resistance by increasing ER protein levels and enhancing chromatin binding in breast cancer.²⁶ In MDA-MB-231 cells, down-regulated lncRNA-ROR could enhance the sensibility of breast cancer cells to tamoxifen by increasing miR-205 expression and suppressing the expressions of ZEB1 and ZEB2.²⁷ Abnormal expression of lncRNAs can mediate drug resistance of breast cancer through a variety of different pathways. Intervention of abnormally expressed lncRNAs can change the drug resistance behavior of breast cancer. Therefore, searching for drug resistance-related lncRNAs and their molecular mechanisms to regulate drug resistance will help improve the clinical efficiency and open up new research fields for exploring the theory of drug resistance in breast cancer. Here, we found that lncRNA PVT1 could promote the drug resistance of MDA-MB-231 cells by preventing Nrf2 protein from degradation via inhibiting the binding of Keap1 and Nrf2. This finding is helpful for the development and application of PVT1 in clinic related to TNBC resistance.

In recent years, the Keap1/Nrf2/ARE signaling pathway, one of the important antioxidant stress systems in the body, has been confirmed to be closely related to drug resistance of tumor cells.²⁸⁻³² Keap1 is a negative regulator of Nrf2, which can bind Nrf2 protein in the form of homodimers and recruit E3 ubiquitin ligase to mediate the ubiquitination degradation of Nrf2.³³ Through bioinformatics analysis, we found that there might be an RNA-protein interaction between PVT1 and Keap1. Here, we revealed that PVT1 could competitively bind Keap1 with Nrf2 to inhibit the degradation of Nrf2 protein, which could activate the expression of downstream drug resistance-associated genes and enhance the drug resistance of breast cancer cells. However, limited by the experimental conditions, we failed to carry out relevant animal experiments and clinical trials to further confirm the relationship between PVT and breast cancer resistance *in vivo*.

Collectively, our study revealed a new molecular mechanisms of PVT1 regulating resistance in triple-negative breast cancer. Specifically, lncRNA PVT1 could improve the resistance of MDA-MB-231 cells to doxorubicin by preventing Nrf2 protein from degradation via inhibiting the binding of

Keap1 and Nrf2. These findings may provide a theoretical basis for the development of PVT1 as a target for diagnosis and treatment in clinical.

Authors' Note

Ying Luo and Wei Zhang contributed equally to this manuscript. Our study did not require an ethical board approval because it did not contain human or animal trials.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the Open Research Fund of Hubei Provincial Key Laboratory of Occurrence and Intervention of Rheumatic Diseases (Hubei Minzu University) (No. PT022005) and National Natural Science Foundation of China (No.81860757).

Supplemental Material

Supplemental material for this article is available online.

ORCID iD

Ying Luo  <https://orcid.org/0000-0003-1878-7641>

References

- Chen W. Cancer statistics: updated cancer burden in China. *Chin J Cancer Res.* 2015; 27(1): 1.
- Feng RM, Zong YN, Cao SM, Xu RH. Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics? *Cancer Commun.* 2019; 39(1):22.
- Liang Y, Song X, Li Y, et al. LncRNA BCRT1 promotes breast cancer progression by targeting miR-1303/PTBP3 axis. *Mol Cancer.* 2020;19(1): 85.
- Yu Y, Chen Q, Zhang X, et al. Long non-coding RNA ANRIL promotes the malignant progression of cholangiocarcinoma by epigenetically repressing ERRFI1 expression. *Cancer Sci.* 2020; 111(7):2297-2309.
- Guo J, Ding Y, Yang H, Guo H, Zhou X, Chen X. Aberrant expression of lncRNA MALAT1 modulates radioresistance in colorectal cancer in vitro via miR-101-3p sponging. *Exp Mol Pathol.* 2020;115:104448.
- Bhan A, Soleimani M, Mandal SS. Long noncoding RNA and cancer: a new paradigm. *Cancer Res.* 2017;77(15):3965-3981.
- Peng WX, Koirala P, Mo YY. LncRNA-mediated regulation of cell signaling in cancer. *Oncogene.* 2017;36(41):5661-5667.
- Gooding AJ, Zhang B, Gunawardane L, Beard A, Valadkhan S, Schiemann WP. The lncRNA BORG facilitates the survival and chemoresistance of triple-negative breast cancers. *Oncogene.* 2019;38(12):2020-2041.
- Wu J, Chen H, Ye M, et al. Long noncoding RNA HCP5 contributes to cisplatin resistance in human triple-negative breast cancer via regulation of PTEN expression. *Biomed Pharmacother.* 2019;122:109789.
- Han J, Han B, Wu X, et al. Knockdown of lncRNA H19 restores chemo-sensitivity in paclitaxel-resistant triple-negative breast cancer through triggering apoptosis and regulating Akt signaling pathway. *Toxicol Appl Pharmacol.* 2018;359:55-61.
- Pan X, Cheng R, Zhu X, et al. Prognostic significance and diagnostic value of overexpressed lncRNA PVT1 in colorectal cancer. *Clin Lab.* 2019;65(12). doi: 10.7754/Clin.Lab.2019.190412
- Wei CM, Zhao XF, Qiu HB, Ming Z, Liu K, Yan J. The long non-coding RNA PVT1/miR-145-5p/ITGB8 axis regulates cell proliferation, apoptosis, migration and invasion in non-small cell lung cancer cells. *Neoplasma.* 2020;67(4):802-812.
- von Minckwitz G, Untch M, Nuesch E, et al. Impact of treatment characteristics on response of different breast cancer phenotypes: pooled analysis of the German Neo-Adjuvant Chemotherapy Trials. *Breast Cancer Res Treat.* 2011;125(1):145-156.
- Andre F, Zielinski CC. Optimal strategies for the treatment of metastatic triple-negative breast cancer with currently approved agents. *Ann Oncol.* 2012; 23(Suppl 6):vi46-51.
- Fujii T, Kogawa T, Dong W, et al. Revisiting the definition of estrogen receptor positivity in HER2-negative primary breast cancer. *Ann Oncol.* 2017; 28(10):2420-2428.
- Carey LA, Dees EC, Sawyer L, et al. The triple-negative paradox: primary tumor chemosensitivity of breast cancer subtypes. *Clin Cancer Res.* 2007;13(8):2329-2334.
- Liedtke C, Mazouni C, Hess KR, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. *J Clin Oncol.* 2008;26(8):1275-1281.
- Galvez M, Castaneda CA, Sanchez J, et al. Clinicopathological predictors of long-term benefit in breast cancer treated with neoadjuvant chemotherapy. *World J Clin Oncol.* 2018;9(2):33-41.
- Silver DP, Richardson AL, Eklund AC, et al. Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. *J Clin Oncol.* 2010;28(7):1145-1153.
- Wang J, Ye C, Xiong H, et al. Dysregulation of long non-coding RNA in breast cancer: an overview of mechanism and clinical implication. *Oncotarget.* 2017;8(3):5508-5522.
- Chen X, Lu P, Wang DD, et al. The role of miRNAs in drug resistance and prognosis of breast cancer formalin-fixed paraffin-embedded tissues. *Gene.* 2016;595(2):221-226.
- Diab TAA, Alkafaas SS, Shalaby TI, et al. Paclitaxel nanoparticles induce apoptosis and regulate txr1, cyp3a4 and cyp2c8 in breast cancer and hepatoma cells. *Anticancer Agents Med Chem.* 2020;20(13):1582-1591.
- Barzaman K, Karami J, Zarei Z, et al. Breast cancer: biology, biomarkers, and treatments. *Int Immunopharmacol.* 2020;84:106535.
- Aggarwal T, Wadhwa R, Gupta R, et al. MicroRNAs as biomarker for breast cancer. *Endocr Metab Immune Disord Drug Targets.* 2020.
- Wu C, Luo J. Long non-coding RNA (lncRNA) urothelial carcinoma-associated 1 (UCA1) enhances tamoxifen resistance in breast cancer cells via inhibiting mTOR signaling pathway. *Med Sci Monit.* 2016;22:3860-3867.
- Xue X, Yang YA, Zhang A, et al. LncRNA HOTAIR enhances ER signaling and confers tamoxifen resistance in breast cancer. *Oncogene.* 2016;35(21):2746-2755.
- Zhang HY, Liang F, Zhang JW, Wang F, Wang L, Kang XG. Effects of long noncoding RNA-ROR on tamoxifen resistance of

- breast cancer cells by regulating microRNA-205. *Cancer Chemother Pharmacol.* 2017;79(2):327-337.
28. Ge W, Zhao K, Wang X, et al. iASPP is an antioxidative factor and drives cancer growth and drug resistance by competing with Nrf2 for Keap1 binding. *Cancer Cell.* 2017;2(5):561-573.
 29. Leinonen HM, Kansanen E, Pölonen P, Heinäniemi M, Levonen AL. Role of the Keap1-Nrf2 pathway in cancer. *Adv Cancer Res.* 2014;122:281-320.
 30. Roh JL, Kim EH, Jang H, Shin D. Nrf2 inhibition reverses the resistance of cisplatin-resistant head and neck cancer cells to artesunate-induced ferroptosis. *Redox Biol.* 2017;11:254-262.
 31. Wan ZH, Jiang TY, Shi YY, et al. RPB5-mediating protein promotes cholangiocarcinoma tumorigenesis and drug resistance by competing with NRF2 for KEAP1 binding. *Hepatology.* 2020; 71(6):2005-2022.
 32. Huang H, Wu Y, Fu W, et al. Downregulation of Keap1 contributes to poor prognosis and axitinib resistance of renal cell carcinoma via upregulation of Nrf2 expression. *Int J Mol Med.* 2019; 43(5):2044-2054.
 33. Lu K, Alcivar AL, Ma J, et al. Nrf2 induction supporting breast cancer cell survival is enabled by oxidative stress-induced DPP3-Keap1 interaction. *Cancer Res.* 2017;77(11):2881-2892.