Long non-coding RNA TMPO-AS1 facilitates chemoresistance and invasion in breast cancer by modulating the miR-1179/TRIM37 axis

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Abstract. Breast cancer has become the most common female tumor in the world. Although great progress has been made in the past decade, the treatment of advanced breast cancer remains unsatisfactory. An increasing number of reports have indicated that long non-coding RNAs (lncRNAs) have a pivotal role in chemoresistance as potential oncogenes in numerous types of cancer. However, the precise mechanisms underlying the action of lncRNAs in breast cancer resistance to chemotherapy have yet to be fully elucidated. In the present study, the function and molecular mechanisms of the lncRNA TMPO-antisense RNA 1 (AS1) in terms of its resistance to docetaxel (DOC) were explored in the MDA-MB-231 and MCF7 breast cancer cell lines. The results obtained suggested that TMPO-AS1 was markedly upregulated in DOC-resistant breast cancer cells compared with the sensitive breast cancer cells. Functionally, TMPO-AS1-knockdown sensitized MDA-231/DOC and MCF-7/DOC cells to DOC and suppressed cell invasion, with increased rates of DOC-induced apoptosis. Mechanistically, TMPO-AS1-downregulation induced DOC-sensitivity in breast cancer cells via depleting tripartite motif-containing protein 37 (TRIM37) by sponging microRNA (miR)-1179. Taken together, the present study has revealed the existence of a novel TMPO-AS1/miR-1179/TRIM37 molecular axis conferring DOC resistance of breast cancer cells, thereby suggesting a promising novel therapeutic target for breast cancer.

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Introduction

Breast cancer is one of the most commonly diagnosed cancer among women and the fifth cause of cancer-associated death worldwide (1). Although systemic therapies, including surgery, chemo/radiotherapy and targeted therapy, lead to marked improvements in clinical outcome, the survival rates of patients with breast cancer remain unsatisfactory in developing countries, considering that breast cancer in developing countries accounts for 50% of all breast cancer cases and 62% of cancer-associated deaths (2). Docetaxel (DOC), an effective chemotherapeutic drug, is indispensable for standard treatment in breast cancer, according to the latest National Comprehensive Cancer Network guidelines (3). However, the presence of chemoresistance is considered as one of the barriers that leads to poor efficacy (4). Therefore, it is imperative to gain a deeper understanding of the key molecular mechanisms associated with chemoresistance in breast cancer.

Long non-coding RNAs (lncRNAs) are a class of non-coding RNAs >200 nucleotides in length (5). Evidence has indicated that lncRNAs have a critical role in tumorigenesis, serving either as oncogenes or tumor suppressors in different types of cancer, such as gastric, liver and colon cancer (6,7). In addition, lncRNAs are also associated with chemoresistance of tumors (8). For example, the lncRNA Colorectal Neoplasia Differentially Expressed has been shown to facilitate colorectal cancer chemoresistance through the Wnt/β-catenin signaling pathway (9). Metastasis-associated lung adenocarcinoma transcript 1 modulates autophagy-associated chemoresistance in gastric cancer (10), whereas the lncRNA Urothelial Cancer Associated 1 promotes cisplatin resistance in oral squamous cell carcinoma by inhibiting miR-184 (11).

There are numerous different forms of lncRNAs that are involved in the processes of biological regulation, including cell proliferation, apoptosis and cell cycle regulation, among which antisense (AS) lncRNA is usually reverse-transcribed from the corresponding gene (7). TMPO-AS1, located on chromosome 12, was previously identified in human lung cancer (12). Emerging evidence has indicated that TMPO-AS1 could act as an indicator of the prognosis of patients with lung cancer through regulation of the cell cycle and cell adhesion (13). Another study demonstrated that TMPO-AS1 served

an oncogenic role and may be a potentially novel prognostic biomarker as well as a therapeutic target in prostate cancer (14). However, the function and mechanism of TMPO-AS1 in DOC resistance and invasion in breast cancer cells has yet to be fully elucidated. In addition, tripartite motif-containing protein 37 (TRIM37) has been shown to be involved in the development of breast cancer (15), but the role of TRIM37 in breast cancer chemotherapy is unclear.

The purpose of the present study was to investigate the association between TMPO-AS1 and breast cancer drug resistance, and to identify the key targets and molecular axes of TMPO-AS1 affecting breast chemotherapy resistance. The current findings may provide novel insights into the mechanism in which breast cancer acquires chemoresistance, facilitating the development of novel therapeutical drugs for breast cancer.

Materials and methods

Cell lines and cell culture. Breast cancer cell lines (MDA-MB-231 and MCF7 cell lines) were acquired from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. Cells were treated with increasing concentrations of DOC (20-1,000 nM) for 48 h. The surviving DOC-resistant sublines, MDA-231/DOC and MCF-7/DOC, were established successfully. The cells cultured in DMEM medium (Nanjing KeyGen Biotech. Co., Ltd.) were supplemented with 10% fetal bovine serum (FBS) (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C in a humidified atmosphere of 5% CO_2.

Cell transfection. pcDNA3.1-TMPO-AS1, pcDNA3.1-TRIM37 and empty vector plasmids (2 μg used for transfection) were acquired from GeneCopoeia, Inc. In total, three siRNAs specifically targeting TMPO-AS1 (10 nM; si-TMPO-AS1 #1, 5'-CCGCCAAACGCCCGCCTTT-3'; si-TMPO-AS1 #2, 5'-AGGTAGAAACGCAGTTTAA-3'; and si-TMPO-AS1 #3, 5'-GAGCCGAACUACGAACCAATT-3') or TRIM37 (10 nM; si-TRIM37, 5'-GGAGAAGATTCAGAATGAA-3') and scrambled siRNA negative control (10 nM; si-NC, 5'-UUCUCC GAACGUGUCACGU-3'), miR-1179 mimic (10 nM; 5'-AAG CAUUCUUUCAUUGGUUGG-3'), scrambled miRNA control (10 nM; miR-NC, 5'-AGGTAGAAACGCAGTTTA A-3'), miR-1179 inhibitor (10 nM; miR-inhibitor, 5'-CAG UACUUUUGUGUAGUACAA-3') and scrambled inhibitor control (10 nM; inhibitor-NC, 5'-CAGUACUUUUGUGUA GUACAA-3') were acquired from Shanghai GenePharma Co., Ltd. All transfection processes were performed with Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 48 h according to the manufacturer's instructions. Cells were subjected to subsequent experimentation 48 h following transfection.

Reverse transcription-quantitative PCR analysis (RT-qPCR). Total RNA from the breast cancer cells was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). First-strand cDNA was synthesized with Reverse Transcriptase XL (Takara Bio, Inc.) in the presence of random primers and oligo(dT) with the following temperature protocol: 30 min at 16˚C, 30 min at 42˚C and 5 min at 85˚C. RT-qPCR was conducted on an ABI 7300 real-time PCR machine (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the SYBR Green reaction mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). β-actin was used as the internal reference of lncRNA and mRNA qPCR, while U6 as the internal reference of miRNA. Primers for TMPO-AS1 were as follows: Forward, 5'-AGCCCACACACTACAGGCA-3' and reverse, 5'-GCACAAAAGCAGTACGACCT-3'. Primers for miR-1179 as follows: Forward, 5'-AAGCATTCTTTCATT GGTTGGA-3' and reverse CTCTACAGCTATATTGCCAGC CAC. Primers for U6 were: Forward, 5'-TGCGGGTGCTCG CTTCGGCAGC-3' and reverse 5'-GTGCAGGGTCCGAGG T-3'. Primers for β-actin were: Forward, 5'-CCTCTCCCAAGT CCACACAG-3' and reverse, 5'-GGGCACGAAGGCTCATCA TT-3'. Primers for TRIM37 were: Forward, 5'-TGGACTTAC TCGCAAATG-3' and reverse: 5'-ATCTGGTGGTGACAA ATC-3'. The following thermocycling conditions were used for PCR: Pre-denaturation at 93˚C for 15 sec, then denaturation at 90˚C for 25 sec, annealing at 58˚C for 25 sec and extension at 72˚C for 30 sec for 35 cycles. The $2^{-\Delta\Delta Cq}$ method was used to analyze gene expression levels (16).

DOC-resistance analysis. In vitro cancer cell proliferation was detected using a Cell Counting Kit-8 (CCK-8) assay. Briefly, the proliferation of non-DOC-treated cancer cells was regarded as the 100% standard. MDA-231/DOC and MCF-7/DOC cells were processed with various concentrations of DOC in order to determine the half-maximal inhibitory concentration (the IC_{50} value). Subsequently, following incubation at room temperature for 48 h, cell viability was detected with CCK-8 (Dojindo Molecular Technologies, Inc.), following the manufacturer's instructions. The absorbance of the breast cancer cells was measured at 450 nm using a microplate reader (Bio-Rad Laboratories, Inc.).

Western blot analysis. The cancer cells were first lysed in RIPA buffer incubated with cocktail protease inhibitors (both from Beyotime Institute of Biotechnology) in order to extract the total protein. Subsequently, proteins (30 μg/lane) were separated via 10% SDS-PAGE and then blotted onto a PVDF membrane (EMD Millipore). Subsequently, the PVDF membrane was probed with primary antibodies (anti-TRIM37, dilution 1:1,000; Abcam) was incubated at 4˚C overnight. The membrane was then incubated with the HRP-conjugated goat anti-rabbit IgG secondary antibody (1:1,000; cat. no. ab205718; Abcam) at room temperature for 2 h. Finally, blots were detected and visualized with an ECL detection kit (Santa Cruz Biotechnology, Inc.).

Transwell assay. The transfected MDA-231/DOC and MCF-7/DOC cells were rinsed with PBS and incubated at 37˚C for 1 h with serum-free DMEM. Subsequently, the cell suspension ($5x10^4$ cells) was transferred to the upper chamber of a Boyden apparatus (BD Biosciences), with the lower chamber filled with Complete™ medium containing 20% FBS (Gibco; Thermo Fisher Scientific, Inc.). After the non-migrating cells had been cleared from the upper chamber, the lower chamber of migrating cells were incubated with 4% methanol for 20 min at room temperature, followed by staining with 1% crystal violet for 20 min at

room temperature. The stained cells were then observed and counted using an inverted light microscope (magnification, x200; Olympus Corporation).

Apoptosis analysis. MDA-231/DOC and MCF-7/DOC cells were incubated in six-well plates ($2x10^4$ cells/well) for 24 h, followed by treatment with 50 ng/l DOC at room temperature for 24 h. The harvested cells were subsequently washed thrice with pre-cooled PBS. The cells were treated with 3 ml annexin V for 10 min at room temperature in the dark, followed by an incubation with 2 ml propidium iodide (PI) at room temperature for 5 min. Subsequently, the extent of apoptosis with annexin V and PI was determined in the dark through analysis using an Attune NxT flow cytometer (Thermo Fisher Scientific, Inc.). The apoptosis results were analyzed using FlowJo version 10.4 (FlowJo LLC).

Target prediction and luciferase reporter assay. Starbase version 3.0 (http://starbase.sysu.edu.cn/) and TargetScan (www.targetscan.org; Human 7.2) databases were used to predict the potential binding sites. The pMIR-REPORT Luciferase vector (Promega Corporation) of TMPO-AS1-wild-type (Wt) or TMPO-AS1-mutant (Mut) reporters were co-transfected with NC mimics or miR 1179 mimics into 293T cells (The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences) with Lipofectamine 2000 as aforementioned. Similarly, pMIR-TRIM37-Wt or pMIR-TRIM37-Mut reporters were co-transfected with NC mimics or miR-1179 mimics into 293T cells with Lipofectamine 2000. After 36 h, the relative luciferase activity was detected using a Dual Luciferase Reporter assay system (Promega Corporation) and normalized to *Renilla* luciferase activity.

Statistical analysis. The data are presented as the mean ± SD with three independent experiments. All data were analyzed using GraphPad Prism software, version 7.0 (GraphPad Software, Inc.). Unpaired Student's t-test or one-way ANOVA analyses followed by Tukey's post hoc test were performed to distinguish differences between two and three or more groups, respectively. P<0.05 was considered to indicate a statistically significant difference.

Results

TMPO-AS1 levels are markedly elevated in DOC-resistant breast cancer cells. To confirm whether TMPO-AS1 was associated with DOC resistance in breast cancer, DOC-resistant MDA-231/DOC and MCF-7/DOC breast cancer cell lines were constructed. The IC_{50} values of DOC in MDA-231/DOC and MCF-7/DOC cells were significantly increased compared with that of their parental cells (both P<0.05; Fig. 1C), demonstrating the successful establishment of breast cancer cells resistant to DOC. Furthermore, the level of TMPO-AS1 was significantly increased in MDA-231/DOC and MCF-7/DOC cells compared with their parental cells (both P<0.05; Fig. 1D). In invasion assays, MDA-231/DOC and MCF-7/DOC exhibited an enhanced invasion capability compared with that of their parental MDA-231 and MCF-7 cells (both P<0.05; Fig. 1E). Taken together, these results suggested that TMPO-AS1 was overexpressed in DOC-resistant breast cancer cells, and this may provide one of the reasons underlying the resistance of breast cancer cells to DOC.

TMPO-AS1 knockdown enhances sensitivity to DOC and suppresses migration in DOC-resistant breast cancer cells. To further confirm the functional role of TMPO-AS1 in breast cancer cells, MDA-231/DOC and MCF-7/DOC cells were transfected with TMPO-AS1 siRNAs (si-TMPO-AS1#1, si-TMPO-AS1#2 or si-TMPO-AS1#3) or si-NC. As shown in Fig. 2A and B, transfection with TMPO-AS1 siRNAs (especially si-TMPO-AS1#3) led to a significant reduction in TMPO-AS1 expression in MDA-231/DOC and MCF-7/DOC cells (P<0.05). Therefore, si-TMPO-AS1 #3 was selected for the subsequent experiments. The CCK-8 assay revealed that TMPO-AS1-knockdown led to a marked decrease in the proliferative activity of MDA-231, MCF-7, MDA-231/DOC and MCF-7/DOC cells (Fig. 2C-F). TMPO-AS1 knockdown also caused a marked decrease in the IC_{50} value of MDA-231, MCF-7, MDA-231/DOC and MCF-7/DOC cells (Fig. 2G), demonstrating that TMPO-AS1-knockdown increased the sensitivity of MDA-231/DOC and MCF-7/DOC cells to DOC. To confirm the mechanism by which TMPO-AS1-knockdown reversed DOC resistance, flow cytometric analysis was performed following transfection in MDA-231/DOC and MCF-7/DOC cells treated with DOC. The results indicated that TMPO-AS1-knockdown significantly increased DOC-induced apoptosis in MDA-231/DOC and MCF-7/DOC cells (both P<0.05; Fig. 2H). In invasion assays, TMPO-AS1-knockdown attenuated the rate of invasion of MDA-231/DOC and MCF-7/DOC cells (both P<0.05; Fig. 2I). Collectively, these results demonstrated that knockdown of TMPO-AS1 diminished DOC resistance and invasion in MDA-231/DOC and MCF-7/DOC cells.

TMPO-AS1 knockdown sensitizes DOC-resistant OC cells to DOC by sponging miR-1179. Searches of the Starbase and TargetScan databases revealed that miR-1179, miR-198, -205-5p and -425-5p may interact with TMPO-AS1; however, our pre-experimental results showed that knockdown of TMPO-AS1 only affected miR-1179 (data not shown), so miR-1179 was therefore selected for subsequent studies. According to the bioinformatics analysis, miR-1179 was predicted to share binding sites with TMPO-AS1 (Fig. 3A). A dual luciferase reporter assay demonstrated that forced expression of miR-1179 markedly mitigated the luciferase activity of TMPO-AS1-Wt reporter, but not the TMPO-AS1-Mut reporter in 293T cells (Fig. 3B and Fig. S1A). To further confirm the effect of TMPO-AS1 on miR-1179 expression, MDA-231/DOC and MCF-7/DOC cells were transfected with si-TMPO-AS1#3 or si-NC, and the results obtained demonstrated that TMPO-AS1-knockdown increased the miR-1179 expression levels in MDA-231/DOC and MCF-7/DOC cells (both P<0.05; Fig. 3C). All the aforementioned data demonstrated that TMPO-AS1 directly sponged miR-1179. Subsequently, function and rescue experiments were conducted to investigate whether the actions of TMPO-AS1 were mediated through sponging miR-1179. The results obtained suggested that forced expression of miR-1179 or silencing TMPO-AS1 increased DOC-sensitivity in MDA-231/DOC and MCF-7/DOC cells; however, miR-1179 inhibition reversed the chemosensitization

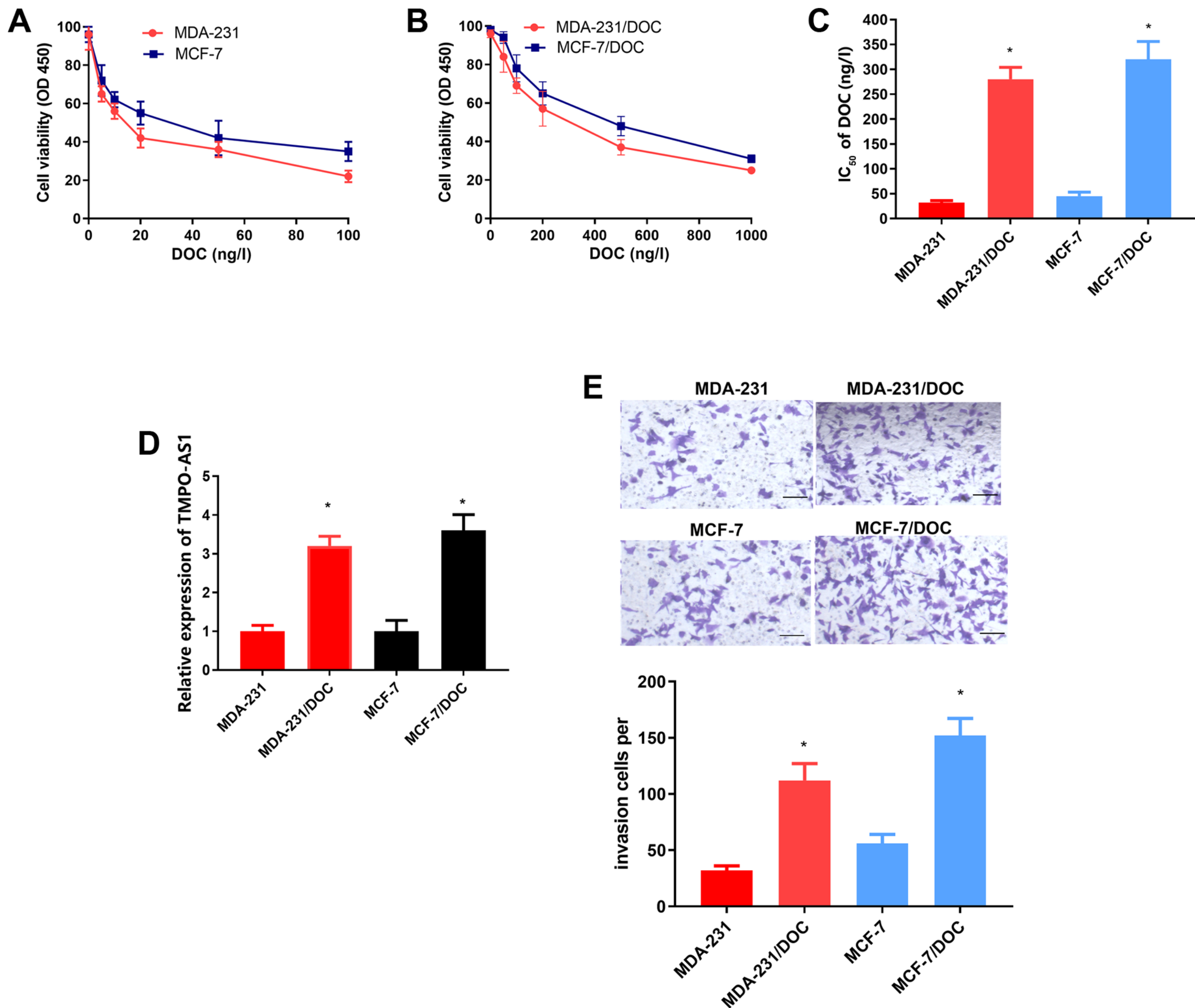

Figure 1. TMPO-AS1 is markedly elevated in DOC-resistant breast cancer cells. (A-C) Cell viability and IC_{50} values of DOC in MDA-231/DOC and MCF-7/DOC cells and their parental cells (MDA-231 and MCF-7) exposed to various concentrations of DOC for 48 h were detected using a Cell Counting Kit-8 assay. (D) TMPO-AS1 level was determined using reverse transcription-quantitative PCR analysis in MDA-231/DOC and MCF-7/DOC cells and their parental cells. (E) Transwell assay showed the invasion of MDA-231/DOC and MCF-7/DOC and their parental cells (scale bar, 100 μm). $^*P<0.05$ vs. respective parental cells. DOC, docetaxel; AS1, antisense 1.

effect of TMPO-AS1-knockdown (Fig. 3D and E and Fig. S1B). In addition, miR-117 expression or TMPO-AS1-silencing increased DOC-induced apoptosis in MDA-231/DOC and MCF-7/DOC cells; nevertheless, miR-1179 inhibition reversed the co-operative effect of TMPO-AS1-knockdown on DOC-induced apoptosis (Fig. 3F). Taken together, these data demonstrated that TMPO-AS1-knockdown led to decreased DOC resistance in DOC-resistant breast cancer cells through promoting miR-1179.

TRIM37 levels increased by the TMPO-AS1/miR-1179 axis contribute to DOC resistance in DOC-resistant breast cancer cells. The TRIM family of proteins may have an important role in tumor regulation (15), although the role of the TRIM protein family in breast cancer drug resistance has yet to be fully established. Using the Starbase and TargetScan databases, it was shown that only TRIM37 of the TRIM family of proteins was predicted to be a potential target of miR-1179 (Fig. 4A). To confirm the interaction, a dual luciferase reporter assay was conducted, which revealed that forced expression of miR-1179 led to a marked attenuation of the luciferase activity of the TRIM37-Wt reporter, which was rescued by overexpression of TMPO-AS1 (Fig. 4B). Nevertheless, the luciferase activity of TRIM37-Mut reporter exhibited no significant changes (Fig. 4B). Subsequently, TRIM37 mRNA and protein levels were further detected in MDA-231/DOC and MCF-7/DOC cells. Silencing TMPO-AS1 or forced expression of miR-1179 led to a clear decrease in the levels of TRIM37 mRNA and protein expression; however, miR-1179 inhibition mitigated the inhibition of TRIM37 mRNA induced by TMPO-AS1-knockdown (Fig. 4C). Western blot analysis revealed that TMPO-AS1 silencing or miR-1179-overexpression clearly decreased the TRIM37 protein levels (Fig. 4D). These results suggested that TMPO-AS1 promoted TRIM37 expression by sponging miR-1179. Subsequently, the effect of TRIM37 on DOC resistance was investigated in MDA-231/DOC and MCF-7/DOC cells. The IC_{50} assay showed that inhibition of TRIM37 markedly promoted

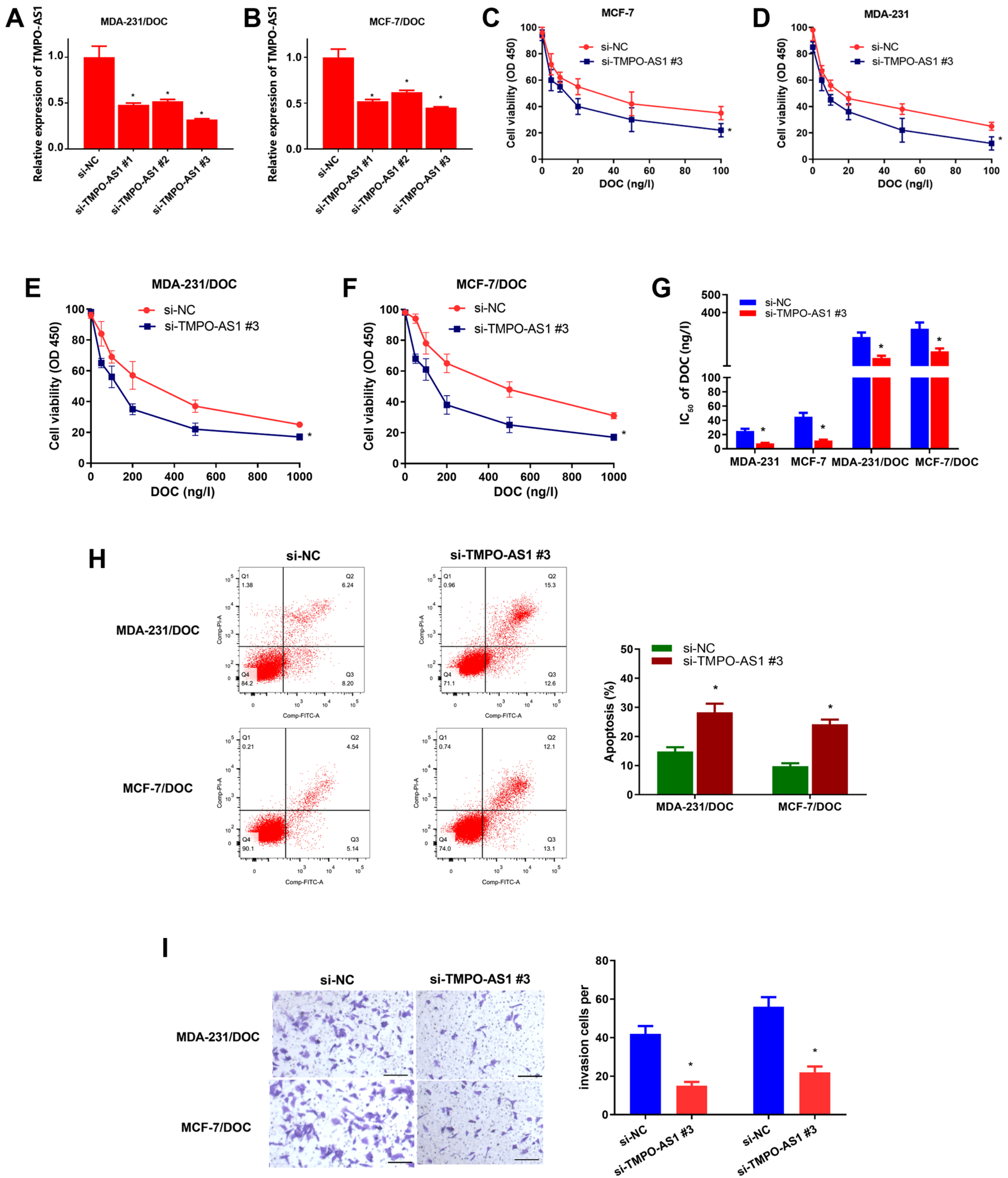

Figure 2. Inhibition of TMPO-AS1 promotes DOC sensitivity in DOC-resistant DOC cells. (A and B) TMPO-AS1 expression in MDA-231/DOC and MCF-7/DOC cells was assessed using reverse transcription-quantitative PCR analysis. (C-F) Cell survival curve of MDA-231, MCF-7, MDA-231/DOC and MCF-7/DOC cells before and after inhibition of TMPO-AS1. (G) IC_{50} values of DOC were detected in transfected MDA-231, MCF-7, MDA-231/DOC and MCF-7/DOC cells treated with different concentrations of DOC. (H) Apoptotic rate was detected by flow cytometric analysis in transfected MDA-231/DOC and MCF7/DOC cells treated with 50 ng/l DOC. (I) Transwell assays for the determination of the effects of si-TMPO AS1 on the invasion of breast cancer cells (scale bar, 100 μm). *P<0.05 vs. si-NC. DOC, docetaxel; si, small interfering; NC, negative control; AS1, antisense 1.

DOC-sensitivity in MDA-231/DOC and MCF-7/DOC cells, and TRIM37-overexpression reversed the TMPO-AS1 knockdown-induced chemosensitization effect (Fig. 4E and F and Fig. S1C and D). Additionally, TRIM37-knockdown led to an increased rate of DOC-induced apoptosis of MDA-231/DOC and MCF-7/DOC cells, and TRIM37-overexpression caused a diminution of the co-operative effect of inhibition of TMPO-AS1 on DOC-induced apoptosis (Fig. 4G). Considered

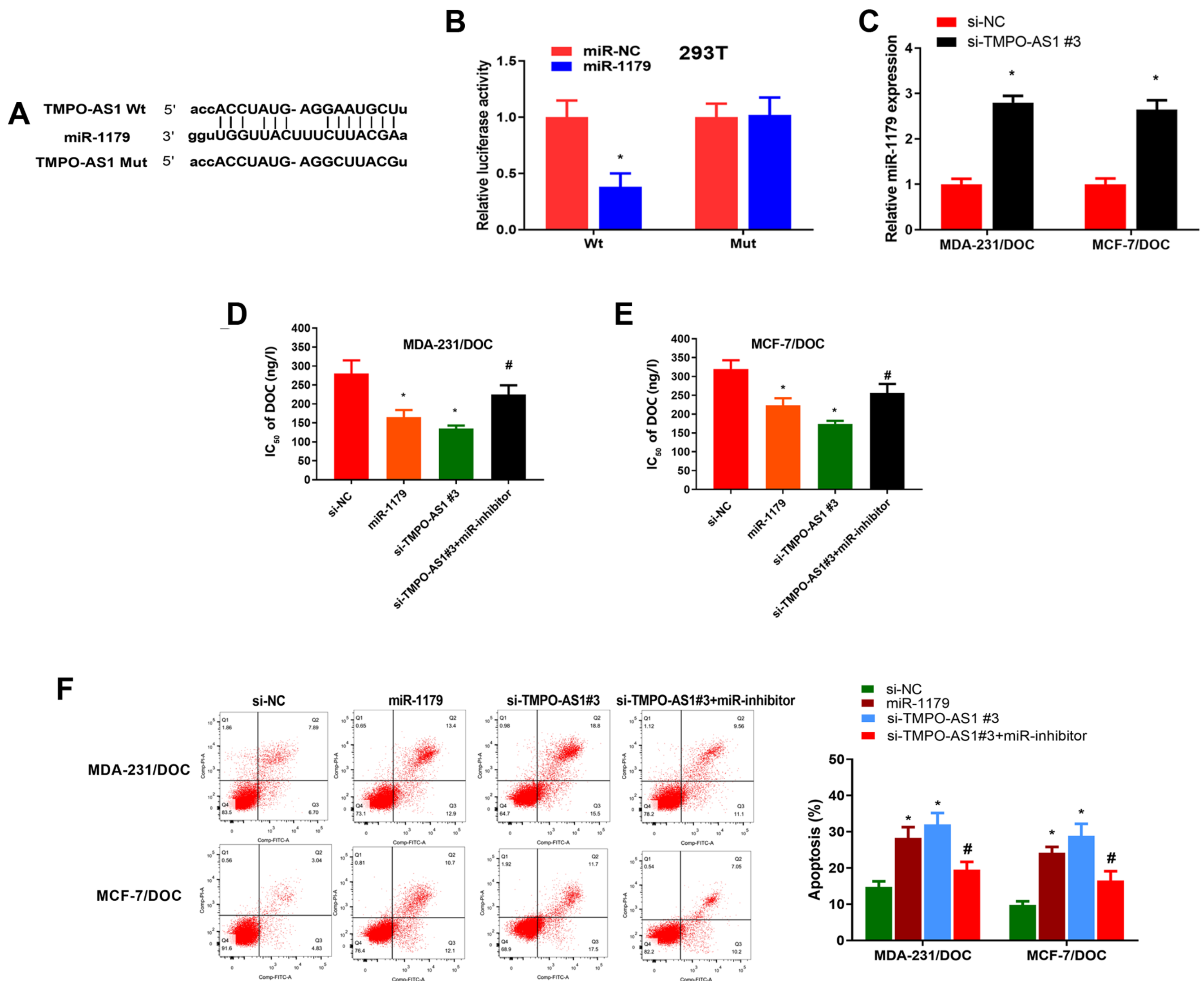

Figure 3. Inhibition of TMPO-AS1 attenuates DOC resistance in DOC-resistant breast cancer cells. (A) Predicted binding sites between miR-1179 and TMPO-AS1. (B) Luciferase reporter assay was conducted in 293T cells transfected with TMPO-AS1-Wt or TMPO-AS1-Mut reporter vector and miR-1179 or miR-NC. (C) miR-1179 was detected using reverse transcription-quantitative PCR analysis in si-TMPO-AS1 #3 or si-NC in MDA-231/DOC and MCF-7/DOC cells. (D and E) Cell Counting Kit-8 assay was performed to measure IC_{50} values of DOC in MDA-231/DOC and MCF-7/DOC cells following transfection with miR-1179, si-TMPO-AS1 #3 or si-TMPO-AS1 #3 plus miR-1179 inhibitor and different doses of DOC treatment. (F) Apoptotic rate was detected by flow cytometry in MDA-231/DOC and MCF-7/DOC cells transfected with miR-1179, si-TMPO-AS1 #3 or si-TMPO-AS1 #3 plus anti-miR-1179, and exposed to DOC. *P<0.05 vs. si-NC; #P<0.05 cf. si-TMPO-AS1 #3. DOC, docetaxel; Wt, wild-type; Mut, mutated; small interfering; NC, negative control; AS1, antisense 1; miR, microRNA.

together, the aforementioned results demonstrated that inhibition of TMPO-AS1 sensitized DOC-resistant OC cells to DOC via inhibition of TRIM37, and this was mediated via inhibiting miR-1179 expression.

Discussion

The poor prognosis of patients with breast cancer is predominantly due to DOC resistance and chemotherapy failure (1). Evidence has suggested that the mechanisms underlying this chemoresistance are modulated by several factors and are complex (17). In present study, the results obtained revealed that the levels of TMPO-AS1 were raised in DOC-resistant breast cells, and TMPO-AS1-knockdown sensitized DOC-resistant breast cancer cells to DOC, thereby attenuating the invasion capability of breast cancer cells through modulating the miR-1179/TRIM37 axis.

lncRNAs have been widely identified as molecules crucial for the chemoresistance and invasion of different cancer types, including breast cancer (18). For example, BMP/OP-responsive gene confers chemoresistance in triple-negative breast cancer (19). Arf-GAP with GTPase, ANK repeat and PH domain-containing protein 2-AS1 was shown to facilitate chemoresistance of breast cancer through regulation of myeloid differentiation primary response 88 (20). Furthermore, Ferritin Heavy Chain 1 Pseudogene 3 promotes paclitaxel resistance in breast cancer through sponging the miR-206/ABCB1 axis (21). Additionally, Cancer Susceptibility 9 was shown to facilitate doxorubicin-resistant breast cancer via binding to enhancer of zeste homolog 2 (22). A recent study indicated that TMPO-AS1, as an oncogenic lncRNA, may function as a potential diagnostic and prognostic biomarker, and as a

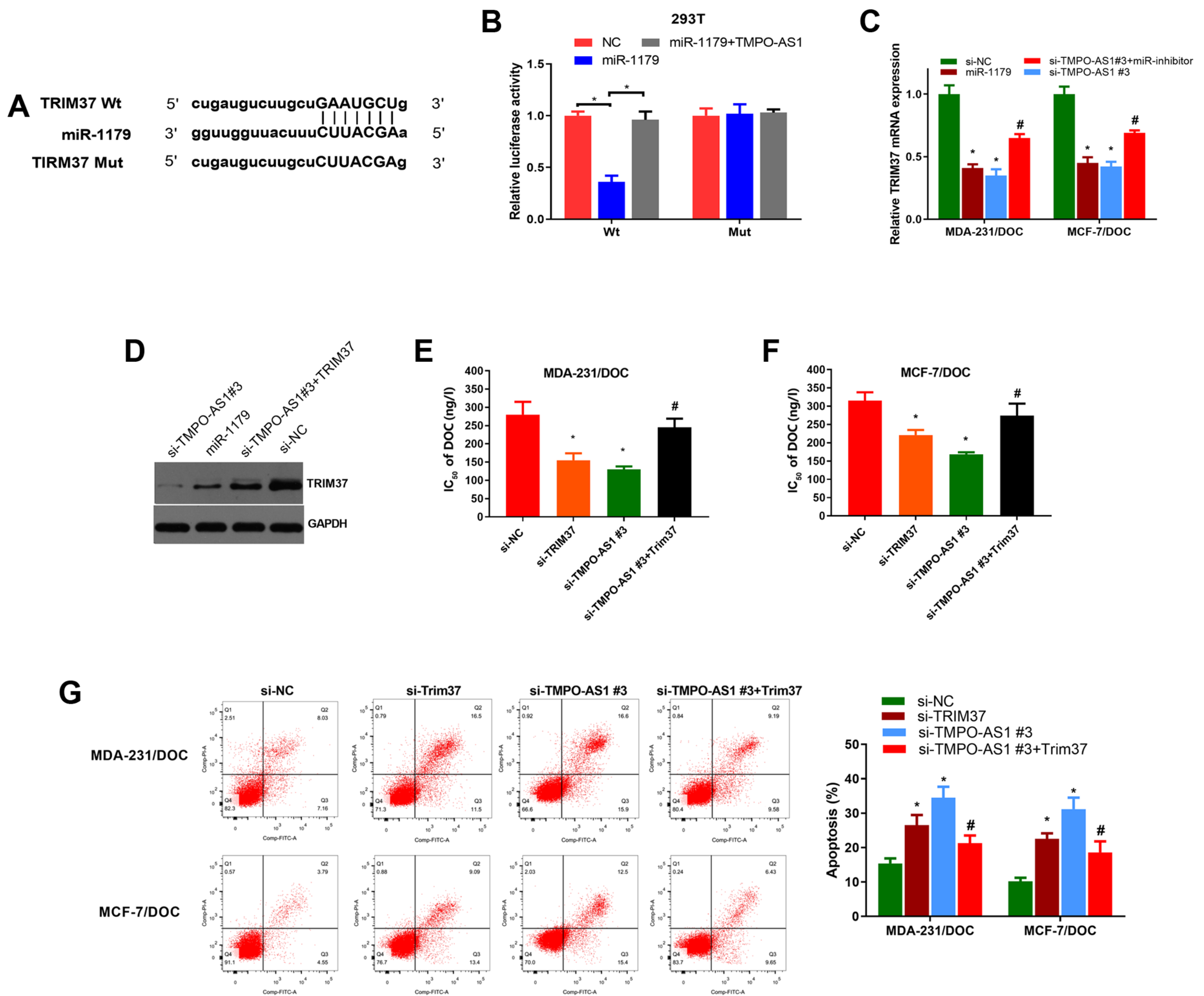

Figure 4. TRIM37 positively modulated by the TMPO-AS1/miR-1179 axis promotes DOC resistance in DOC-resistant breast cells. (A) Predicted binding sites between miR-1179 and TRIM37. (B) Luciferase reporter assay was conducted in 293T cells transfected with TRIM37-Wt or TRIM37-Mut reporter vector and miR-1179, miR-NC or pcDNA-TMPO-AS1. (C) TRIM37 mRNA was detected by reverse-transcription-quantitative PCR analysis in transfected MDA-231/DOC and MCF-7/DOC cells. (D) TRIM37 protein was detected using western blot analysis in transfected MDA-231/DOC and MCF-7/DOC cells. (E and F) Cell Counting Kit-8 assay was conducted to compare IC_{50} values of DOC in MDA-231/DOC and MCF-7/DOC cells after transfection. (G) Apoptotic rate was measured via flow cytometry in MDA-231/DOC and MCF-7/DOC cells following transfection. *P<0.05 vs. si-NC, $^{\#}$P<0.05 vs. si-TMPO-AS1 #3. DOC, docetaxel; TRIM37, tripartite motif-containing protein 37; Wt, wild-type; Mut, mutated; si, small interfering; NC, negative control; AS1, antisense 1; miR, microRNA.

therapeutic target in prostate cancer (13). However, prior to that study, the functional and molecular role of TMPO-AS1 in the regulation of DOC resistance in different types of breast cancer was poorly understood. In the present study, it was observed that TMPO-AS1 was markedly upregulated in DOC-resistant breast cancer cells. TMPO-AS1 silencing sensitized DOC-resistant breast cancer cells to DOC and led to an improvement in DOC-induced apoptosis; moreover, TMPO-AS1-knockdown decreased the number of invasive cells. Therefore, collectively these results demonstrated that TMPO-AS1 could both enhance resistance to DOC and enhance invasion in breast cancer cells. To the best of the authors' knowledge, the present study is the first to have shown the promotional role of TMPO-AS1 in DOC resistance and invasion in breast cancer, thereby revealing a potentially novel therapeutic target for DOC resistance in breast cancer. In terms of mechanism of lncRNA dysfunction in chemoresistance, there is evidence indicating that transcription factors (TFs) take a key role. For instance, lncRNA AGAP2-AS1 is induced by transcription factor SP1 in breast cancer cells to promote chemoresistance (20). In lung adenocarcinoma cells, TMPO-AS1 could be activated by E2F transcription factor 1 (23). Therefore, the authors speculated that the abnormal expression of TMPO-AS1 caused by transcription factors plays an important role in chemotherapy resistance of breast cancer. However, the specific mechanism of the increased expression of TMPO-AS1 needs to be confirmed in the future.

lncRNAs have been shown to serve as regulators of miRNAs that function via modulating cell proliferation and

participating in chemoresistance. The mechanism through which they accomplish these roles is usually via adsorbing and interacting with miRNAs, and they are thereby said to act as 'molecular sponges' (24). For example, lncRNAPVT1 sponging miR-152 enhances the chemoresistance of osteosarcoma to gemcitabine through the c-MET/PI3K/AKT pathway (25). Additionally, Small Nucleolar RNA Host Gene 16 restrained hepatocellular carcinoma (HCC) cell proliferation and chemoresistance by sponging miR-93 (26). A recent study showed that TMPO-AS1 may promote the proliferation and invasion of cervical cancer cells by regulating the miR-143-3p/zinc finger E-box-binding homeobox 1 axis (27); however, the role of TMPO-AS1 in drug resistance of breast cancer has yet to be fully elucidated. In the present study, TMPO-AS1 was first described to directly inhibit miR-1179 expression. Furthermore, overexpression of miR-1179 mitigated DOC resistance in DOC-resistant breast cancer cells. Consistently, miR-1179 inhibition reversed the inhibitory effect on DOC resistance induced by TMPO-AS1-knockdown. These findings showed that silencing TMPO-AS1 increased DOC-sensitivity via direct inhibition of miR-1179. A recent study demonstrated that miR-1179 acts as a tumor suppressor that inhibits cell invasion via the Notch signaling pathway in breast cancer cells (28). Another study demonstrated that miR-1179 inhibits the proliferation, migration and invasion of human pancreatic cancer cells by targeting E2F5 (29). In the present study, using the Starbase and TargetScan databases, it was shown that only TRIM37 was predicted to be a potential target of miR-1179. Moreover, the results indicated that miR-1179 could attenuate DOC resistance in DOC-resistant breast cancer cells by targeting TRIM37.

TRIM37, a recently identified E3 ubiquitin ligase and a member of the TRIM protein family, is dysfunctional in a number of cancer types, and an increase in the level of TRIM37 expression is notably associated with cancer progression. For instance, TRIM37 facilitates cell migration and metastasis in HCC through activation of the Wnt/β-catenin signaling pathway (30). TRIM37 enhances the epithelial-mesenchymal transition in colorectal cancer (31). Furthermore, TRIM37 overexpressed in a subset of breast cancer types was shown to promote tumorigenesis by the silencing of tumor suppressors (32). However, to date, the role of TRIM37 in chemoresistance of breast cancer and invasion has not been reported. In the present study, knockdown of TRIM37 via silencing of TMPO-AS1 significantly promoted DOC sensitivity and apoptosis, with decreased invasion of breast cancer cells. Importantly, TRIM37-overexpression rescued the inhibition of invasion and sensitization effects attributable to TMPO-AS1-knockdown, which demonstrated that TRIM37 is a key regulator in DOC resistance and invasion in breast cancer. To the best of our knowledge, the present study is the first to suggest the involvement of a TMPO-AS1/miR-1179/TRIM37 molecular axis in DOC resistance and invasion in breast cancer. However, there are some limitations to the current study, such as the lack of further confirmation using animal experiments and the lack of an argonatue-2-RIP experiment to further confirm the interaction between TMPO-AS1, miR-1179 and TRIM37 mRNA. The present results may be helpful to further confirm the role of TMPO-AS1 in chemotherapy resistance of breast cancer.

In conclusion, the present study revealed that TMPO-AS1 facilitated DOC resistance and invasion in breast cancer cells via an miR-1179 sponging-induced increase in the level of TRIM37, revealing a novel understanding of the mechanisms underlying chemoresistance in breast cancer, and also providing a potential therapeutic target for breast cancer.

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Availability of data and materials

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

Authors' contributions

XN, BL and JR designed the present study. XN, JZ, FH and YY performed all the experiments, analyzed the data and prepared the figures. XN drafted the initial manuscript. BL and JR reviewed and revised the manuscript. XN and JR confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Merino Bonilla JA, Torres Tabanera M and Ros Mendoza LH: Breast cancer in the 21st century: From early detection to new therapies. Radiologia 59: 368-379, 2017 (In English, Spanish).
2. Fan L, Strasser Weippl K, Li JJ, St Louis J, Finkelstein DM, Yu KD, Chen WQ, Shao ZM and Goss PE: Breast cancer in China. Lancet Oncol 15: e279-e289, 2014.
3. Goetz MP, Gradishar WJ, Anderson BO, Abraham J, Aft R, Allison KH, Blair SL, Burstein HJ, Dang C, Elias AD, *et al*: NCCN guidelines insights: Breast cancer, version 3.2018. J Natl Compr Canc Netw 17: 118-126, 2019.
4. Yang M, Li Y, Shen X, Ruan Y, Lu Y, Jin X, Song P, Guo Y, Zhang X, Qu H, *et al*: CLDN6 promotes chemoresistance through GSTP1 in human breast cancer. J Exp Clin Cancer Res 36: 157, 2017.
5. Jarroux J, Morillon A and Pinskaya M: History, discovery, and classification of lncRNAs. Adv Exp Med Biol 1008: 1-46, 2017.
6. Huarte M: The emerging role of lncRNAs in cancer. Nat Med 21: 1253-1261, 2015.

7. Peng WX, Koirala P and Mo YY: LncRNA-mediated regulation of cell signaling in cancer. Oncogene 36: 5661-5667, 2017.
8. Xiao J, Lv Y, Jin F, Liu Y, Ma Y, Xiong Y, Liu L, Zhang S, Sun Y, Tipoe GL, *et al*: LncRNA HANR promotes tumorigenesis and increase of chemoresistance in hepatocellular carcinoma. Cell Physiol Biochem 43: 1926-1938, 2017.
9. Han P, Li JW, Zhang BM, Lv JC, Li YM, Gu XY, Yu ZW, Jia YH, Bai XF, Li L, *et al*: The lncRNA CRNDE promotes colorectal cancer cell proliferation and chemoresistance via miR-181a-5p-mediated regulation of Wnt/β-catenin signaling. Mol Cancer 16: 9, 2017.
10. YiRen H, YingCong Y, Sunwu Y, Keqin L, Xiaochun T, Senrui C, Ende C, XiZhou L and Yanfan C: Long noncoding RNA MALAT1 regulates autophagy associated chemoresistance via miR-23b-3p sequestration in gastric cancer. Mol Cancer 16: 174, 2017.
11. Fang Z, Zhao J, Xie W, Sun Q, Wang H and Qiao B: LncRNA UCA1 promotes proliferation and cisplatin resistance of oral squamous cell carcinoma by sunppressing miR-184 expression. Cancer Med 6: 2897-2908, 2017.
12. Li DS, Ainiwaer JL, Sheyhiding I, Zhang Z and Zhang LW: Identification of key long non-coding RNAs as competing endogenous RNAs for miRNA-mRNA in lung adenocarcinoma. Eur Rev Med Pharmacol Sci 20: 2285-2295, 2016.
13. Peng F, Wang R, Zhang Y, Zhao Z, Zhou W, Chang Z, Liang H, Zhao W, Qi L, Guo Z and Gu Y: Differential expression analysis at the individual level reveals a lncRNA prognostic signature for lung adenocarcinoma. Mol Cancer 16: 98, 2017.
14. Huang W, Su X, Yan W, Kong Z, Wang D, Huang Y, Zhai Q, Zhang X, Wu H, Li Y, *et al*: Overexpression of AR-regulated lncRNA TMPO-AS1 correlates with tumor progression and poor prognosis in prostate cancer. Prostate 78: 1248-1261, 2018.
15. Bhatnagar S and Green MR: TRIMming down tumor suppressors in breast cancer. Cell Cycle 14: 1345-1346, 2015.
16. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
17. Lu Y, Yang Y, Liu Y, Hao Y, Zhang Y, Hu Y, Jiang L, Gong Y, Wu K and Liu Y: Upregulation of PAG1/Cbp contributes to adipose-derived mesenchymal stem cells promoted tumor progression and chemoresistance in breast cancer. Biochem Biophys Res Commun 494: 719-727, 2017.
18. Guttman M and Rinn JL: Modular regulatory principles of large non-coding RNAs. Nature 482: 339-346, 2012.
19. Gooding AJ, Zhang B, Gunawardane L, Beard A, Valadkhan S and Schiemann WP: The lncRNA BORG facilitates the survival and chemoresistance of triple-negative breast cancers. Oncogene 38: 2020-2041, 2019.
20. Dong H, Wang W, Mo S, Chen R, Zou K, Han J, Zhang F and Hu J: SP1-induced lncRNA AGAP2-AS1 expression promotes chemoresistance of breast cancer by epigenetic regulation of MyD88. J Exp Clin Cancer Res 37: 202, 2018.
21. Wang R, Zhang T, Yang Z, Jiang C and Seng J: Long non-coding RNA FTH1P3 activates paclitaxel resistance in breast cancer through miR-206/ABCB1. J Cell Mol Med 22: 4068-4075, 2018.
22. Jiang B, Li Y, Qu X, Zhu H, Tan Y, Fan Q, Jiang Y, Liao M and Wu X: Long noncoding RNA cancer susceptibility candidate 9 promotes doxorubicin-resistant breast cancer by binding to enhancer of zeste homolog 2. Int J Mol Med 42: 2801-2810, 2018.
23. Wei L, Liu Y, Zhang H, Ma Y, Lu Z, Gu Z and Ding C: TMPO-AS1, a novel E2F1-regulated lncRNA, contributes to the proliferation of lung adenocarcinoma cells via modulating miR-326/SOX12 axis. Cancer Manag Res 12: 12403-12414, 2020.
24. Ding B, Lou W, Xu L and Fan W: Non-coding RNA in drug resistance of hepatocellular carcinoma. Biosci Rep 38: BSR20180915, 2018.
25. Sun ZY, Jian YK, Zhu HY and Li B: lncRNAPVT1 targets miR-152 to enhance chemoresistance of osteosarcoma to gemcitabine through activating c-MET/PI3K/AKT pathway. Pathol Res Pract 215: 555-563, 2019.
26. Xu F, Zha G, Wu Y, Cai W and Ao J: Overexpressing lncRNA SNHG16 inhibited HCC proliferation and chemoresistance by functionally sponging hsa-miR-93. Onco Targets Ther 11: 8855-8863, 2018.
27. Gang X, Yuan M and Zhang J: Long non-coding RNA TMPO-AS1 promotes cervical cancer cell proliferation, migration, and invasion by regulating miR-143-3p/ZEB1 axis. Cancer Manag Res 12: 1587-1599, 2020.
28. Li WJ, Xie XX, Bai J, Wang C, Zhao L and Jiang DQ: Increased expression of miR-1179 inhibits breast cancer cell metastasis by modulating Notch signaling pathway and correlates with favorable prognosis. Eur Rev Med Pharmacol Sci 22: 8374-8382, 2018.
29. Lin C, Hu Z, Yuan G, Su H, Zeng Y, Guo Z, Zhong F, Jiang K and He S: MicroRNA-1179 inhibits the proliferation, migration and invasion of human pancreatic cancer cells by targeting E2F5. Chem Biol Interact 291: 65-71, 2018.
30. Jiang J, Yu C, Chen M, Tian S and Sun C: Over-expression of TRIM37 promotes cell migration and metastasis in hepatocellular carcinoma by activating Wnt/β-catenin signaling. Biochem Biophys Res Commun 464: 1120-1127, 2015.
31. Hu CE and Gan J: TRIM37 promotes epithelial-mesenchymal transition in colorectal cancer. Mol Med Rep 15: 1057-1062, 2017.
32. Bhatnagar S, Gazin C, Chamberlain L, Ou J, Zhu X, Tushir JS, Virbasius CM, Lin L, Zhu LJ, Wajapeyee N and Green MR: TRIM37 is a new histone H2A ubiquitin ligase and breast cancer oncoprotein. Nature 516: 116-120, 2014.

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