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Original Article

LncRNA TTN-AS1 confers tamoxifen resistance in breast cancer via sponging miR-107 to modulate PI3K/AKT signaling pathway

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Abstract: Objective: Tamoxifen resistance of breast cancer (BC) is a significant hindrance in clinical therapy. The long-noncoding RNA (lncRNA) TTN-AS1 has been reported as a crucial tumor promoting factor in various cancers. In this study, we set out to discover the specific pathologic regulatory mechanisms of tamoxifen-resistance in breast cancer. Methods: MTT assay was conducted to evaluate the cell viability of the breast cancer cell lines MCF-7 and MCF-7/TAM. QRT-PCR and western blot assay were performed to estimate the expression of TTN-AS1, miR-107 and related proteins. Flow cytometry was conducted to identify degree of apoptosis and cell cycle. The invasive ability was estimated by transwell chamber assay. Results: Our findings revealed that TTN-AS1 can enhance tamoxifen-resistance in BC cells and augment the invasive ability of tamoxifen-resistant breast cancer cells by down-regulating miR-107, and thereby encourage the development of drug-resistant BC. Further investigation indicates that lncRNA TTN-AS1 worsens the course of tamoxifen-resistant BC by regulating zinc and ring finger 2 (ZNRF2) via miR-107 and activating the PI3K/AKT pathway. Conclusion: Our findings suggest that the lncRNA TTN-AS1 can encourage tamoxifen-resistance in BC by modulating the miR-107/ZNRF2 axis and stimulating the PI3K/AKT pathway.

Keywords: Tamoxifen resistance, breast cancer, TTN-AS1, ZNRF2, PI3K/AKT

Introduction

Breast cancer, a common malignant tumor which significantly endangers women's health, is ranked as the second leading cause of cancer-related deaths [1]. Nowadays, surgery, chemotherapy and endocrine therapy are still predominantly used in breast cancer therapy. Although the progress of diagnostic and treatment technology has improved the efficiency of breast cancer treatment and significantly reduces mortality [2], the majority of patients only have a good response to the initial remedy. However, with progression of treatment, 70% of breast cancer patients develop drug resistance to chemotherapy, which eventually leads to the recurrence of disease. This phenomenon has become the main hindrance faced by the current cancer treatment [3, 4]. Undoubtedly, both initial and acquired drug resistance significantly decrease the disease-free survival (DFS) and overall survival (OS) of breast cancer patients. Drug resistance in breast cancer treatment is regulated through several mechanisms, including somatic mutations [5], epigenetic modifications [6], abnormal signal pathways, metabolism [7, 8], and the effect of a modified tumor microenvironment on drug transport. Therefore, drug resistance in breast cancer is still a big problem for chemotherapy, which urges further study to improve the survival rate and prognosis of breast cancer patients.

To date, with the continued development of oncology research, non-coding RNAs (ncRNAs) have been transformed from "junk" transcription products into functional regulatory molecules that mediate cell processes, including chromatin remodeling, transcription, post transcriptional modification and signal transduction [9-13]. Additionally, the contribution of ncRNAs to the process of tumorigenesis has gradually become the focus of many researchers. Long non-coding RNAs (lncRNAs), a kind of regulatory

ncRNA with a length of more than 200 nucleotides (nt), have been confirmed to be involved in the occurrence, development, metastasis and drug resistance of tumors [14-17]. The correlation and mechanism between lncRNAs and drug resistance of breast cancer cells are of great significance for prevention of treatment failure of breast cancer caused by drug resistance in the later stages of the disease.

TTN-AS1 (lncRNA titin antisense RNA1) is a transcriptional product of oncogene, which is related to the development of tumor pathology. Recently, studies have found that TTN-AS1 participates in the development of esophageal squares cell carcinoma [18], lung adenocarcinoma [19], colorectal cancer [20] and other tumors, and promotes tumor resistance. Based on our previous study, we showed that TTN-AS1 promotes progression of breast cancer by regulating miR-139-5p/ZEB1 axis [21]. For further in-depth study, we study the role of TTN-AS1 in the drug resistance development of breast cancer, which might help elaborate the mechanism of the prime drug resistance (PDR) in breast cancer, and could possibly act as a strategy for treatment of breast cancer resistance.

MicroRNAs (miRNAs), a kind of endogenous non-coding RNA, are composed of 20-25 nucleotides. Current evidence shows that miRNAs can induce abnormal gene expression and cause chemotherapy resistance of breast cancer [22, 23]. For example, mir-129-5p may inhibit adriamycin resistance in breast cancer by directly targeting SOX2 [24]. Additionally, miR-129-3p was upregulated in docetaxel-resistant breast cancer cells (MDA-MB-231), which promotes drug resistance of docetaxel by directly acting on cp110 [25]. Furthermore, mir-1307 may play a role in cisplatin resistance of breast cancer by targeting MDM4 [26].

In this study, we found that miR-107 is negatively regulated by TTN-AS1, which was then validated as a favorable repression factor for the progression and drug resistance in breast cancer. Additionally, ZNRF2, concurrent with TTN-AS1, was discovered to be a negative regulated element of miR-107. Our finding suggests that TTN-AS1/miR-107/ZNRF2 axis are a potential strategy for improving chemotherapy prognosis and rescuing tamoxifen resistance in breast cancer.

Materials and methods

Cell culture

The human breast cancer cell line (MCF-7) was purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China), and cultured using DMEM medium containing 10% fetal bovine serum and penicillin (100 U/mL) at 37°C and 5% CO_2. In order to construct a tamoxifen-resistant breast cancer cell line (MCF-7/TAM), MCF-7 was treated with 0.3 μM Tamoxifen (Sigma-Aldrich, St. Louis, MO) for 6 months. Other culture conditions were the same as normal MCF-7 cells.

Total RNA extraction

MCF-7 and MCF-7/TAM cells were collected in the logarithmic growth period and added to 1 mL of TRIzol to lyse and preserve RNA integrity. Chloroform was added for 15-min at room temperature, and the mixture was then added to isopropyl alcohol and centrifuged to obtain the RNA sediment. Finally, the RNA extraction was dried and stored at -80°C till further experiments.

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

QRT-PCR analyses of TTN-AS1, miR-107 and ZNRF2 were performed using PrimeScript RT reagent Kit and SYBR Prime Script RT-PCR Kits based on manufacturer's guidelines. Transcriptional expression was calculated using the $2^{-\Delta\Delta Ct}$ method. TTN-AS1 and ZNRF2 expression was calculated relative to GAPDH, and miR-107 was measured relative to U6. The primer sequences used in the assay are as provided in **Table 1**.

Cell viability through MTT assay

MCF-7 and MCF-7/TAM cells were seeded onto 96-well plate (5×10^3 cells per well) with 100 μL DMEM medium under regular culture conditions (37°C, 5% CO_2). Cells were divided into 4 groups including MCF-7, MCF-7+TAM, MCF-7/TAM and MCF-7/TAM+TAM. The MCF-7+TAM and MCF-7/TAM+TAM groups were treated with 5 μM of tamoxifen, while the other two groups were added the same volume of media. All cells were incubated for 12 h, 24 h, 36 h, 48 h, 60 h and 72 h, and were then treated with 20 μL

Table 1. The sequences of primers used in this study

Primers	Forward (Sense)	Reverse (Antisense)
TTN-AS1	CGGGAACAAGCCCTGTG	CCGGCCCAAAGATGATG
miR-107	ATGATGAGCAGCATTGTACAGG	GCAGGGTCCGAGGTATTC
ZNRF2	CCTGGTGATCGGCTCCTTAC	TGCATAGACAAGGCAGTCGTG
U6	CTCGCTTCGGCAGCA CA	AACGCTTCACGAA TTTGCGT
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

MTT reagent and cultured for another 4 h. Next, 150 μL of DMSO was mixed and the absorbance under 490 nm (OD490) was measured using an enzyme analyzer within 10 min.

Cell transfection

The related small interference RNAs (si-NC, si-TTN-AS1), miR-107 inhibitors and si-ZNRF2 were transfected into human breast cancer cell lines (MCF-7, MCF-7/TAM) using Lipofectamine 2000 according to manufactures' instructions.

Western blotting

The total protein from MCF-7 and MCF-7/TAM cells were extracted using RIPA lysis buffer with 1% DMSO. Total protein quantities were calculated using the BCA protein assay kit. After the addition of loading buffer, the mixture was boiled for 5 min to denature proteins. Antibodies against ABCB1, ABCC1, ABCG2, ZNRF2, p-PI3K, PI3K, p-AKT, AKT and GAPDH used in the assay were purchased from Abcam, and GAPDH represented internal control.

Flow cytometry

Cell cycle and apoptosis levels were examined by flow cytometry using the Annexin V-FITC Apoptosis Detection Kit and Cell Cycle Detection Kit (Beyotime, Shanghai, China). Cells were treated using the manufacture's protocol.

Transwell assay

Further examination of the cell's invasion ability was performed using the transwell chamber assay. In brief, 200 μL of cell suspension (1×10^5 cells) was transferred onto the upper compartment coated with Matrigel (BD Biosciences, San Jose, CA, USA) of a Transwell chamber (Corning, Corning, NY, USA). The upper compartment has an 8 μm pore size and a 24-well insert. In the upper chamber of each well, 50 μL of serum-free medium that contained 10 g/L bovine serum albumin was added. All lower chambers were loaded with 10% FBS. The invasion ability was assessed using the number of cells that reached the lower chamber.

Dual-luciferase reporter assay

Synthetic TTN-AS1 and ZNRF2 with wild-type (WT) and mutated (Mut) sequences, respectively, were used to construct the expression reporter plasmids with pGL3. Subsequently, the reporter plasmids were co-transfected with miR-107 mimics or miRNA negative control (miR-NC) using Lipofectamine 2000 under the manufacture's guidelines. After incubating for 24 h, the luciferase activities of Renilla and Firefly were measured using dual-luciferase reporter assay system and luminometer.

Statistical analyses

Data was expressed as mean of triplicate measurements plus standard deviation (SD). Statistically significant differences between two groups were evaluated using a two-tailed Student's t-test. Multiple comparisons between groups was performed using analysis of variance (ANOVA) followed by Tukey's post hoc test using SPSS (13.0) or GraphPad Prism 7.0. $P < 0.05$ represented the threshold for statistical significance.

Results

Confirmation of tamoxifen-resistant breast cancer (TAM-resistant BC) cell line

To investigate the biological differences between normal breast cancer and tamoxifen-resistant breast cancer, we constructed a tamoxifen-resistant breast cancer (TAM-resistant BC) cell line by giving continuous tamoxifen stimulation. The MTT assay was performed to evaluate cell viability with or without TAM in

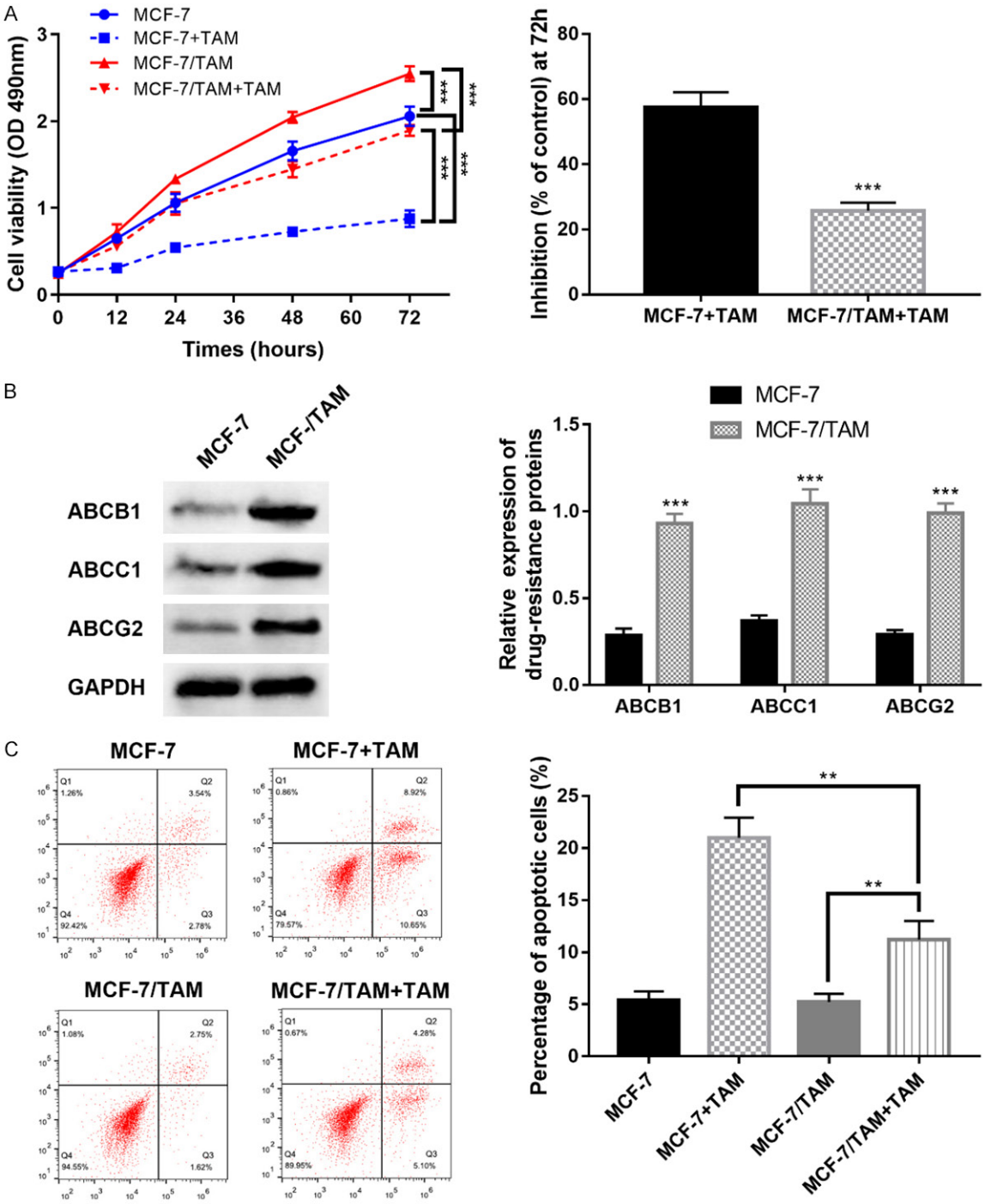

Figure 1. Confirmation of TAM-resistant BC cell line. A. Cell viability measured using the MTT assay. B. Western blotting demonstrated that the drug resistance-related transport proteins were higher expressed in MCF-7/TAM. C. Flow cytometry assay illustrated that MCF-7 had higher percentage of apoptotic cells compared to MCF-7/TAM upon treatment with 5 μM TAM. All data are represented as mean ± SD. Each experiment was conducted in triplicate. **P < 0.01; ***P < 0.001.

MCF-7 and MCF-7/TAM, respectively. Results indicated that MCF-7/TAM cells have significantly higher viability than MCF-7 cells, and that TAM-treated MCF-7/TAM cells demonstrated significantly increased growth compared to MCF-7 cells with TAM treatment (**Figure 1A**).

Further mechanisms of TAM-resistance in MCF-7 was explored through western blotting assay. The exogenous transport proteins ABCB1, ABCC1 and ABCG2 were conspicuously upregulated in the MCF-7/TAM cell line (**Figure 1B**), which suggests that the tamoxifen resistance in breast cancer is implemented by overexpressing a related exogenous transporter.

Flow cytometry was conducted to study the mechanism of cellular proliferation. Upon treatment with TAM, MCF-7/TAM demonstrated lower proportion of apoptotic cells than MCF-7 (**Figure 1C**), which suggests that MCF-7/TAM developed tamoxifen-resistance with a lower cellular apoptotic rate compared to normal MCF-7 cells.

Overexpression of lncRNA TTN-AS1 resulted in augmented invasive and proliferative ability in TAM-resistant BC cell line

The lncRNA TTN-AS1 has been shown to promote tumor progression, including cell growth, metastasis and migration. In order to further explore whether lncRNA TTN-AS1 has a pathological association with drug resistance in breast cancer, we evaluated its expression across two different breast cancer cell lines (MCF-7, MCF-7/TAM) using qRT-PCR. TTN-AS1 was found to be expressed in higher quantities in TAM-resistant BC cell line (MCF-7/TAM) than normal MCF-7 cells (**Figure 2A**), which illustrates that TTN-AS1 might have a functional role in mediating the development of tamoxifen-resistance in breast cancer.

In order to validate our hypothesis and evaluate the specific function of TTN-AS1 in regulating TAM-resistant breast cancer, we constructed two small interference RNAs (si-TTN-AS1#1, si-TTN-AS1#2) and transfected them into the MCF-7/TAM cell line in order to inhibit the expression of TTN-AS1 and develop a TTN-AS1 downregulated MCF-7/TAM cell line. Compared to the siRNA negative control (si-NC), treatment with si-TTN-AS1#1 and si-TTN-AS1#2 led to significantly lower TTN-AS1 expression in the MCF-7/TAM cell line (**Figure 2B**). Therefore, si-TTN-AS1#2 was chosen as the ideal small interfering RNA that can inhibit TTN-AS1 in the continual studies. First, we examined the cell viability discrepancy in relation to different TTN-AS1 expression levels among cells treated with tamoxifen using MTT assay. Concurrent with our hypothesis, downregulation of TTN-AS1 significantly increased the sensitivity of MCF-7/TAM toward TAM (**Figure 2C**).

Subsequently, a transwell assay was conducted to evaluate the role of TTN-AS1 in regulating the invasive ability of MCF-7/TAM. Compared to the MCF-7/TAM, lower expression of TTN-AS1 (MCF-7/TAM+si-TTN-AS1) led to a reduction in invasive capacity, suggesting that lncRNA TTN-AS1 possibly augments the invasive ability of TAM-resistant BC cells. Next, in order to further investigate the mechanism of TTN-AS1 in increasing cell growth of MCF-7/TAM, flow cytometry was utilized to scrutinize changes in cell cycle and apoptosis. Indeed, downregulation of TTN-AS1 in MCF-7/TAM increases proportion of apoptotic cells when compared to MCF-7 and MCF-7/TAM control cells (**Figure 2E**). Additionally, MCF-7/TAM transfected with si-TTN-AS1 was found to induce the highest proportion of cells arrested in the G0/G1 cell cycle phase (**Figure 2F**). Hence, flow cytometry results demonstrate that the existence of TTN-AS1 leads to increased proliferative and invasive ability in TAM-resistant BC cells.

TTN-AS1 down-regulates miR-107 in TAM-resistant BC cell line

LncRNA-targeted miRNAs were screened using the miRanda, LncBase and Starbase databases, from which miR-107 was chosen as a high score target gene of TTN-AS1. The intersection of predicted targets showed that presence of continuous TTN-AS1 wild type binding sites in the miR-107 gene sequence (**Figure 3A**). It was also reported that miR-107 played an inhibitory role in tumor-promotion and drug resistance across various cancers, including breast cancer [27], gastric cancer [28], glioma [29] and pancreatic cancer [30], and esophageal squamous cell carcinoma [31]. To validate these computational predictions, dual-luciferase reporter assay was performed which would elucidate the interaction between TTN-AS1 and miR-107. Results of the luciferase assay indicated evidence of direct binding of miR-107 to TTN-AS1 (**Figure 3B**). However, the exactly regulatory relationship between miR-107 and TTN-AS1 in tamoxifen-resistance BC cells is still unknown. Hence, we next determined levels of miR-107 in MCF-7/TAM cell line through qRT-PCR. We found that miR-107 significantly lowly expressed in MCF-7/TAM cells compared with

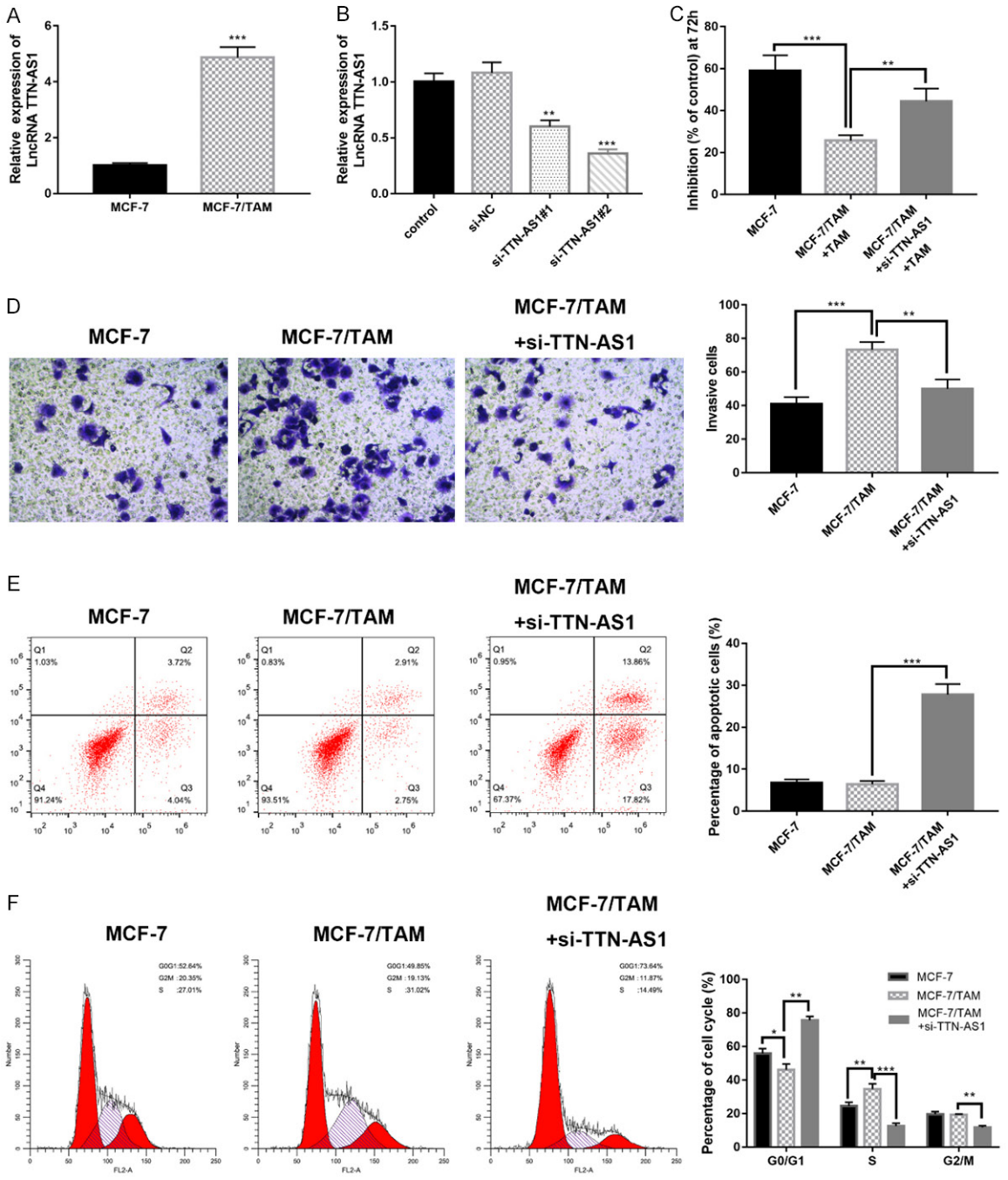

Figure 2. Knockdown of TTN-AS1 inhibits MCF-7/TAM cells proliferation and invasion, promotes cell apoptosis and G0/G1 cell arrest. A. MCF-7/TAM indicated significantly higher expression of TTN-AS1 versus MCF-7. B. The siRNA si-TTN-AS1#2 induced the lowest expression of TTN-AS1 in MCF-7/TAM, and was selected as the optimal siRNA to develop TTN-AS1-deficient cell line. C. MTT assay showed downregulation of TTN-AS1 led to higher inhibition in MCF-7/TAM. D. TTN-AS1 silencing impaired the invasive ability of MCF-7/TAM. E, F. Downregulation of TTN-AS1 accelerated cell cycle arrest and apoptosis in MCF-7/TAM. All data are represented as mean ± SD. Each assay was performed in triplicate. *P < 0.05; **P < 0.01; ***P < 0.001.

that MCF-7 cells (**Figure 3C**). Furthermore, we conducted qRT-PCR to assess the specific regulation between miR-107 and TTN-AS1. Again, we found that miR-107 expressed in lower-than-usual quantities in MCF-7/TAM compared with that in MCF-7, and previous experiments

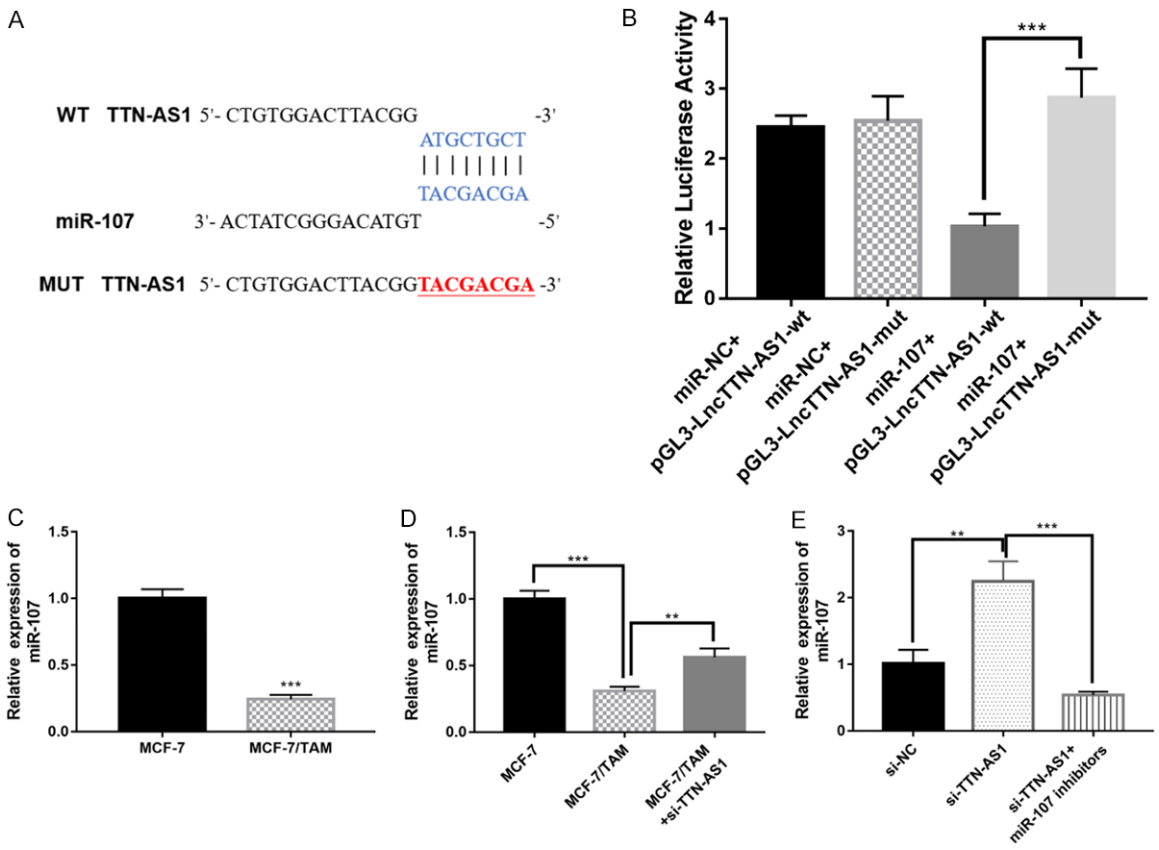

Figure 3. miR-107 is negatively regulated by TTN-AS1. A. The predicted binding site between TTN-AS1 and miR-107. B. Luciferase assay results demonstrated direct binding of TTN-AS1 and miR-107. C. MiR-107 was expressed at lower levels in MCF-7/TAM versus MCF-7. D, E. QRT-PCR results indicate miR-107 is negatively regulated by TTN-AS1. All data are represented as mean ± SD. Each assay was performed in triplicate. **P < 0.01; ***P < 0.001.

have validated that TTN-AS1 is overexpressed in the MCF-7/TAM cell line, which indirectly suggests that overexpression of TTN-AS1 led to a down-regulation of miR-107. On the other hand, transfection of si-TTN-AS1 in MCF-7/TAM reversed the expression of miR-107, which indicates that miR-107 is negatively regulated by lncRNA TTN-AS1 (**Figure 3D**, **3E**).

MiR-107 inhibitor reversed the effect of si-TTN-AS1 in MCF-7/TAM

In order to further study the influence of miR-107 in MCF-7/TAM cells, we conducted transwell assay, as well as flow cytometry to analyze cell cycle and apoptosis. Similar to results from our previous investigation, a decrease in TTN-AS1 indicated lowest cell invasion compared to si-NC transfected MCF-7/TAM (**Figure 4A**). In comparison, MCF-7/TAM treated co-transfection with si-TTN-AS1 and miR-107 inhibitor demonstrated a significantly higher proportion of invasive cells than those transfected only with si-TTN-AS1. Furthermore, miR-107 knockdown induced a significantly lower percentage of apoptotic cells and a descent proportion of cell cycle arrest in the G0/G1 phase that induced by si-TTN-AS1 (**Figure 4B**, **4C**).

ZNRF2 is negatively regulated by miR-107

Bioinformatics analysis indicated that E3 ubiquitin-protein ligase zinc and ring finger 2 (ZNRF2) was a putative target of miR-107, the binding site was shown in **Figure 5A**. Next, we performed a dual-luciferase assay to verify the interaction between miR-107 and ZNRF2, results from which indicated that miR-107 can directly bind to ZNRF2 (**Figure 5B**). ZNRF2 has recently been recognized as an oncogene that mediates tumors progression, metastasis, cell growth and invasion [32]. To understand the role of ZNRF2 in TAM-resistant breast cancer,

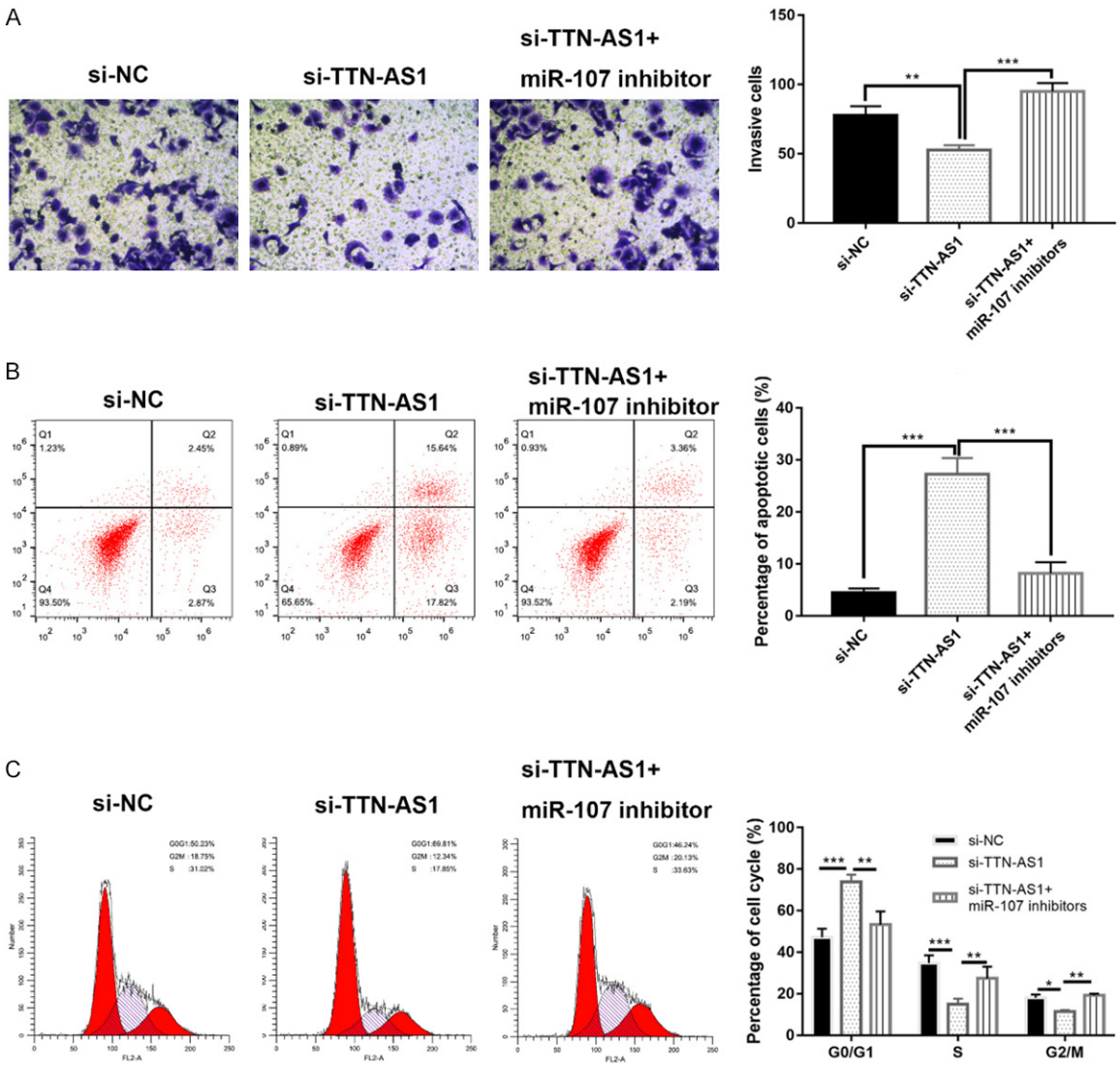

Figure 4. MiR-107 inhibitor reversed the effect of si-TTN-AS1 in MCF-7/TAM. A. MiR-107 knockdown led to augmented invasion of MCF-7/TAM induced by si-TTN-AS1. B, C. miR-107 inhibitor reduced proportion of apoptotic cells and G0/G1 cell cycle arrest influenced by si-TTN-AS1. All data are represented as mean ± SD. Each assay was performed in triplicate. *P < 0.05, **P < 0.01, ***P < 0.001.

we explored the related assays in the MCF-7/TAM cell line. Firstly, the expression of ZNRF2 in MCF-7 and MCF-7/TAM was examined by qRT-PCR and western blotting. Results indicated that ZNRF2 was overexpressed in TAM-resistant BC cell line (**Figure 5C**, **5D**). Next, ZNRF2 expression was evaluated in the TTN-AS1-silenced MCF-7/TAM cell line to determine the relationship between the lncRNA TTN-AS1 and ZNRF2. Western blotting results indicated that ZNRF2 was highly expressed in MCF-7/TAM, while ZNRF2 was downregulated in TTN-AS1-silenced MCF-7/TAM cells (**Figure 5E**, **5F**). Additionally, ZNRF2 was upregulated in miR-107-silenced MCF-7/TAM cells (**Figure 5G**, **5H**). Hence, all data collectively suggests that ZNRF2 is involved in the TTN-AS1/miR-107 axis to regulate the progression of breast cancer.

Knockdown of ZNRF2 reversed the effect of miR-107 inhibitor in MCF-7/TAM via mediating the PI3K/AKT pathway

We conducted further investigation on ZNRF2 by studying the role of ZNRF2 in mediating the invasion and cell growth of TAM-resistant breast cancer cells. Results from the transwell chamber assay and flow cytometry demonstrat-

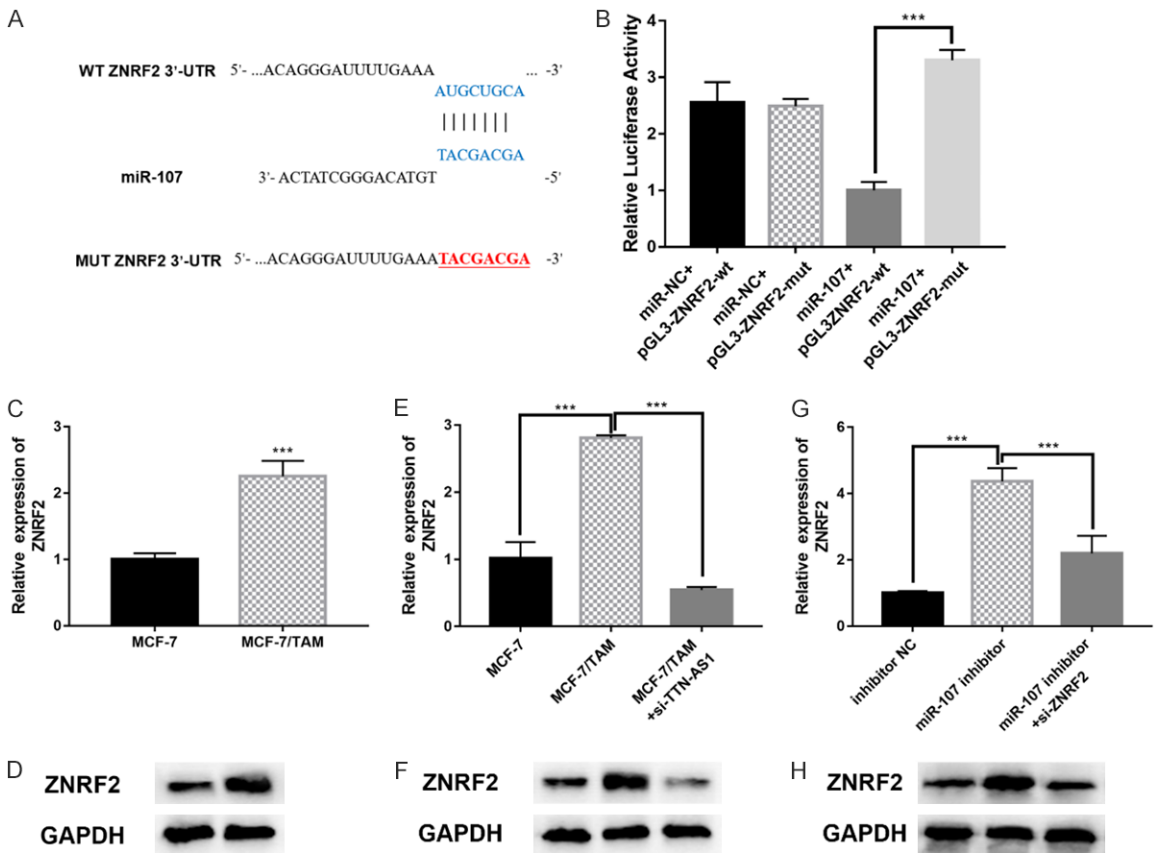

Figure 5. ZNRF2 is a direct target of miR-107. A. The predicted binding site of miR-107 on ZNRF2 sequence. B. Dual-luciferase assay results showed direct binding between miR-107 and ZNRF2. C, D. Western blot results showed ZNRF2 is higher expressed in MCF-7/TAM than MCF-7. E, F. The expression of ZNRF2 is proportional to TTN-AS1. G, H. ZNRF2 is negatively regulated by miR-107. All data are represented as mean ± SD. Each assay was performed in triplicate. ***P < 0.001.

ed that down-regulation of ZNRF2 in the miR-107 inhibitor-treated MCF-7/TAM cell line was associated with weakened invasive ability (**Figure 6A**). The results from flow cytometry suggested that co-transfection with si-ZNRF2 led to higher proportion of apoptotic cells compared to only miR-107-inhibitor treated group (**Figure 6B**). Western blot results also indicated that down-regulation of ZNRF2 led to inactivate PI3K/AKT pathway that activated by miR-107 inhibitor (**Figure 6C**).

Discussion

Recently, endocrine therapy has gradually become the main type of treatment for breast cancer, among which tamoxifen has attracted the most attention due to its significant efficacy and reduced side effects. Tamoxifen has been proven to reduce the incidence of breast cancer among women that are at high risk of breast cancer by 49% [33]. Nevertheless, acquired drug resistance caused by long-term use of tamoxifen limits its clinical use. Therefore, further study of the pathological mechanism of tamoxifen resistance is urgent.

Current studies have demonstrated that the lncRNA TTN-AS1 works to enhance the progression of various tumors, including esophageal squamous cell carcinoma [18], lung adenocarcinoma [19] and colorectal cancer [20]. For instance, it has been reported that TTN-AS1 can promote colorectal cancer development by sponging miR-376a-3p, and upregulating KLF15 [20]. Additionally, TTN-AS1 has been shown to facilitate papillary thyroid cancer through regulating the miR-153/ZNRF2 axis [32]. However, the specific function of TTN-AS1 in the progression of breast cancer is still under exploration. In this study, we determined that TTN-AS1 possibly participates in the develop-

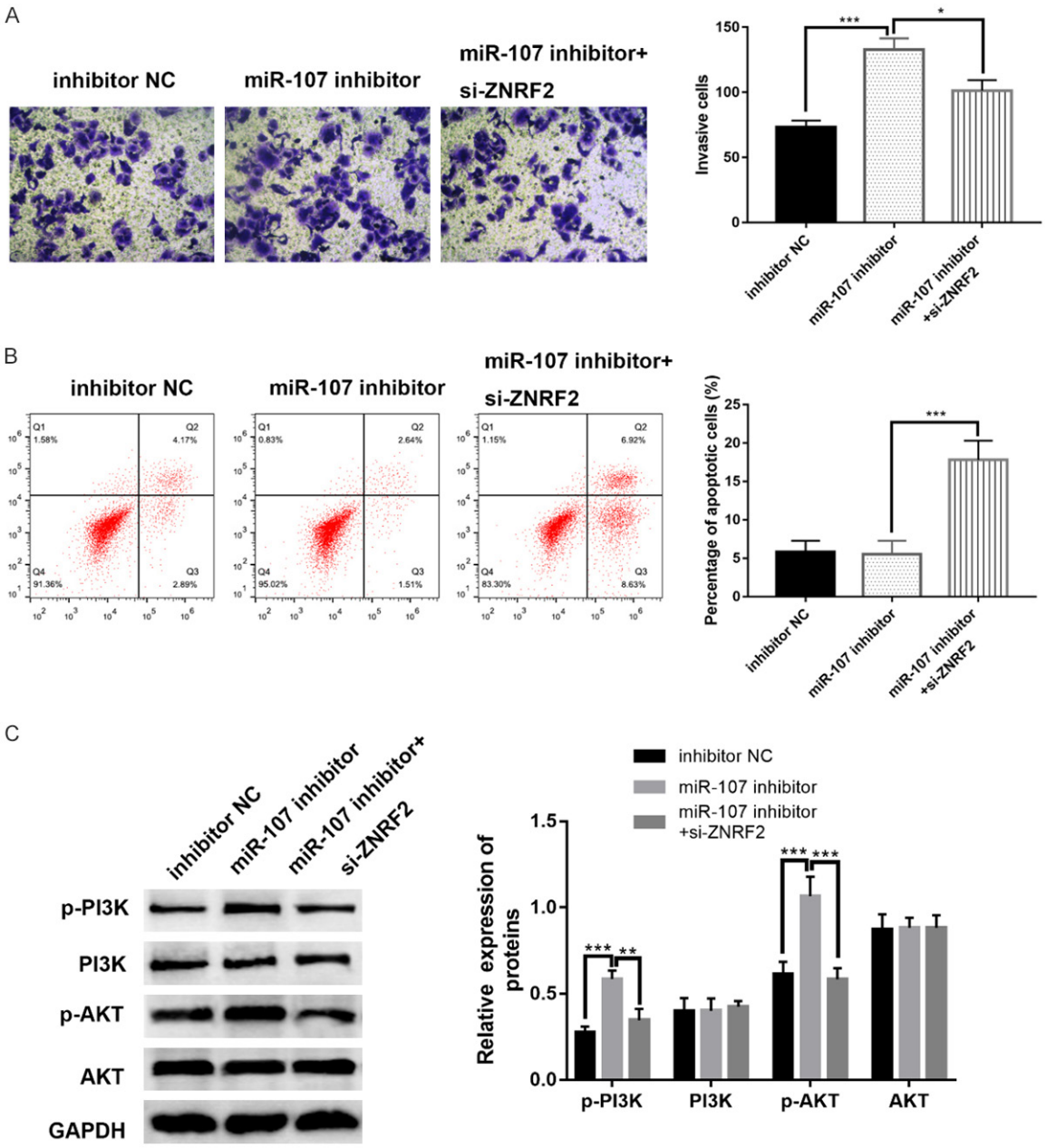

Figure 6. Knockdown of ZNRF2 reversed the effect of miR-107 inhibitor in MCF-7/TAM via mediating the PI3K/AKT pathway. A. Results of cell invasion by the transwell chamber assay. B. Cell apoptosis by flow cytometry. C. Western blotting assay was performed to examine the proteins that participate in the PI3K/AKT pathway. All data are represented as mean ± SD. Each assay was performed in triplicate. *P < 0.05, ***P < 0.001.

ment of tamoxifen-resistance in breast cancer, since TTN-AS1 was discovered to be highly expressed in TAM-resistant BC cell line (MCF-7/TAM), when compared to normal MCF-7 cells. This result indicates that TTN-AS1 might have a pathological association with tamoxifen resistance in breast cancer. To further understand the specific role of TTN-AS1, we transfected si-TTN-AS1 into MCF-7/TAM cells to knockdown TTN-AS1. Compared to MCF-7/TAM cells, si-TTN-AS1-treated MCF-7/TAM cells demonstrated increased invasive and proliferative capacity in TAM-resistant breast cancer *in vitro*.

Recently, miRNAs have been proven to have a role in modulating tumorigenesis. Investigation of miRNA expression in breast cancer, as well as their relationship with tumor occurrence,

development and drug resistance in breast cancer have become a research hotspot. For example, one study found that miR-107 can regulate pancreatic and gastric cancer development via mediating the expression of CDK6 [34, 35], leading to cell cycle arrest in non-small cell lung cancer (NSCLC) [36]. Hence, studying miRNA-associated drug-resistance in breast cancer might of great help in resolving the drug-resistance in breast cancer therapy. In this study, we found that miR-107 was negatively regulated by TTN-AS1 using a dual-luciferase reporter assay. In addition, miR-107 expression leads to a reduction in cell growth and invasion, which indicates that the lncRNA TTN-AS1 enhances cell proliferation and invasion in TAM-resistant breast cancer cell line via down-regulation of miR-107.

ZNRF2 was has been known as a factor that participates in regulation of various tumorigenesis. Recent studies have illustrated that ZNFR2 is a functional element in modulating the progression of papillary thyroid cancer, through the regulation of downstream PI3K/AKT pathway. Nevertheless, whether ZNRF2 also involved in tamoxifen-resistance in breast cancer remains unknown. In this study, we explored the specific function of ZNRF2 in drug-resistant breast cancer. Our results demonstrated that ZNRF2 is a negatively regulated factor of miR-107 by qRT-PCR and Western blotting assay. We also showed that ZNRF2 has the ability to promote cell growth and invasion. Furthermore, knockdown of ZNRF2 in TAM-resistant BC cell line induced cell apoptosis. Additional mechanisms by which ZNRF2 prevented cell growth in breast cancer was studied through western blot to discover the proteins expression of downstream regulated pathways. Western blot results indicated that knockdown of ZNRF2 caused the activation of the PI3K/AKT pathway, which suggests that ZNRF2 promotes tamoxifen-resistance in BC cells via stimulating PI3K/AKT pathway. This result showed that the PI3K/AKT pathway also participates in the drug-resistance and cell progression of TAM-resistant breast cancer. The collective data from our research demonstrates that the lncRNA TTN-AS1 enhances tamoxifen resistance, cell growth and invasion in breast cancer via modulating the miR-107/ZNRF2 axis.

In summary, our study reported the lncRNA TTN-AS1 as a promoter of tumorigenesis and tamoxifen-resistance via regulation of the miR-107/ZNRF2 axis. Also, TTN-AS1/miR-107/ZNRF2 regulates cell growth and invasion through downstream PI3K/AKT pathway.

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Disclosure of conflict of interest

None.

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