Docetaxel-resistant triple-negative breast cancer cell-derived exosomal lncRNA LINC00667 reduces the chemosensitivity of breast cancer cells to docetaxel *via* targeting miR-200b-3p/Bcl-2 axis

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ABSTRACT

Development of docetaxel (TXT) resistance is a major obstacle for triple-negative breast cancer (TNBC) treatment. Additionally, chemoresistant cell-derived exosomes were able to change the chemo-response of chemosensitive recipient cells *via* transportation of lncRNAs. It has been shown that lncRNA LINC00667 level was significantly elevated in breast cancer tissues. Therefore, we explored whether LINC00667 level is increased in TXT-resistant TNBC cell-derived exosomes. In addition, whether exosomal LINC00667 derived from TXT-resistant TNBC cell could affect TXT sensitivity in TXT-sensitive TNBC cells was investigated as well. In the present study, exosomes were isolated from the TXT-resistant TNBC cells and from TXT-sensitive TNBC cells. Next, the level of LINC00667 in the isolated exosomes was detected with RT-qPCR. We found that LINC00667 expression was obviously elevated in TXT-resistant TNBC cell-derived exosomes compared to that in TXT-sensitive TNBC cell-derived exosomes. In addition, LINC00667 could be transferred from TXT-resistant TNBC cells to TNBC cells *via* exosomes. Moreover, TXT-resistant TNBC cell secreted exosomal LINC00667 markedly reduced the sensitivity of TNBC cells to TXT *via* upregulation of Bcl-2. Meanwhile, downregulation of LINC00667 notably enhanced the sensitivity of TXT-resistant TNBC cells to TXT through downregulation of Bcl-2. Additionally, LINC00667 was considered to be a ceRNA to sponge miR-200b-3p, thereby elevating Bcl-2 expression. Collectively, TXT-resistant TNBC cell-derived exosomal LINC00667 could decrease the chemosensitivity of TNBC cells to TXT *via* regulating miR-200b-3p/Bcl-2 axis. These findings suggested that LINC00667 might serve as a promising target for enhancing sensitivity of TNBC cells to TXT therapy.

Key words: Breast cancer; docetaxel; chemosensitivity; exosomes; long non-coding RNAs.

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Introduction

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer due to its aggressive phenotype, high drug resistance, high heterogeneity, and lack of clear therapeutic targets.[1-3] In addition, TNBC is characterized by the lack of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2).[4] Thereby, there is still no effective therapy for the treatment of TNBC.[5] Clinically, nonspecific chemotherapy is a primary treatment method for TNBC;[6] however, the effectiveness of nonspecific chemotherapy is hampered by the emergence of drug resistance.[7]

Docetaxel (TXT), an anti-microtubule chemotherapeutic agent, has been used for treating various cancers including TNBC.[8-10] TXT exerts its anti-tumor effects through triggering cell cycle arrest in cancer cells.[11] Although TXT showed promising anti-tumor effects, acquired resistance to TXT is a main obstacle for TNBC treatment clinically;[12,13] Thus, exploring the underlying mechanism of TXT resistance in TNBC may facilitate the development of new therapeutic methods.

Long non-coding RNAs (lncRNAs) exert a key role in multiple cellular processes such as cell proliferation, migration and chemoresistance.[14,15] Du *et al*. found that lncRNA DLX6-AS1 enhanced cisplatin resistance in TNBC cells *via* miR-199b-5p/PXN signaling.[16] Li *et al*. showed that lncRNA OTUD6B-AS1 facilitate paclitaxel resistance in TNBC *via* miR-26a-5p/MTDH axis.[17] Additionally, evidence has reported that lncRNAs play a key function in intracellular communication as well as in the tumor microenvironment.[18,19]

Exosomes exert a key role in mediating intercellular communications.[20] Exosomes are extracellular nanovesicles (30-150 nm in size) secreted by multiple cells.[21-23] Meanwhile, exosomes are able to mediate intercellular communication *via* transferring functional cargos, including mRNA, miRNAs, lncRNAs and proteins.[24-26] Additionally, Dong *et al*. showed that chemo-resistant cell-derived exosomes could influence the chemo-response of chemo-sensitive recipient cells *via* transportation of lncRNAs.[27] Zhu *et al.* reported that LINC00667 was obviously elevated in breast cancer tissues.[28] However, whether exosomal LINC00667 could regulate TNBC progression and TXT sensitivity remains largely unknown.

In this study, we found that LINC00667 level was greatly elevated in TXT-resistant TNBC cell-derived exosomes. In addition, TXT-resistant TNBC cells were able to enhance the proliferation of MDA-MB-231 cells and reduced TXT sensitivity through transferring exosomal LINC00667. These findings might provide different insights for the treatment of TNBC.

Materials and Methods

Cell culture and transfection

TNBC cell line MDA-MB-231 and MDA-MB-468 were purchased from Procell Life Science & Technology Co., Ltd. (Wuhan, China). TXT-resistant TNBC cell line (MDA-MB-231/TXT) was established by continuous exposure of MDA-MB-231 cells to a stepwise gradually concentration of TXT (from 0.25 to 16 μg/mL; MedChemExpress, Princeton, NJ, USA) for more than 3 months.

MDA-MB-231, MDA-MB-468, and MDA-MB-231/TXT cells were grown on plastic plates and were maintained in DMEM supplemented with 10% FBS and 1% penicillin/streptomycin at 37°C with 5% CO_2.

Human siRNA negative control (siRNA-ctrl, 5'-CAGCCCTAGAGCTTAATCAGGGTGTA-3'), LINC00667 siRNA1 (si-LINC00667-1, 5'-CAGCATCACCTAGGGAGCTTATGTA-3'), LINC00667 siRNA2 (si-LINC00667-2, 5'-CCACATTAGTGAGGGTGGATCTTCT-3'), LINC00667 siRNA3 (si-LINC00667-3, 5'-CAGTCTAGTTTGACTAGGTCCTGTA-3') obtained from RIBOBIO were transfected into TNBC cells using Lipofectamine 2000.

Cell counting kit-8 (CCK-8) assay

Cells (1×10^5/well) were seeded onto a 96-well plate (Corning Inc., Cornig, NY, USA) overnight. After the indicated treatments, 10 μL CCK-8 reagent (Beyotime Institute of Biotechnology, Jiangsu, China) was added to each well, followed by incubation for 2 h. Next, the absorbance of each well was evaluated at 450 nm with a microplate reader (DR-200Bs; DiaTek Co., Wuxi, China).

Exosomes isolation and identification

Using ultracentrifugation method, exosomes were isolated from the conditioned medium (CM) of MDA-MB-231/TXT cells, as described previously.[29] Next, the isolated pellets were resuspended in PBS. The number and size of the exosomes were tracked using a ZetaView nanoparticle tracking analyzer (Particle Metrix). Next, exosomes were fixed in 2.5% glutaraldehyde, dropped onto a carbon-coated copper grids, and then stained with 1% phosphotungstic acid for 3 min. Next, a transmission electron microscope (TEM; HT7700, HITACHI) was applied to capture micrographs.

RT-qPCR assay

Total RNA was isolated from with the TRIpure Total RNA Extraction Reagent (No. EP013) and the purified RNA was reverse-transcribed to form cDNA using a M-MLV Reverse Transcriptase kit (No. EQ002). Next, qPCR experiments were carried out using the QuFast SYBR Green PCR (No. EQ001). All kits were obtained from ELK Biotechnology Co., Ltd (Wuhan, China). The thermocycling parameters were 95°C for 1 min, followed by 40 cycles of 95°C for 15 s, 58°C for 20 s, 72°C for 45 s. β-actin was used to normalize lncRNA levels. β-actin forward, 5'-GTCCACCGCAAATGCTTCTA-3' and reverse, 5'- TGCTGTCACCTTCACCGTTC-3'; LINC00667 forward, 5'-GATAGTTCAGGCCCAGAGACG-3' and reverse, 5'-TTTTTTCCTGGTAGTGGCCAG-3'; Bcl2 forward, 5'-TGGGGTCATGTGTGTGGAGAG-3' and reverse, 5'- AATCAAACAGAGGCCGCATG-3'; Linc00461 forward, 5'- GATCCTTGAATCAGCTGTCACTT-3' and reverse, 5'- GCTATTAAGGTGCAGCAAGC-3'; EPB41L4A-AS2 forward, 5'- GTGTGTAGCCAAGTTCAAGCTCT-3' and reverse, 5'- TTTGCTGAATGTCCTGAAGAAG-3'; TMPO-AS1 forward, 5'- TGGATGTAGGGTGTGTTAATCAT-3' and reverse, 5'-CAATTTGAATAAAAGTAAGTCGC-3'.

Exosome labeling and uptake

TNBC cells were seed onto a 6 well plate overnight. After that, the PKH26 red dye (Sigma-Aldrich, St. Louis, MO, USA) was used to label exosomes. Later on, TNBC cells were treated with PKH26-labeled exosomes (50 μg/mL) for 24 h. Phalloidin (1:1000 dilution in 1%BSA; YEASEN Biotechnology, Shanghai, China) was used to stain actin filaments (F-actin) at 37°C for 30 min, and DAPI (10 μg/mL; YEASEN Biotechnology) was used to stain cell nuclei at room temperature for 5 min. Next, exosomes absorbed by TNBC cells were captured under a confocal laser scanning microscope (ZEISS LSM880, light: laser; objective: 40x; Zeiss, Jena, Germany). Green fluorescence, excitation wavelength/emission wavelength: 490 nm/530 nm; blue fluorescence: 364 nm/454 nm; red fluorescence, 540 nm/615 nm.

TUNEL assay

TUNEL assay was performed using the In Situ Cell Death Detection Kit (Roche Diagnostics Co., Indianapolis, IN, USA). TNBC cells were fixed with 4% paraformaldehyde for 20 min and then incubated with the TUNEL reaction mixture (solution A and solution B) for 1 h in darkness at 37°C. Finally, analysis was performed by a fluorescence microscopy (Nikon, Eclipse Ci-L, light: mercury lamp; objective: 20x; Nikon, Tokyo, Japan). Green fluorescence, 490 nm/530 nm; blue fluorescence: 364 nm/454 nm. TUNEL-positive cells (green color) were counted in 3 random fields using the Image-Pro Plus software.

Western blot assay

Equal amounts of protein were subjected to 10% SDS-PAGE and transferred onto a PVDF membrane. Next, the membrane was incubated with primary antibodies including anti-Bcl-2 (1:1000, cat. no. AF6139, Affinity Biosciences, Cincinnati, OH, USA), anti-cleaved caspase 9 (1:1000, cat. no. ab32539, Abcam, Cambridge, UK), anti-cleaved caspase 3 (1:1000, cat. no. ab32042, Abcam), anti-β-actin (1:10000, cat. no. 66009-1-Ig, Proteintech Group, Rosemont, IL, USA) at 4°C overnight, followed by incubation with HRP-labeled secondary antibody for 1 h at room temperature. Subsequently, the immunoreactive bands were visualized by an ECL reagent.

Dual-luciferase reporter assay

The mutant-type (MT) of LINC00667 or Bcl-2 3′-UTR and wild type (WT) of LINC00667 or Bcl-2 3′-UTR were amplified and cloned into the pGL6-miR-based luciferase reporter plasmids. After that, these above plasmids were co-transfected into MDA-MA-231 cells with miR-200b-3p mimics using Lipofectamine 2000 for 48 h. Next, the luciferase activity was measured using a dual luciferase reporter assay kit (Promega Co., Madison, WI, USA).

Fluorescence *in situ* hybridization analysis

The LINC00667 probe was designed and synthesized by RiboBio and then labeled with FAM fluorescent dye. In addition, a Cy3-conjugated miR-200b-3p probe was designed and obtained from RiboBio. MDA-MB-231 cells were incubated with FAM-labelled LINC00667 probes and Cy3-conjugated miR-200b-3p probes overnight. Subsequently, the subcellular location of LINC00667 and miR-200b-3p were captured by a confocal laser scanning microscope (ZEISS LSM880, light: laser; objective: 40x). Green fluorescence, 490 nm/530 nm; blue fluorescence: 364 nm/454 nm; red fluorescence, 540 nm/615 nm.

Statistical analyses

All experiments were conducted at least three times. Data are presented as the mean ± standard deviation. The difference among multiple groups was performed by one-way analysis of variance (ANOVA). Unpaired Student's *t*-test was used for comparison between two groups. The statistical significance was $p<0.05$.

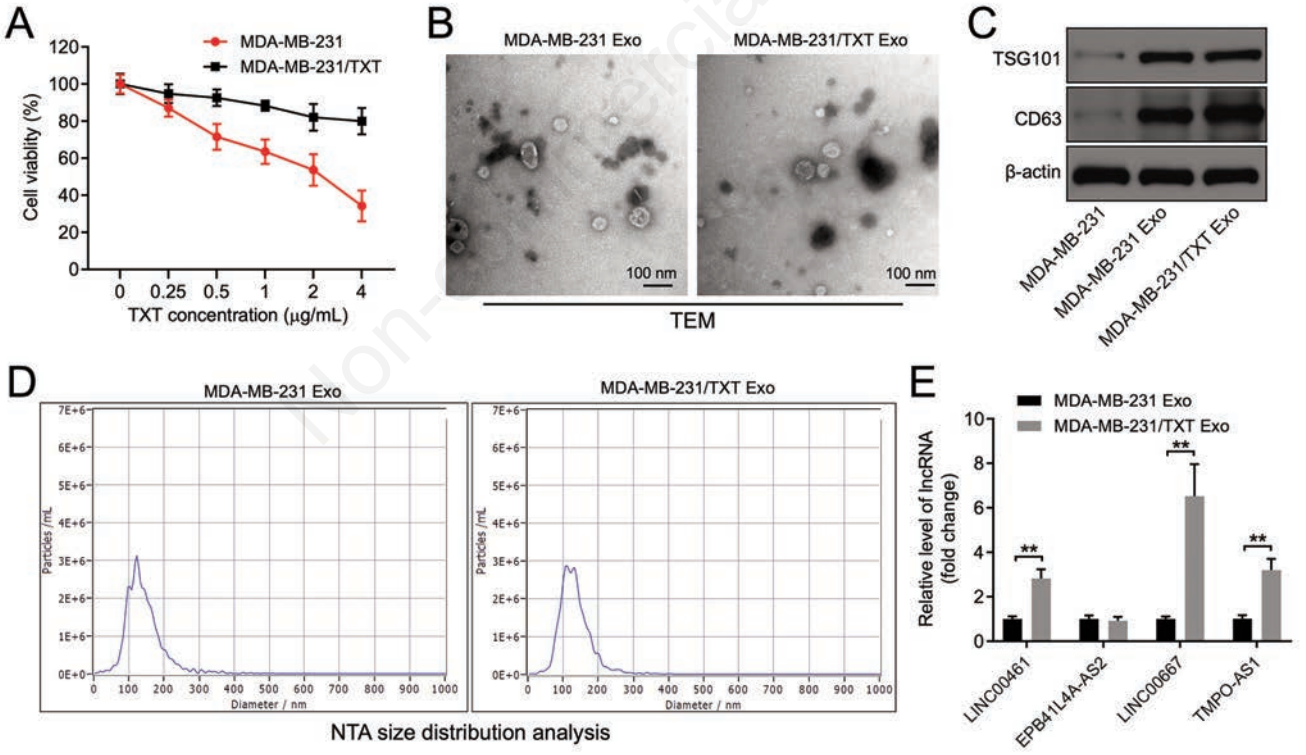

Figure 1. The level of LINC00667 was increased in MDA-MB-231/TXT cell-derived exosomes. A) MDA-MB-231 and MDA-MB-231/TXT cells were treated with different concentration (0, 0.25, 0.5, 1, 2 or 4 g/mL) of docetaxel (TXT) for 48 h; cell viability was determined by CCK-8 assay. B) Exosomes were isolated from the supernatant of MDA-MB-231 and MDA-MB-231/TXT cells; the morphology of MDA-MB-231-Exo and MDA-MB-231/TXT-Exo was captured by TEM. C) Western blot assay was used to detect the TSG101 and CD63 level in MDA-MB-231 cells, MDA-MB-231-Exo and MDA-MB-231/TXT-Exo. D) NTA analysis was performed to determine the size distribution of MDA-MB-231-Exo and MDA-MB-231/TXT-Exo. E) RT-qPCR analysis of LINC00461, EPB41L4A-AS2, LINC00667 and TMPO-AS1 level in MDA-MB-231-Exo and MDA-MB-231/TXT-Exo. **p<0.01.

Results

The level of LINC00667 was increased in TXT-resistant TNBC cell-derived exosomes

To explore the effects of TXT on the viability of MDA-MB-231 and MDA-MB-231/TXT cells, CCK-8 assay was performed. The result of CCK-8 indicated 2 μg/mL of TXT induced about 50% growth inhibition in MDA-MB-231 cells, while it induced about 20% growth inhibition in MDA-MB-231/TXT cells (Figure 1A). Thus, 2 μg/mL of TXT were utilized in the following experiments. As we know, exosome-derived from chemo-resistant cancer cells could influence the sensitivity of chemo-sensitive recipient cells to chemotherapy agents *via* transportation of lncRNAs.[27,30] In order to investigate whether TXT-resistant TNBC cell-derived exosomes could regulate TNBC progression in TXT-sensitive TNBC cells, exosomes were isolated from the CM of MDA-MB-231 cells (MDA-MB-231-Exo) and from MDA-MB-231/TXT cells (MDA-MB-231/TXT-Exo), respectively. Then, the isolated vesicles were identified by TEM, nanoparticle tracking analysis (NTA), and Western blot assay. These two isolated vesicles had a cup-shaped structure with diameters ranged from 30 to 150 nm and expressed exosomal markers TSG101 and CD63 (Figure 1 B-D), indicating these vesicles were exosomes. Evidence has shown that LINC00461, EPB41L4A-AS2, LINC00667 and TMPO-AS1 were dysregulated in breast cancer.[31-34] To investigate whether these lncRNAs are involved in the regulation of TNBC progression and TXT sensitivity, LINC00461, EPB41L4A-AS2, LINC00667 and TMPO-AS1 level in MDA-MB-231-Exo and MDA-MB-231/TXT-Exo were detected by RT-qPCR. As revealed in Figure 1E, LINC00667 level was markedly elevated in MDA-MB-231/TXT-Exo compared to MDA-MB-231-Exo. Thus, we focus on exploring the role of LINC00667 in chemosensitivity of TNBC.

Exosomes released by TXT-resistant TNBC cells could be internalized by TNBC cells

Next, to determine if MDA-MB-231/TXT-Exo could be internalized by TNBC cells, cells were incubated with PKH26-labeled MDA-MB-231/TXT-Exo. The results showed that PKH26 dye was observed in TNBC cells (Figure 2 A,B). In addition, MDA-MB-231/TXT-Exo significantly elevated LINC00667 level in TNBC cells (Figure 2 C,D). To sum up, LINC00667 is contained in MDA-MB-231/TXT-Exo and can be transferred to TNBC cells.

TXT-resistant TNBC cell secreted exosomal LINC00667 reduced the sensitivity of TNBC cells to TXT

To explore the interaction between LINC00667 and chemosensitivity of TNBC, TNBC cells were transfected with si-LINC00667-1, si-LINC00667-2, or si-LINC00667-3. As shown in Figure 3A, si-LINC00667-1 notably reduced LINC00667 level in

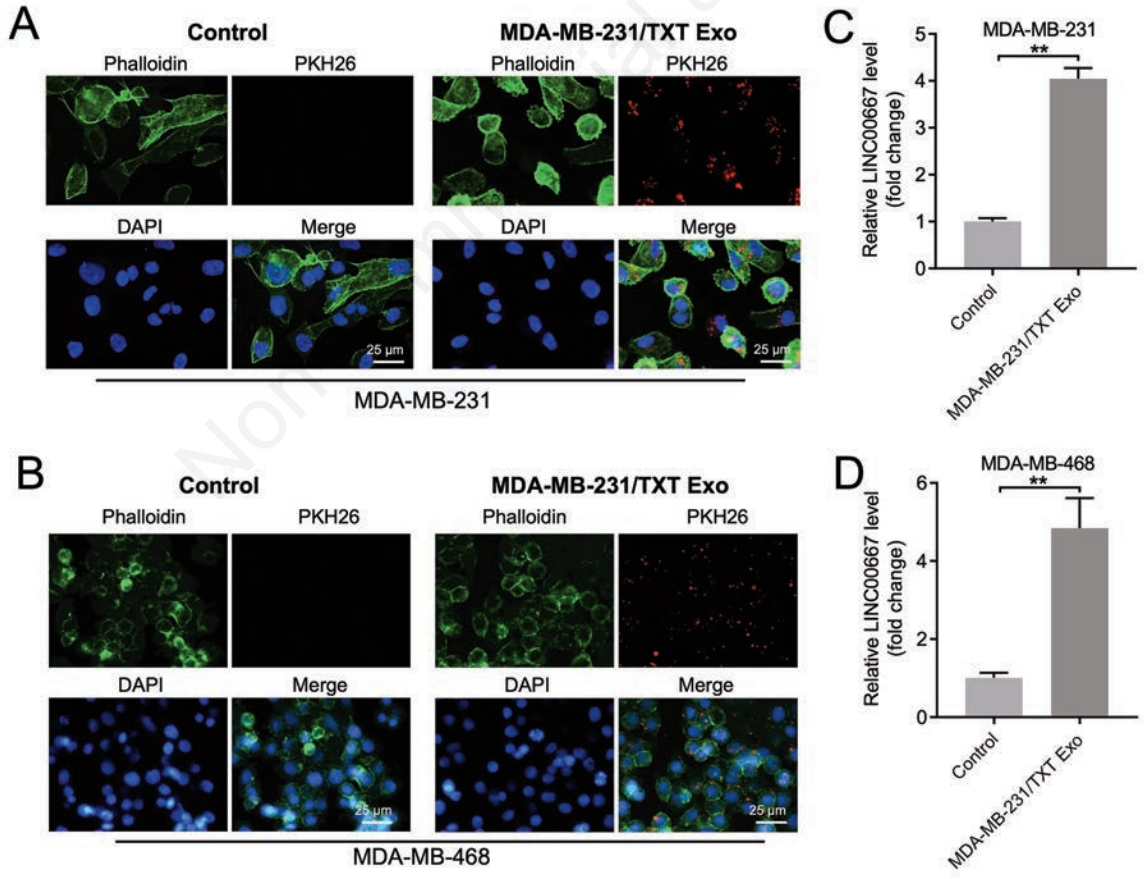

Figure 2. Exosomes released by MDA-MB-231/TXT cells could be internalized by TNBC cells. A,B) Immunofluorescence images showed the internalization of PKH26-labeled MDA-MB-231-Exo and MDA-MB-231/TXT-Exo (red color) by TNBC cells; phalloidin (green color) was used to stain cytoskeleton, and DAPI (blue color) was used to stain cell nuclei. C,D) RT-qPCR analysis of LINC00667 in TNBC cells treated with or without MDA-MB-231/TXT-Exo. **p<0.01.

MDA-MB-231 cells. In addition, TXT significantly reduced the viability and triggered the apoptosis of TNBC cells (Figure 3 B-D). However, MDA-MB-231/TXT-Exo markedly abolished the anti-tumor effects of TXT in TNBC cells, whereas these changes were reversed by si-LINC00667-1 (Figure 3 B-D). Moreover, compared to TXT treatment group, MDA-MB-231/TXT-Exo markedly upregulated Bcl-2 level and downregulated cleaved caspase 3 and cleaved caspase 9 level in TXT-treated TNBC cells; however, downregulation of LINC00667 in TNBC cells reversed these effects (Figure 4 A-D). Collectively, MDA-MB-231/TXT cell secreted exosomal LINC00667 could reduce the sensitivity of TNBC cells to TXT.

TXT-resistant TNBC cell secreted exosomal LINC00667 reduced the sensitivity of TNBC cells to TXT *via* miR-200b-3p/Bcl-2 axis

LncRNAs can act as sponges for miRNA to regulate mRNA expression.[35] The lncRNA-miRNA-mRNA axis plays important roles in tumor progression.[36,37] Thus, Starbase database was used to identify the miRNAs interacting with LINC00667. The result of database suggested miR-200b-3p is a potential miRNA target of LINC00667 (Figure 5A). Additionally, FISH results showed the co-localization of LINC00667 and miR-200b-3p in the cytoplasm of TNBC cells (Figure 5B). Furthermore, miR-200b-3p mimics notably depleted the luciferase activity in TNBC cells co-transfect-

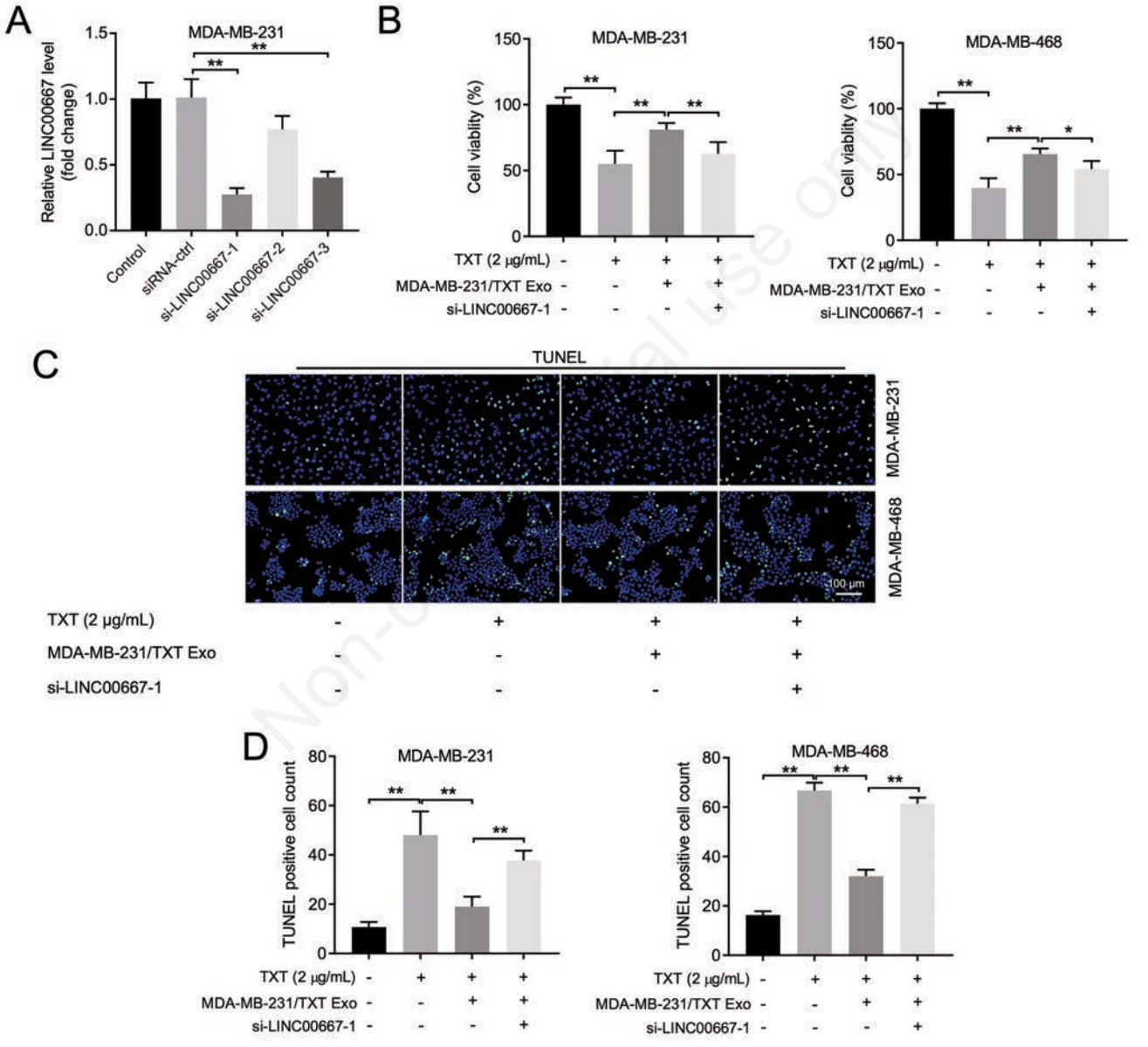

Figure 3. MDA-MB-231/TXT cell secreted exosomal LINC00667 reduced the sensitivity of TNBC cells to TXT. A) LINC00667 level in MDA-MB-231 cells transfected with siRNA-ctrl, si-LINC00667-1, si-LINC00667-2, or si-LINC00667-3 was detected by RT-qPCR. B-D) MDA-MB-231 and MDA-MB-468 cells were treated with 2 μg/mL TXT, TXT plus MDA-MB-231/TXT Exo, or TXT, MDA-MB-231/TXT Exo plus si-LINC00667-1 for 48 h; cell viability and apoptosis were detected by CCK-8 and TUNEL assays respectively. *p<0.05; **p<0.01.

ed with LINC00667-WT plasmids (Figure 5C). All these data showed that LINC00667 directly binding to miR-200b-3p in TNBC cells.

Next, the target gene of miR-200b-3p was predicted using Targetscan and Starbase databases. These two databases indicated that Bcl-2 might be a potential target of miR-200b-3p (Figure 5D). Furthermore, miR-200b-3p mimics obviously suppressed the luciferase activity in TNBC cells co-transfected with Bcl-2-WT plasmids (Figure 5E). Moreover, miR-200b-3p overexpression remarkedly downregulated Bcl-2 level in TNBC cells (Figure 5F). Collectively, Bcl-2 is a direct target of miR-200b-3p in TNBC cells.

Downregulation of LINC00667 sensitized TXT-resistant TNBC cells to TXT treatment

We next investigated the role of LINC00667 in TXT-resistant TNBC cells. As revealed in Figure 6 A,B, TXT reduced the viability by inducing the apoptosis of MDA-MB-231/TXT cells. As expected, these effects were further enhanced by si-LINC00667-1 (Figure 6 A,B). In addition, TXT significantly reduced Bcl-2 level and elevated cleaved caspase 3/9 level in MDA-MB-231/TXT cells, and these effects were further enhanced in the presence of si-LINC00667-1 (Figure 6 C-F) as well. To sum up, downregulation of LINC00667 could sensitize TXT-resistant TNBC cells to TXT treatment.

Discussion

TXT is a commonly used chemotherapy drug in TNBC.[38] However, acquired chemotherapy resistance is an obstacle toward the successful treatment of TNBC.[39,40] Thus, improving the sensitivity of TNBC cells to TXT may be a novel approach to overcome TXT resistance and to improve the therapeutic outcome. Our results showed that TXT-resistant TNBC cell-derived exosomal LINC00667 remarkably reduced the sensitivity of TNBC cells to TXT *via* miR-200b-3p/Bcl-2 axis. In addition, downregulation of LINC00667 strengthened the sensitivity of TXT-resistant TNBC cells to TXT. These studies showed that lncRNAs play an important role in regulating the sensitivity of TNBC cells to TXT.

Exosomes play crucial roles in intercellular communications.[20,41] In addition, exosomes participate in the regulation of the chemosensitivity of the cancer cells.[20,41] Moreover, lncRNAs are enriched in exosomes and exosomal lncRNAs play vital roles in the chemoresistance and chemosensitivity of human cancers including TNBC.[42-45] LncRNA-HOTTIP-enriched exosomes derived from cisplatin-resistant gastric cancer cells could enhance cisplatin resistance in gastric cancer cells.[46] Meanwhile, exosomal AFAP1-AS1 derived from trastuzumab-resistant breast cancer cells was able to confer trastuzumab resistance in breast cancer cells.[45] Our results showed that LINC00667 level was markedly upregulated in TXT-resistant TNBC cell-derived exosomes compared to that in TXT-sensitive TNBC cell-derived exosomes. In addition, LINC00667 can be transferred from TXT-resistant TNBC cells to TXT-sensitive TNBC cells *via* exosomes. Significantly, exosomal LINC00667 reduced the sensitivity of TNBC cells to

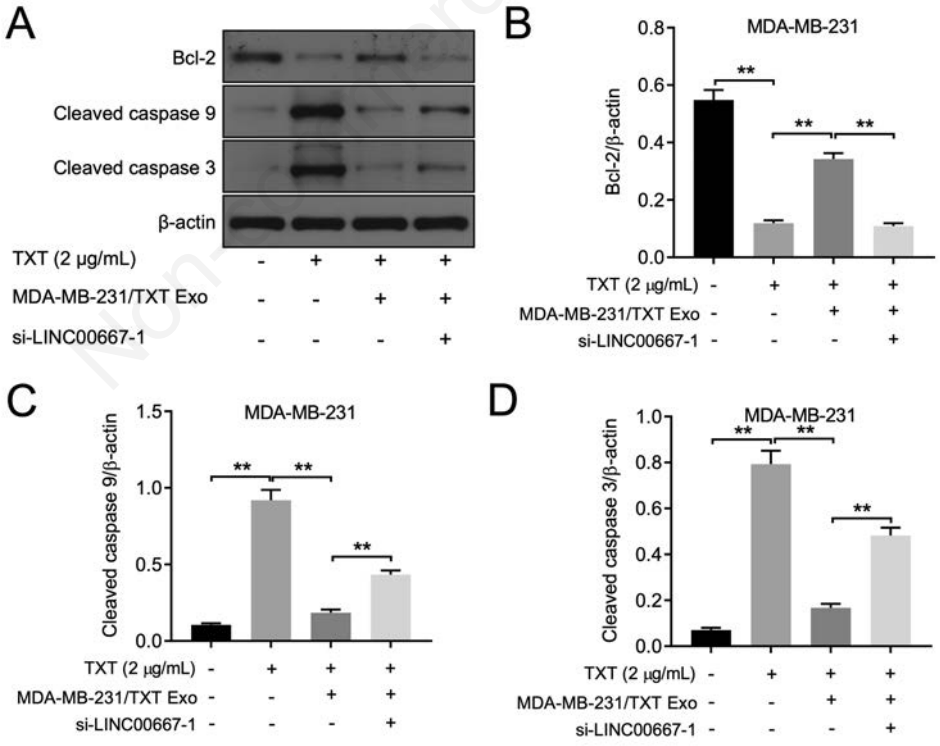

Figure 4. MDA-MB-231/TXT cell secreted exosomal LINC00667 reduced the sensitivity of TNBC cells to TXT *via* suppressing cell apoptosis. MDA-MB-231 cells were treated with 2 μg/mL TXT, TXT plus MDA-MB-231/TXT Exo, or TXT, MDA-MB-231/TXT Exo plus si-LINC00667-1 for 48 h. Western blot assay was used to measure Bcl-2, cleaved caspase 9, and cleaved caspase 3 protein expressions in MDA-MB-231 cells. The relative expressions of Bcl-2, cleaved caspase 9, and cleaved caspase 3 were quantified *via* normalization to β-actin. **p<0.01.

TXT. These results showed that exosomes from chemo-resistant cancer cells are able to alter the chemosensitivity of chemo-sensitive cells *via* delivering lncRNAs. Sheng *et al*. showed that lncRNA CARMN could increase the sensitivity of TNBC cells to cisplatin.[47] Ning *et al*. showed that downregulation of lncRNA TMPO-AS1 was able to sensitize TXT-resistant breast cancer cells to TXT through regulating the miR-1179/TRIM37 axis.[48] Here, we found that downregulation of LINC00667 could elevate TXT sensitivity in TXT-resistant TNBC cells, indicating that LINC00667 plays a key role in retention of a TXT-sensitive status in TNBC cells.

LncRNAs could act as miRNA sponges to regulate gene expression.[49] In this study, we found that LINC00667 could bind to miR-200b-3p in TNBC cells. Moreover, Bcl-2 is a potential binding target of miR-200b-3p in TNBC cells. Meanwhile, we identified miR-200b-3p that showed complementary sequence to both LINC00667 and Bcl-2. Bcl-2, an anti-apoptotic protein, has been found to confer resistance to treatment with anti-cancer agents.[50] Gu *et al.* found that lncRNA NONHSAT141924 was able to strengthen tumor resistance to paclitaxel in breast cancer through upregulation of Bcl-2.[51] Our results showed that, compared to TXT treatment group, TXT-resistant TNBC cell-derived exosomes could upregulate Bcl-2 level in TNBC cells upon TXT treatment, while that effect was reversed by si-LINC00667-1. Additionally, LINC00667 positively modulated the expression of Bcl-2 by sponging miR-200b-3p. These data suggested that TXT-resistant TNBC cell secreted exosomal LINC00667 could reduce the sensitivity of TNBC cells to TXT *via* regulating miR-200b-3p/Bcl-2 axis. Our research still has some limitations. First, LINC00667 has been found to target different miRNAs except for miR-200b-3p, such as miR-34c and miR-130a-3p.[52,53] Thus, other potential targets of LINC00667 are needed to be further investigated in the future. Second, our results found that downregulation of LINC00667 significantly enhanced the pro-apoptotic effect of TXT in TXT-resistant TNBC cells *via* downregulating Bcl-2. Further studies are needed to explore the role and molecular mechanism of LINC00667 on the migration and invasion of TXT-resistant TNBC cells.

Collectively, TXT-resistant TNBC cell-derived exosomal LINC00667 could decrease the chemosensitivity of TNBC cells to TXT *via* targeting miR-200b-3p/Bcl-2 axis. These findings suggested that LINC00667 might be a promising target to enhance sensitivity of TNBC cells to TXT therapy.

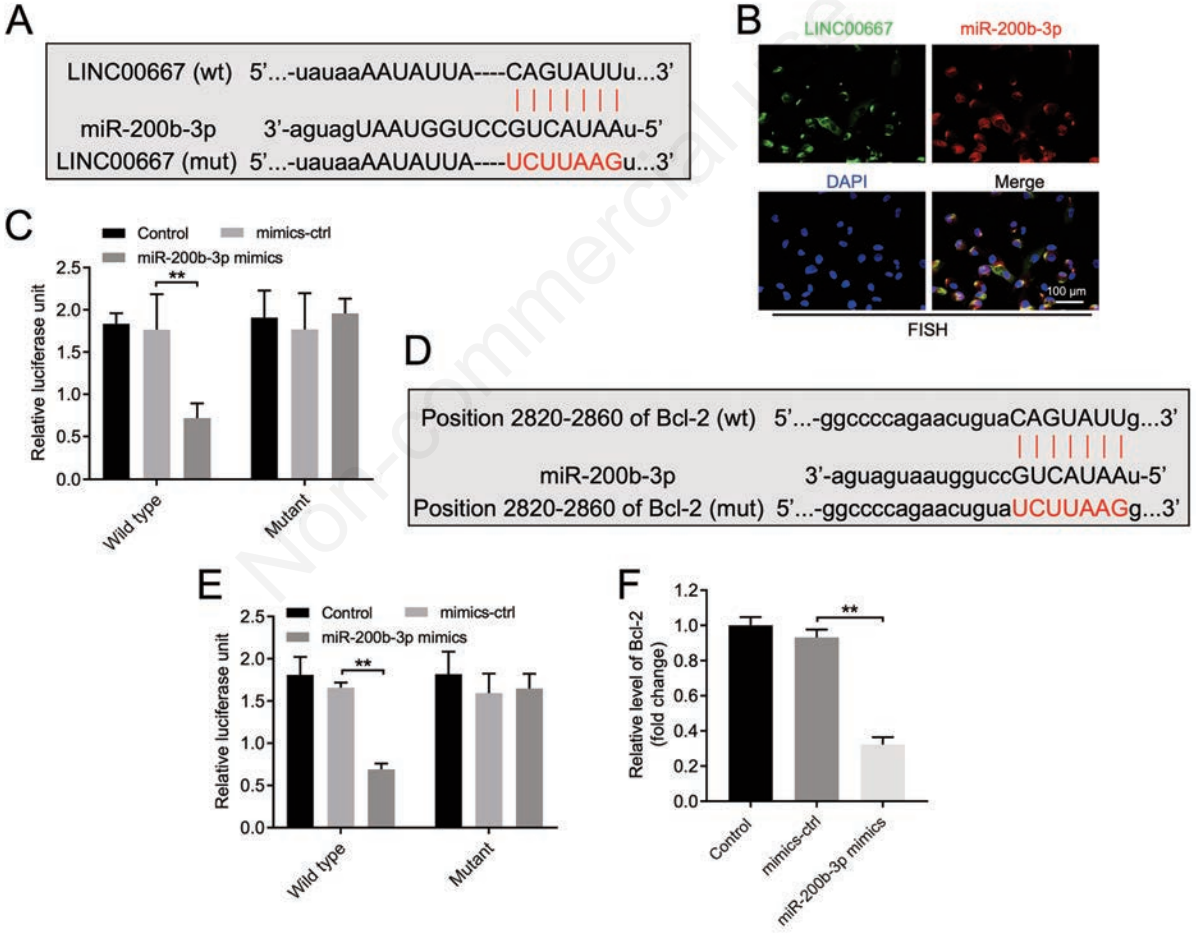

Figure 5. MDA-MB-231/TXT cell secreted exosomal LINC00667 reduced the sensitivity of TNBC cells to TXT *via* miR-200b-3p/Bcl-2 axis. A) The complementary sequences or mutant binding sites between LINC00667 and miR-200b-3p. B) FISH was used to determine the subcellular location of LINC00667 (green color) and miR-200b-3p (red color). C) Dual-luciferase reporter assay was used to determine whether miR-200b-3p is associated with LINC00667. D) The complementary sequences or mutant binding sites between Bcl-2 and miR-200b-3p. E) Dual-luciferase reporter assay was used to determine whether miR-200b-3p is associated with Bcl-2. F) Bcl-2 level in MDA-MB-231 cells transfected with miR-200b-3p mimics was assessed by RT-qPCR. **p<0.01.

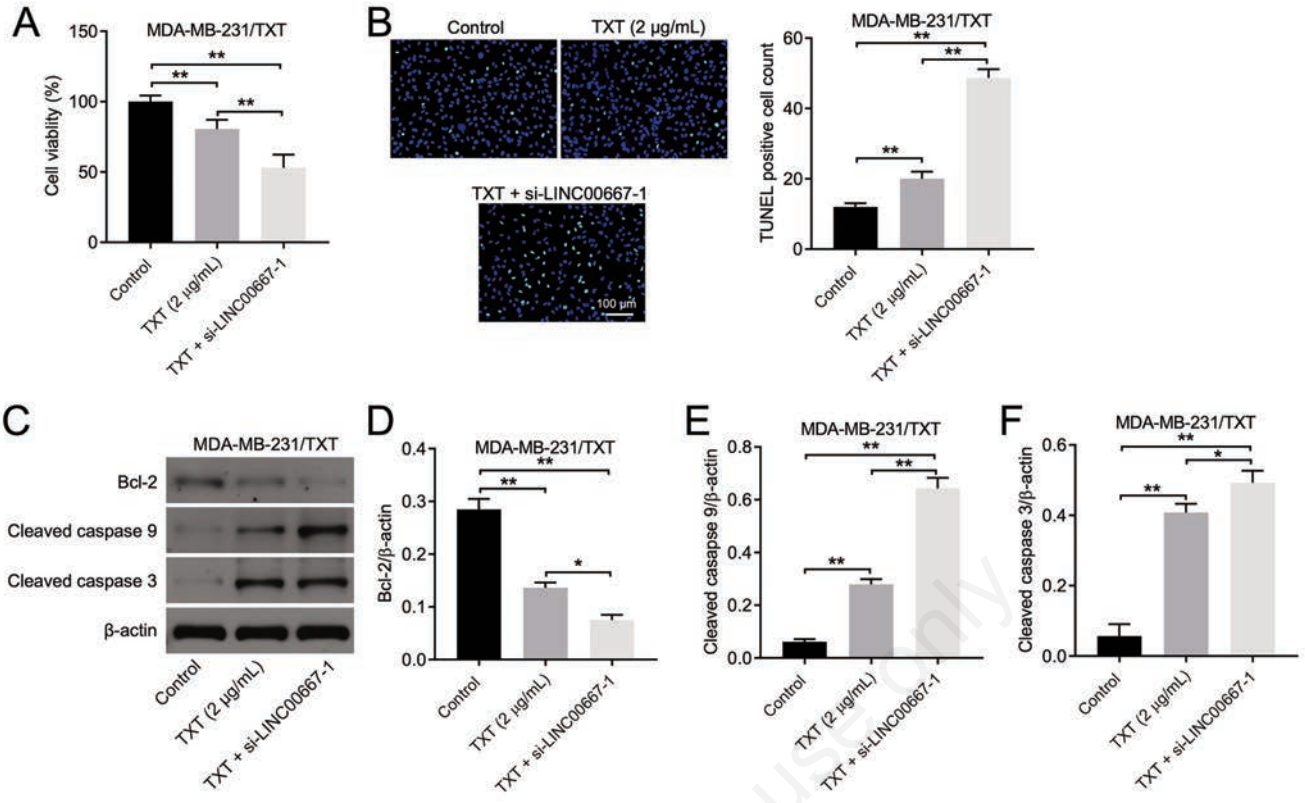

Figure 6. Downregulation of LINC00667 increased the sensitivity of MDA-MB-231 cells to TXT. MDA-MB-231 cells were treated with 2 μg/mL TXT, or TXT plus si-LINC00667-1 for 48 h. A) CCK-8 assay was used to determine cell viability. B) TUNEL assay was used to detect cell apoptosis. C-F) Western blot assay was used to measure the expressions of Bcl-2, cleaved caspase 9, and cleaved caspase 3 in MDA-MB-231 cells; the relative expressions of Bcl-2, cleaved caspase 9, and cleaved caspase 3 were quantified *via* normalization to β-actin. *p<0.05; **p<0.01.

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