NT21MP negatively regulates paclitaxel-resistant cells by targeting miR-155-3p and miR-155-5p via the CXCR4 pathway in breast cancer

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Abstract. Evidence has shown that microRNAs (miRNAs) are vital in cell growth, migration, and invasion by inhibiting their target genes. A previous study demonstrated that miRNA (miR)-155-3p and miR-155-5p exerted opposite effects on cell proliferation, apoptosis, migration and invasion in breast cancer cell lines. An miRNA microarray was used to show that miR-155-3p was downregulated whereas miR-155-5p was upregulated in paclitaxel-resistant (PR) cells compared with parental breast cancer cells. However, the role of miR-155 in breast cancer cell invasion and metastasis remains to be elucidated. A 21-residue peptide derived from the viral macrophage inflammatory protein II (NT21MP), competes with the ligand of CXC chemokine receptor 4 (CXCR4) and its ligand stromal cell-derived factor-1α, inducing cell apoptosis in breast cancer. The present study aimed to identify the underlying mechanism of action of miR-155-3p/5p and NT21MP in PR breast cancer cells. Quantitative polymerase chain reaction, western blotting, wound-healing, cell cycle and apoptosis assays, and Cell Counting kit-8 assay were used to achieve this goal. The combined overexpression of miR-155-3p with NT21MP decreased the migration and invasion ability and increased the number of apoptotic and arrested cells in the G0/G1 phase transition *in vitro*. The knockdown of miR-155-5p combined with NT21MP had a similar effect on PR breast cancer cells. Furthermore, the ectopic expression of their target gene myeloid differentiation primary response gene 88 (*MYD88*) or tumor protein 53-induced nuclear protein 1 (*TP53INP1*) combined with NT21MP enhanced the sensitivity of the breast cancer cells to paclitaxel. Taken together, these findings suggested that miR-155-3p/5p and their target genes *MYD88* and *TP53INP1* may serve as novel biomarkers for NT21MP therapy through the CXCR4 pathway for improving sensitivity to paclitaxel in breast cancer.

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Introduction

Breast cancer is one of the most frequently diagnosed types of cancer and is the leading cause of mortality in Western women (1). However, the specific developmental mechanisms of this cancer remain to be fully elucidated (2). Chemotherapy is a basic strategy for treating breast cancer (3,4), however, this approach has several limitations, including limited drug delivery due to the blood-brain barrier and drug resistance (5,6). Paclitaxel, as one of the most effective chemotherapeutic drugs, is used to treat breast cancer (7,8), however, its failure in treatment and poor prognosis are mainly due to the development of drug resistance (9).

In previous years, small non-coding RNAs, known as microRNAs (miRNAs), have appeared as a pivotal regulators in human tumorigenesis, including breast cancer (10,11). miRNAs bind to the 3'-untranslated regions (3'-UTRs) of the target gene, thus inhibiting expression of the target gene at the transcriptional or posttranscriptional level. Dysregulated miRNAs have significant roles in tumor occurrence (12-14). Additionally, miRNA (miR)-3p and miR-5p, which are processed from the 5' and 3' precursors of pre-miRNA, have been identified to be involved in different regulatory loops and exert the same or different effects on tumors (15,16). For example, miR-409-3p/-5p have an oncogenic effect on prostate cancer bone metastasis and can serve as a therapeutic target (17). Deep sequencing revealed that the opposite strands of miR-144-5p/-3p, miR-145-5p/-3p, and miR-139-5p/-3p can function as dual-strand tumor-suppressor miRNAs (18-20). Sakaguchi *et al* also found that the miR-199 family (miR-199a-3p/5p and miR-199b-3p/5p) may function as tumor suppressors by regulating common

the target gene integrin α3 (21). Despite this, miR-3p and miR-5p can have opposite effects on carcinogenesis. For example, a previous study showed that mature miR-96-5p was significantly upregulated in cirrhosis and dysplastic nodules in hepatocellular carcinoma, whereas the expression of passenger strand miR-96-3p was detectable in cirrhosis and dysplastic nodules (22). Based on the previous studies, the miR-155 family was found to be involved in the regulation of corresponding biological activity in breast cancer. miR-155-3p was found to be downregulated whereas miR-155-5p acted as an oncogenic gene in breast cancer cell lines. However, the mechanisms involving 21-residue N-terminal of viral macrophage inflammatory protein II (vMIP-II), termed NT21MP, and the miR-155 family remains to be fully elucidated.

Previous studies have demonstrated that NT21MP, derived from vMIP-II, efficiently inhibits proliferation, invasion, cell cycle, and apoptosis in breast cancer cells by inhibiting CXC chemokine receptor 4 (CXCR4) and its ligand stromal cell-derived factor-1α (SDF-1α; also known as CXCL12) *in vitro* and *in vivo* (23-25). Although NT21MP has been shown to reverse breast cancer, the underlying specific molecular mechanism requires further investigation.

The present study aimed to determine whether the miR-155 family can be regulated using NT21MP in breast cancer cells and whether the overexpression of miR-155-3p or downregulation of miR-155-5p combined with NT21MP can reverse paclitaxel-resistant (PR) breast cancer cells more than the single treatment group. In addition, by analyzing the respective target genes of miR-155-3p and miR-155-5p, the present study aimed to verify whether NT21MP combined with the downregulation of myeloid differentiation primary response gene 88 (*MYD88*), the target gene of miR-155-3p, or upregulation of tumor protein 53-induced nuclear protein 1 (*TP53INP1*), the target gene of miR-155-5p, can significantly inhibit carcinogenesis *in vitro*. The findings provided novel insight into the potential efficacy of NT21MP as an adjuvant chemotherapy for breast cancer through regulating the miRNA family.

Materials and methods

Cell culture. The MCF-7 human breast cancer cell line was obtained from the Shanghai Cell Institute of Chinese Academy of Science (Shanghai, China). The corresponding PR cells (MCF-7/PR) were treated with 25 μg/ml paclitaxel. All cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; HyClone; GE Healthcare Life Sciences, Logan, UT, USA) maintained at 37˚C in a saturated humidity atmosphere containing 5% CO_2.

Transfection. Cell transfections were performed using Lipofectamine 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. Final concentrations of 50 nM miRNA mimics and 0.75 μg/ml plasmids were added into a 6-well plate with 2 ml of culture medium. MYD88 small interfering (si)RNA (si-MYD88) and pcDNA-TP53INP1 (GenePharma, Shanghai, China) were used for stable transfection. The pcDNA-TP53INP1 was constructed using G418 (200 μg/ml). The sequences for the siRNAs and RNA oligoribonucleotides were as follows: si-MYD88-1, 5'-CCCAUCAGAAGCGACUGAUTTAUCAGUCGCUUCU GAUGG GTT-3'; si-MYD88-2, 5'-GGCAACUGGAACAGAC AAATTUUUGUCUGUUCCAGUUGCCTT-3'; si-MYD88-3, 5'-GCCUGUCUCUGUUCUUGAATTUUCAAGAACAGAG ACAGGCTT-3'.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) analysis. The total RNA was isolated from breast cancer cells using TRIzol reagent according to the manufacturer's protocol (Invitrogen; Thermo Fisher Scientific, Inc.). cDNA was synthesized using the First-Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc.). The qPCR analysis was performed using the SYBR premix Ex TaqII kit (Takara Biotechnology, Co., Ltd., Dalian, China) through an ABI 7500 fast real-time PCR system (Applied Systems, Thermo Fisher Scientific, Inc.) following the manufacturer's protocol. Additionally, glyceraldehyde 3-phosphate dehydrogenase was used as an internal control. The PCR procedure [SYBR Premix (2X), 10 μl; forward and reverse primer (10 μM), 0.8 μl; ROX reference dye II (50X), 0.4 μl; cDNA, 2 μl; sterile purified water, 6 μl; total volume, 20 μl) was performed under the following conditions: 95.0˚C for 30 sec, followed by 40 cycles at 95.0˚C for 15 sec, 57˚C for 30 sec and 72˚C for 34 sec, and a final extension step at 72˚C for 5 min. Data were processed using the $2^{-\Delta\Delta Cq}$ method (26). The corresponding primers are listed in Table I.

Western blot analysis. Cell lysates were prepared in lysis buffer containing 150 mM NaCl, 50 mM Tris, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 0.5% sodium deoxycholate, 0.02% sodium azide, 1 mM sodium vanadate, and protease inhibitors (10 μg/ml leupeptin, 10 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). The protein concentration was measured using a Bio-Rad protein assay. Equal quantities of proteins were subjected to 10% SDS-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The membranes were blocked using phosphate-buffered saline (PBS) with 5% non-fat milk and incubated overnight at 4˚C with primary antibodies. Following incubation with peroxidase-conjugated AffiniPure goat anti-mouse immunoglobulin G (IgG) or peroxidase-conjugated affinipure goat anti-rabbit IgG for 2 h at 37˚C. The immune complexes were detected using an Enhanced Chemiluminescence Western Blotting kit (EMD Millipore, Billerica, MA, USA) and Bio-Rad Image-Lab software 5.2.1 (Bio-Rad Laboratories, Inc.). The relative protein expression was determined using ImageJ V1.8.0 (National Institutes of Health, Bethesda, MD, USA), with GAPDH used as the internal reference. The following antibodies and dilutions were used: *MYD88* (1:2,000; cat. no. ab2068, Abcam, Cambridge, MA, USA), *TP53INP1* (1:2,000; cat. no. ab154877, Abcam), B-cell lymphoma 2 (Bcl-2; 1:1,500; cat. no. ab196495, Abcam), caspase-3 (1:5,000; cat. no. ab13586, Abcam), Bcl-2-associated X protein (Bax; 1:1,000; cat. no. 23931-1-AP, ProteinTech Group, Inc., Chicago, IL, USA), β-actin (1:3,000; cat. no. sc-130065, Santa Cruz Biotechnology Co., Ltd., Dallas, TX, USA), goat anti-rabbit IgG-horseradish peroxidase (1:5,000; cat. no. sc-2004, Santa Cruz Biotechnology, Inc.), and goat anti-mouse

Table I. Sequences of primers.

Name	Forward primer (5'-3')	Reverse primer (5'-3')
MYD88	CTGCCTCCTCCTTTCGTTGTAG	GCTCTGCTGGTCCTTCTTAGTC
TP53INP1	GACTTCATAGATACTTGCAC	ATTGGACATGACTCAAACTG
Bax	GGGGACGAACTGGACAGTAA	CAGTTGAAGTTGCCGTCAGA
Caspase-3	ACAAATGGACCTGTTGACCTGA	ACACCACTGTCTGTCTCAATGC
Bcl-2	ATGTGTGTGGAGAGCGTCAA	ACAGTTCCACAAAGGCATCC
GAPDH	CAGCCTCAAGATCATCAGCA	TGTGGTCATGAGTCCTTCCA

MYD88, myeloid differentiation primary response gene 88; TP53INP1, tumor protein 53-induced nuclear protein 1; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

IgG-horseradish peroxidase (1:5,000; cat. no. sc-2005, Santa Cruz Biotechnology, Inc.).

Wound-healing assay. The transfected breast cancer cells were seeded into 6-well plates and then wounded by scratching with a sterile 10-μl pipette tip. The detached cells were removed by washing twice with PBS, and fresh culture medium without serum was added. The wound closure was monitored at 0 and 24 h using a fluorescence microscope (x40 magnification; IX71; Olympus Corporation, Tokyo, Japan). The wound surface area was quantified by image analysis (Image J V1.8.0; National Institutes of Health).

Cell cycle and apoptosis assays. The cells were seeded into 6-well plates. Following transfection for 24 h, the cells were collected by trypsinization and then analyzed with a flow cytometer (Muse Cell Analyzer, Merck Millipore, Darmstadt, Germany) using an Annexin V and Dead Cell kit and a Cell Cycle Detection kit (Merck Millipore). All experiments were repeated three times.

Cell counting kit-8 (CCK-8) assay. The MCF-7 and MCF-7/PR cells were trypsinized and seeded into 96-well plates at $1x10^5$ cells/ml. After 24 h, various concentrations of paclitaxel (0, 20, 40, 60, 80, and 100 μmol/l) were added, and 50 nmol/l miR-155-3p mimics/miR-155-5p inhibitor/NT21MP was transfected into each well and incubated at 37˚C for 72 h. Subsequently, 10 μl of CCK-8 (Beyotime Institute of Biotechnology, Shanghai, China) was added to 100 μl of DMEM medium containing 10% FBS. The values of absorbance were measured at 450 nm.

Statistical analysis. All analyses were performed in triplicate. Statistical analysis was performed using one-way analysis of variance and the post hoc Least Significant Difference test with SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference. The data are reported as the mean ± standard deviation.

Results

NT21MP reverses the SDF-1α-induced decrease of miR-155-3p and increase of miR-155-5p in MCF-7 and MCF-7/PR cells. Previous studies have confirmed that NT21MP can inhibit SDF-1α-induced proliferation, migration and invasion, and promote apoptosis by downregulating the expression of CXCR4 in breast cancer cells. The results of the RT-qPCR analysis showed that NT21MP (1 μg/ml) inhibited the SDF-1α-induced decrease of miR-155-3p in the parental and PR cells compared with the control group (Fig. 1A). By contrast, SDF-1α (0.1 μg/ml) promoted the expression level of miR-155-5p, whereas NT21MP inhibited its effect (Fig. 1B). These results suggested that miR-155-3p/5p was involved in the regulation of NT21MP, not only in breast cancer parental cells, but also in drug-resistant cells.

NT21MP inhibits the SDF-1α-induced increase of MYD88 and decrease of TP53INP1 in MCF-7 and MCF-7/PR cells. Previous studies have demonstrated that *MYD88* functions as the target gene of miR-155-3p and *TP53INP1* functions as the target gene of miR-155-5p using TargetScan v7.1, miRanda, and miRTarbase (27). The same experiments for miR-155-3p/5p were performed in the present study to further elucidate whether the targets of miR-155-3p/5p were also involved in the regulatory effect of NT21MP in drug resistance in breast cancer. The results showed that SDF-1α promoted the expression level of *MYD88* whereas NT21MP suppressed this effect in the MCF-7 and MCF-7/PR cells (Fig. 2A). Additionally, NT21MP inhibited the SDF-1α-induced decrease of *TP53INP1* (Fig. 2B). The corresponding protein levels are shown in Fig. 2C.

NT21MP, combined with the overexpression of miR-155-3p, inhibits target gene MYD88 and biological activities in MCF-7/PR cells. A wound-healing assay was performed to assess the ability of SDF-1α to promote cell migration and the ability of miR-155-3p or NT21MP to weaken this effect, particularly in the combined groups, in order to examine the molecular effect of NT21MP and the overexpression of miR-155-3p in breast cancer-resistant cells (Fig. 3A). Subsequent cell cycle and apoptotic analyses were performed using flow cytometry to detect the combined effect of NT21MP and miR-155-3p. The percentage of cells in the G0/G1 phase decreased from 70.2 to 68.4%, whereas the number of cells in the S phase increased from 23.6 to 24.6% following SDF-1α stimulation, suggesting that SDF-1α promoted cell transformation from the G0/G1 phase to the S phase. When the cells were treated with NT21MP or miR-155-3p mimics,

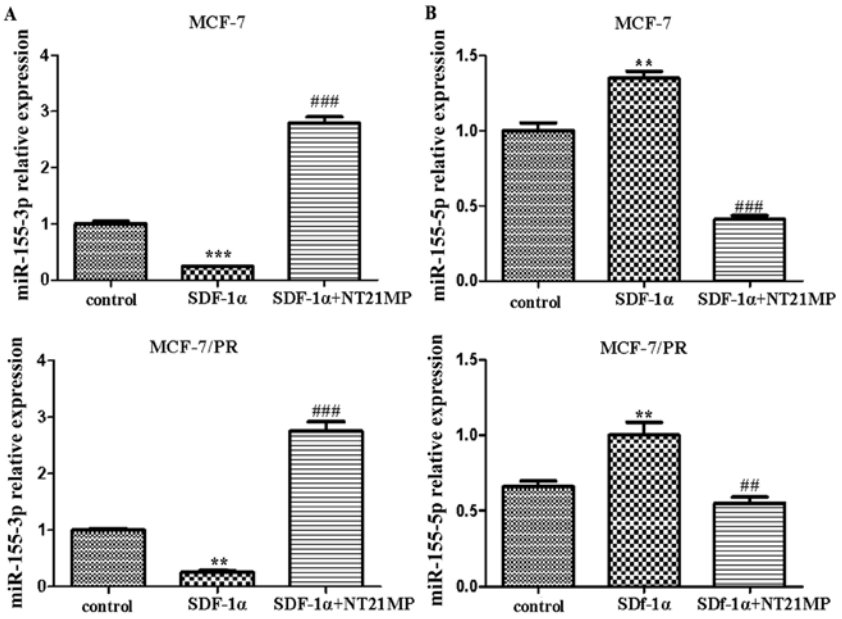

Figure 1. Effects of NT21MP on the expression level of miR-155-3p/5p in MCF-7 and MCF-7/PR cell lines. (A) Effects of NT21MP on the expression level of miR-155-3p in MCF-7 and MCF-7/PR cells, detected using RT-qPCR analysis, compared with control groups. (B) Effects of NT21MP on the expression level of miR-155-5p in MCF-7 and MCF-7/PR cells, detected using RT-qPCR analysis, compared with control groups. **P<0.01, ***P<0.001, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001, compared with SDF-1α treatment. NT21MP; 21-residue peptide derived from viral macrophage inflammatory protein II; miR, microRNA; SDF-1α, stromal cell-derived factor-1α; PR, paclitaxel-resistant; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

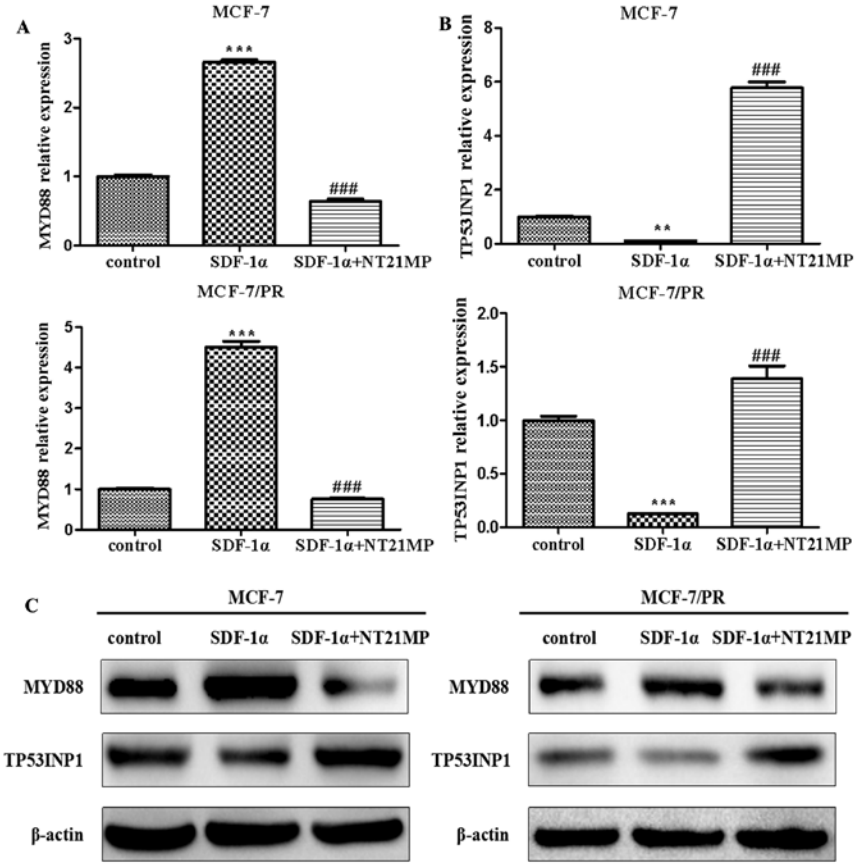

Figure 2. Effects of NT21MP on the expression of *MYD88* or *TP53INP1* in MCF-7 and MCF-7/PR cells. (A) Effects of NT21MP on the expression of *MYD88* using RT-qPCR analysis, compared with the control groups. (B) Effects of NT21MP on the expression of *TP53INP1* using RT-PCR analysis, compared with the control groups. (C) Western blot analysis was performed to identify the effects of NT21MP on the expression of *MYD88* or *TP53INP1* in MCF-7 and MCF-7/PR cells, compared with control groups. The results are representative of three independent experiments. **P<0.01, ***P<0.001 and $^{\#\#\#}$P<0.001, compared with SDF-1α treatment. NT21MP; 21-residue peptide derived from viral macrophage inflammatory protein II; SDF-1α, stromal cell-derived factor-1α; PR, paclitaxel-resistant; MYD88, myeloid differentiation primary response gene 88; TP53INP1, tumor protein 53-induced nuclear protein 1; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

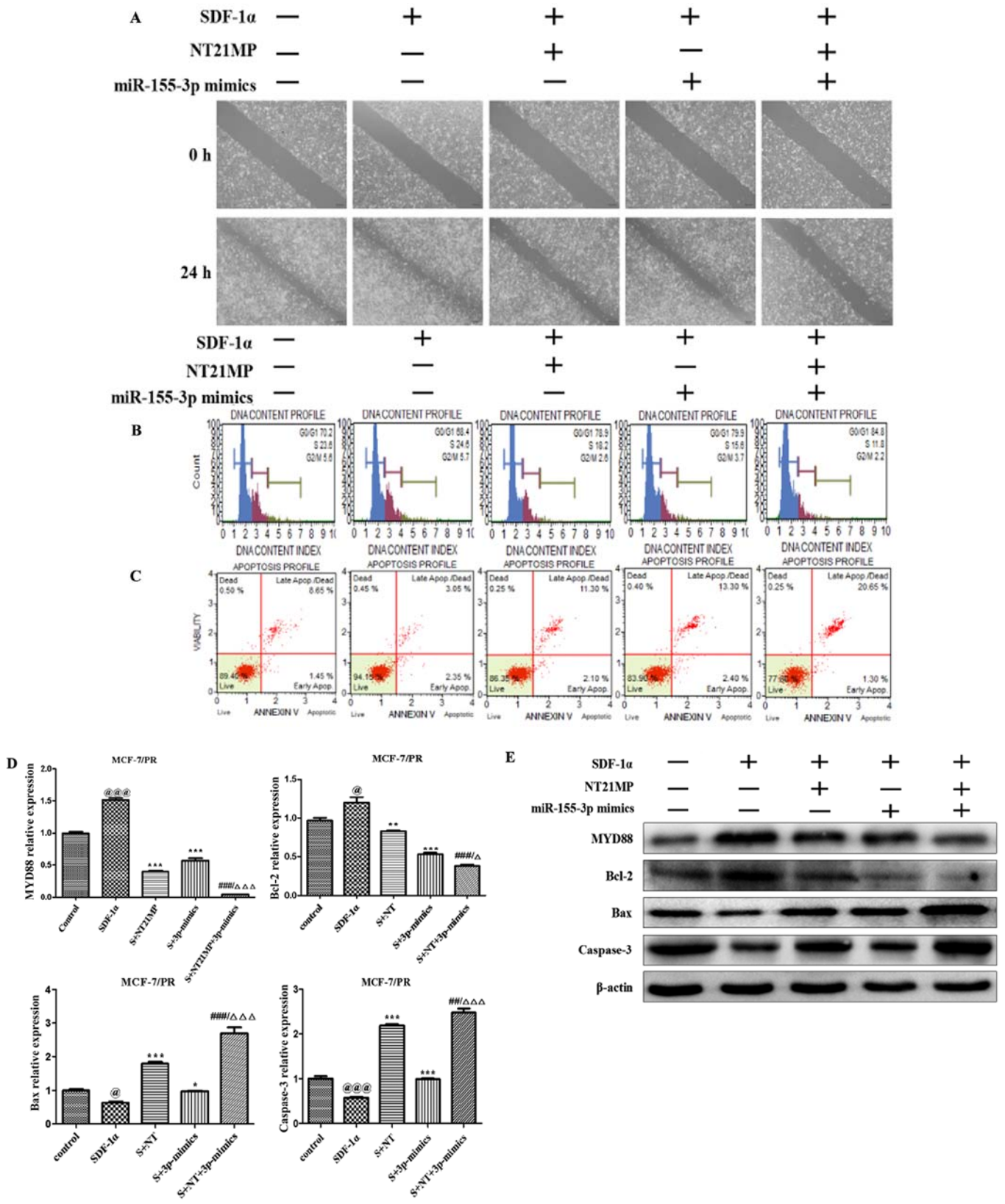

Figure 3. Biological effects of NT21MP and miR-155-3p mimics on PR breast cancer cells. (A) Effects of NT21MP and miR-155-3p mimics on cell migration and invasion were measured using a wound-healing assay. (B) Effects of NT21MP and miR-155-3p mimics on cell cycle were analyzed using PI staining and flow cytometry. (C) Effects of NT21MP and miR-155-3p mimics on cell apoptosis were evaluated using the Annexin V/PI staining and flow cytometry. (D) Reverse transcription-quantitative polymerase chain reaction analysis was used to detect the effects of NT21MP and miR-155-3p mimics on target gene mRNA level, cell cycle, and apoptosis-related factors. (E) Results of western blot analysis of the protein levels of target genes, cell cycle, and apoptosis-related factors in PR cells were consistent with the mRNA results. Data are presented as the mean ± standard deviation of three independent experiments. $^{@}$P<0.05 and $^{@@@}$P<0.001, compared with the control group; *P<0.05, **P<0.01 and ***P<0.001, compared with SDF-1α treatment; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001, compared with S + NT21MP treatment; $^{\Delta}$P<0.05 and $^{\Delta\Delta\Delta}$P<0.001, compared with S + 3p mimics treatment. NT21MP; 21-residue peptide derived from viral macrophage inflammatory protein II; miR, microRNA; S/SDF-1α, stromal cell-derived factor-1α; NT, NT21MP; PR, paclitaxel-resistant; MYD88, myeloid differentiation primary response gene 88; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; PI, propidium iodide.

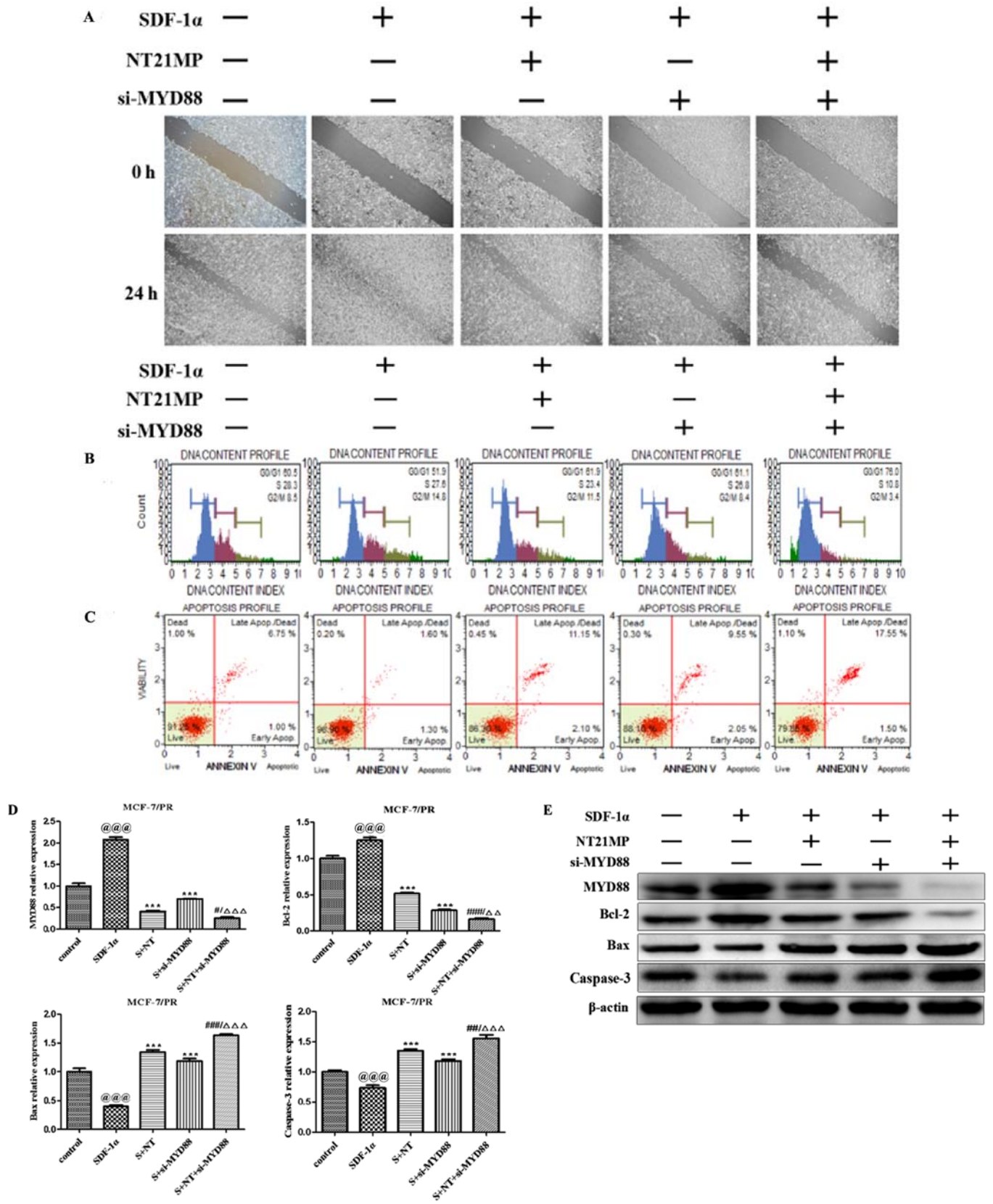

Figure 4. Biological effects of NT21MP and si-MYD88 on PR cells. (A) Effects of NT21MP and si-MYD88 on cell migration and invasion were measured using a wound-healing assay. (B) Effects of NT21MP and si-MYD88 on cell cycle were analyzed using PI staining and flow cytometry. (C) Effects of NT21MP and si-MYD88 on cell apoptosis were evaluated using Annexin V/PI staining and flow cytometry. (D) Reverse transcription-quantitative polymerase chain reaction analysis was used to detect the effect of NT21MP and si-MYD88 on the mRNA levels of target genes, cell cycle, and apoptosis-related factors. (E) Western blot analysis results of the protein level of target gene, cell cycle, and apoptosis-related factors in PR cells were consistent with the mRNA results. The data are presented as the mean ± standard deviation of three independent experiments. $^{@@@}$P<0.001, compared with the control group; ***P<0.001, compared with SDF-1α treatment; $^{\#}$P<0.05, $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001, compared with S + NT21MP treatment; $^{\Delta\Delta}$P<0.01 and $^{\Delta\Delta\Delta}$P<0.001, compared with S + si-MYD88 treatment. NT21MP; 21-residue peptide derived from viral macrophage inflammatory protein II; S/SDF-1α, stromal cell-derived factor-1α; NT, NT21MP; PR, paclitaxel-resistant; MYD88, myeloid differentiation primary response gene 88; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; PI, propidium iodide; si, small interfering RNA.

an increased percentage of cells were arrested in the G0/G1 phase, from 68.4 to 78.9% in the NT21MP group, and from 68.4 to 79.9% in the miR-155-3p mimics group, particularly in the combined group (Fig. 3B). Additionally, the changes in apoptotic cells were in line with the cell cycle results (Fig. 3C). RT-qPCR and western blot analyses were used to analyze the mRNA and protein expression levels of cell cycle-related factors. As shown in Fig. 3D and E, SDF-1α upregulated the expression of target gene *MYD88* and Bcl-2, and downregulated the expression of apoptotic genes Bax and caspase-3. NT21MP inhibited the anti-apoptotic effects of SDF-1α, indicating that NT21MP induced breast cancer-resistant cell apoptosis. Furthermore, *MYD88* was significantly attenuated in the NT21MP and overexpression of miR-155-3p treatment group compared with the univariate treatment group. Bcl-2 was also downregulated under the same treatment. By contrast, Bax and caspase-3 exhibited opposite expression trends. These results indicated that NT21MP and miR-155-3p inhibited the stimulatory effect of SDF-1α on the biological activity in PR cells.

NT21MP, combined with the downregulation of MYD88, inhibited its biological effects in MCF-7/PR cells. The present study further examined the biological effect of *MYD88*, the target gene of miR-155-3p, when presented in NT21MP. The wound-healing assay also showed that the scratch wounds underwent slower closing following the knockdown of *MYD88*, particularly when combined with NT21MP (Fig. 4A). The cell cycle and apoptosis assays showed an increased percentage of cells in the G0/G1 phase but a decreased percentage of cells in the S phase. The number of apoptotic cells increased significantly in the si-MYD88 and NT21MP combined group (Fig. 4B and C). In addition, the results of the RT-q PCR and western blot analyses showed that the expression levels of *MYD88* and Bcl-2 increased in the SDF-1α group but decreased in the NT21MP and/or downregulation of *MYD88* groups. The expression levels of Bax and caspase-3 were decreased in the SDF-1α group but increased in the NT21MP and/or downregulation of MYD88 groups (Fig. 4D and E).

NT21MP, combined with the downregulation of miR-155-5p, induces the expression of its target gene TP53INP1 and biological effects in PR cells. The same functional experiments as in miR-155-3p were performed to examine the association between miR-155-5p and NT21MP in breast cancer. The SDF-1α group underwent faster closing of scratch wounds compared with the negative control group. The NT21MP and miR-155-5p inhibitor groups showed slower closing of scratch wounds compared with the SDF-1α group, particularly in the combination groups (Fig. 5A). The results of cell cycle analysis revealed a decreased number of cells in the G0/G1 phase but an increased number of cells in the S phase when transfected with NT21MP or downregulation of miR-155-5p, compared with the SDF-1α treatment group, and this was more marked in the combination group (Fig. 5B). The ratio of apoptotic cells decreased with SDF-1α treatment but increased with NT21MP or miR-155-5p inhibitor; it also decreased significantly in the combined group (Fig. 5C). The RT-qPCR and western blot analyses verified that the expression level of target gene *TP53INP1* decreased with SDF-1α treatment, but increased significantly with NT21MP or downregulation of miR-155-5p (Fig. 5D and E). The expression level of Bcl-2 increased following SDF-1α treatment but decreased following NT21MP treatment or the downregulation of miR-155-5p, whereas Bax and caspase-3 exhibited the opposite results. These results suggested that NT21MP had a more marked inhibitory effect in reversing PR cells when combined with the expression of miR-155-5p.

NT21MP, combined with the upregulation of TP53INP1, inhibits its biological effects in MCF-7/PR cells. As shown in Fig. 6A, transfection with NT21MP and/or pC-TP53INP1 led to slower closure of wounds. Changes in cell cycle and cell apoptosis showed that the number of cells in the G0/G1 phase increased but the number of cells in the S phase decreased. In addition, the percentage of apoptotic cells increased with NT21MP and/or pC-TP53INP1 treatment (Fig. 6B and C). The RT-qPCR and western blot analyses showed that the expression levels of *TP53INP1*, Bax, and caspase-3 increased with NT21MP treatment and/or the overexpression of *TP53INP1*, whereas the expression level of Bcl-2 showed the opposite effect (Fig. 6D and E). Therefore, these results indicated that miR-155-3p/5p was important in reversing PR, and its target gene had a regulatory effect when combined with NT21MP.

NT21MP, combined with miR-155-3p/5p, promotes PR cell sensitivity in breast cancer. The CCK-8 assay was used to measure the proliferation of cells incubated with paclitaxel (0, 20, 40, 60, 80, and 100 μmol/l) for 72 h to clarify the efficiency of NT21MP in reversing drug resistance. The results showed that NT21MP/miR-155-3p mimics/miR-155-5p inhibitor inhibited the proliferation of PR cells (Fig. 7A and B, $P<0.05$). The efficiency of NT21MP in reversing drug resistance was found to be more marked when combined with the miR-155-3p mimics or miR-155-5p inhibitor.

Discussion

Chemotherapy is currently widely used in breast cancer treatment (28). Despite its impressive therapeutic effect, drug resistance has become a hurdle in several clinical cases (29,30). miRNAs have emerged as pivotal regulators of tumorigenesis, particularly in drug resistance of breast cancer (31-33). However, the molecular mechanism of action of miRNAs in regulating drug resistance remains to be fully elucidated. The present study focused on the mature miR-155 family (miR-155-3p and miR-155-5p), which are expressed abnormally in PR breast cancer cells. A previous study showed that the overexpression of miR-155-3p enhanced cell proliferation and tumorigenesis by inhibiting the expression of F-Box and WD repeat domain containing 7 in hepatocellular carcinoma (34). Yim *et al* showed that the upregulation of miR-155-3p led to an increased number of sub-G1 apoptotic cells and reduced cellular viability, suggesting its tumor-suppressive effects (35). However, investigations on miR-155-3p in terms of the development of breast cancer or drug resistance have not been performed. miR-155-5p has also been shown to be upregulated in triple-negative breast cancer (36) and involved in cell

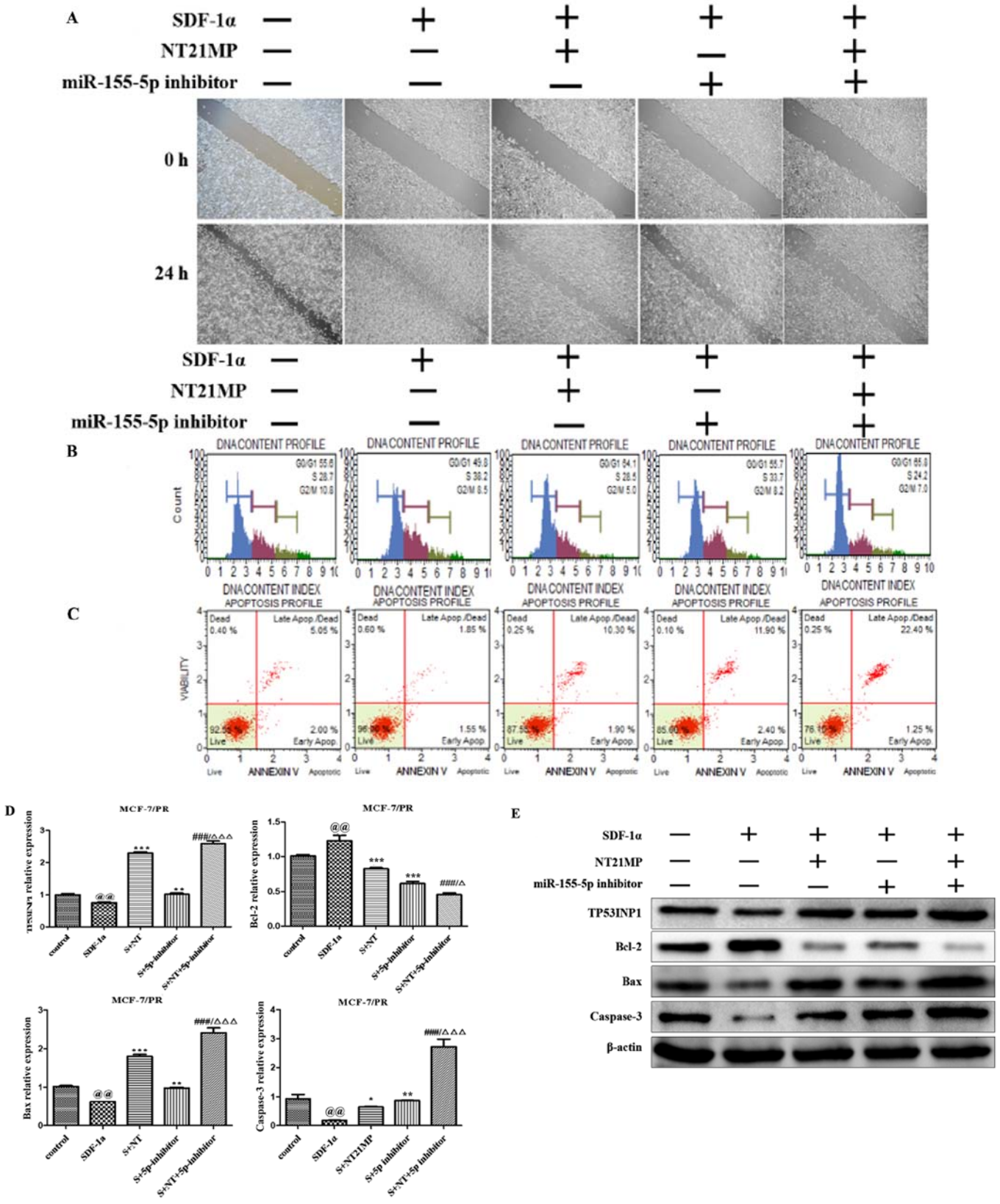

Figure 5. Biological effects of NT21MP and miR-155-5p inhibitor on PR cells. (A) Effects of NT21MP and miR-155-5p inhibitor on cell migration and invasion were measured using the wound-healing assay. (B) Effects of NT21MP and miR-155-5p inhibitor on cell cycle were analyzed using PI staining and flow cytometry. (C) Effects of NT21MP and miR-155-5p inhibitor on cell apoptosis were evaluated using the Annexin V/PI staining and flow cytometry. (D) Reverse transcription-quantitative polymerase chain reaction analysis was used to detect the effect of NT21MP and miR-155-5p inhibitor on the mRNA levels of target gene, cell cycle, and apoptosis-related factors. (E) Western blot analysis results of the protein levels of target genes, cell cycle, and apoptosis-related factors in PR cells were consistent with the mRNA results. Data are presented as the mean ± standard deviation of three independent experiments. $^{@@}$P<0.01, compared with the control group; *P<0.05, **P<0.01 and ***P<0.001, compared with SDF-1α treatment; $^{\#\#\#}$P<0.001, compared with S + NT21MP treatment; $^{\Delta}$P<0.05 and $^{\Delta\Delta\Delta}$P<0.001, compared with S + 5p inhibitor treatment. NT21MP; 21-residue peptide derived from viral macrophage inflammatory protein II; miR, microRNA; S/SDF-1α, stromal cell-derived factor-1α; NT, NT21MP; PR, paclitaxel-resistant; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; TP53INP1, tumor protein 53-induced nuclear protein 1; PI, propidium iodide.

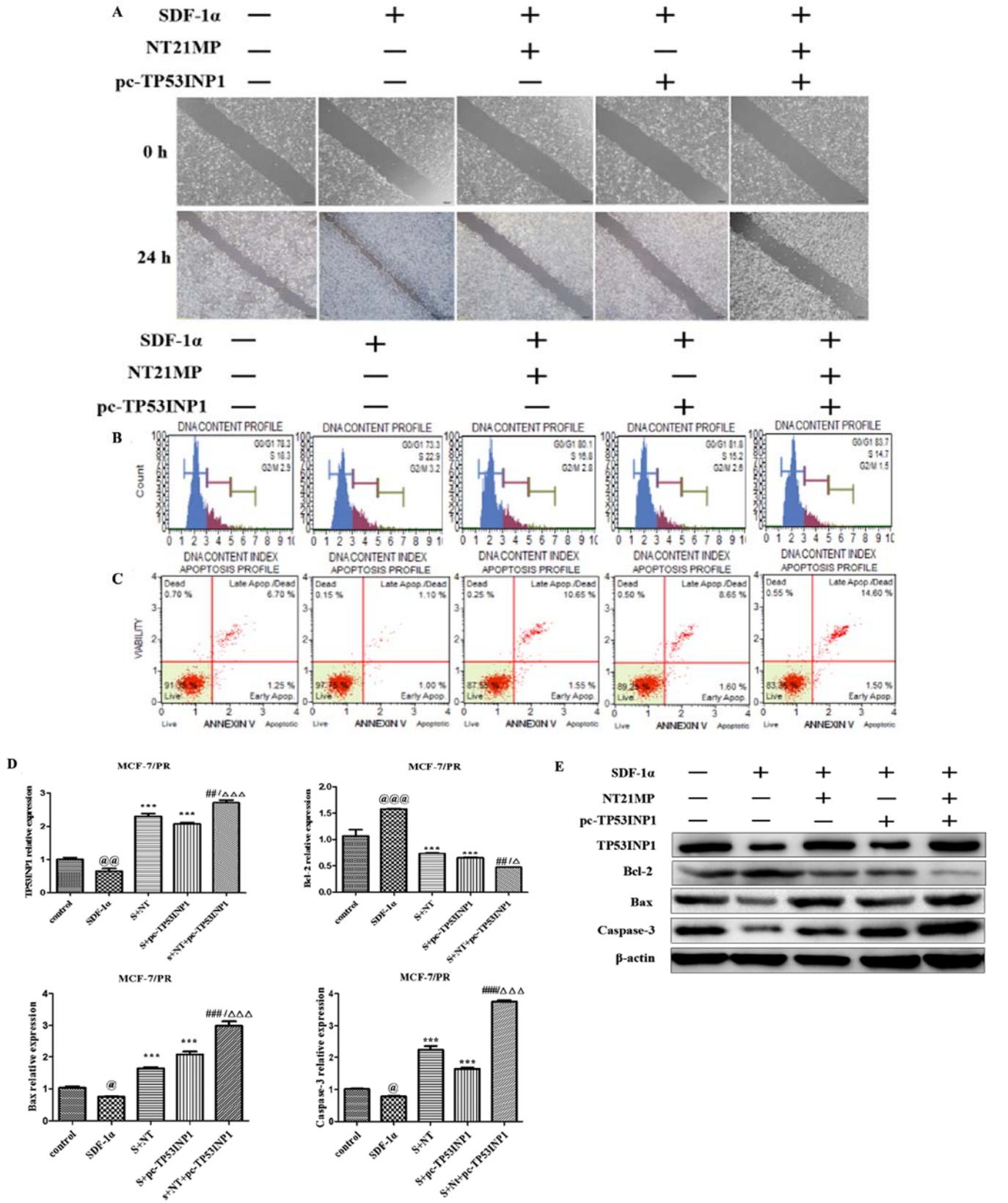

Figure 6. Biological effects of NT21MP and pc-TP53INP1 on PR cells. (A) Effects of NT21MP and pc-TP53INP1 on cell migration and invasion were measured using the wound-healing assay. (B) Effects of NT21MP and pc-TP53INP1 on cell cycle were analyzed using propidium iodide (PI) staining and flow cytometry. (C) Effects of NT21MP and pc-TP53INP1 on cell apoptosis were evaluated using Annexin V/PI staining and flow cytometry. (D) Reverse transcription-quantitative polymerase chain reaction analysis was used to detect the effect of NT21MP and pc-TP53INP1 on the mRNA levels of target genes, cell cycle, and apoptosis-related factors. (E) Western blot analysis results of the protein levels of target genes, cell cycle, and apoptosis-related factors in PR cells were consistent with the mRNA results. Data are presented as the mean ± standard deviation of three independent experiments. $^{@}$P<0.05, $^{@@}$P<0.01 and $^{@@@}$P<0.001, compared with the control group; ***P<0.001, compared with SDF-1α treatment; $^{\#\#}$P<0.01 and $^{\#\#\#}$P<0.001, compared with S + NT21MP treatment; $^{\Delta}$P<0.05 and $^{\Delta\Delta\Delta}$P<0.001, compared with S + pc-TP53INP1 treatment. NT21MP; 21-residue peptide derived from viral macrophage inflammatory protein II; S/SDF-1α, stromal cell-derived factor-1α; NT, NT21MP; PR, paclitaxel-resistant; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; TP53INP1, tumor protein 53-induced nuclear protein 1; PI, propidium iodide.

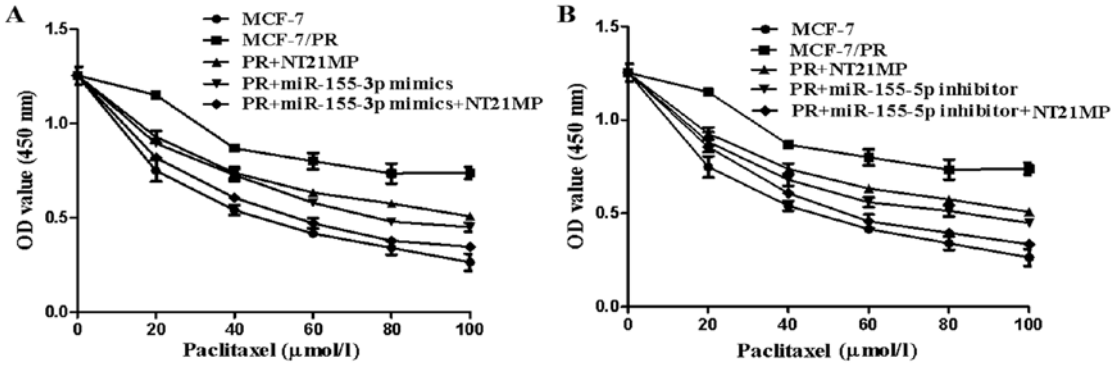

Figure 7. Antiproliferative effects of NT21MP and miR-155-3p/5p on human breast cancer MCF-7 cells. (A) Antiproliferative effects of NT21MP and miR-155-3p mimics on human breast cancer MCF-7 cells. (B) Antiproliferative effects of NT21MP and miR-155-5p inhibitor on human breast cancer MCF-7 cells. The cells were treated with various concentrations of paclitaxel for 72 h, and then analyzed for viability using a Cell Counting kit-8 assay. Absorbance at 450 nm is presented as the mean ± standard deviation. The results are representative of three independent experiments performed in triplicate. NT21MP; 21-residue peptide derived from viral macrophage inflammatory protein II; PR, paclitaxel-resistant; miR, microRNA.

progression and the epithelial-mesenchymal transition process sponged by myocardial infarction-associated transcript (37). The present study demonstrated that not only miR-155-3p, a tumor-suppressor gene, but also miR-155-5p, an oncogenic gene, were involved in the biological activities of PR breast cancer, including cell invasion, cell cycle and cell apoptosis.

Previous studies have shown that these two miRNAs have opposite effects in breast cancer cells and predicted that their target genes were *MYD88* and *TP53INP1*, using bioinformatics software and dual-luciferase assays, respectively. *MYD88* was previously reported to be pivotal in anticancer treatment via activation of the Toll-like receptor-mediated MYD88 signaling pathway in tumor biology, providing a novel potential target for cancer immunotherapy (38-42). Studies have reported that *MYD88* is involved in the regulation of drug resistance in breast cancer cells (43,44). For example, using antibody microarrays, *MYD88* was found to be differentially expressed as a predictive biomarker of neoadjuvant chemotherapy resistance in breast cancer (45). Xiang *et al* suggested that the downregulation of *MYD88* reduced the proliferation, migration and invasion of breast cancer cells, and increased tumor cell sensitivity to paclitaxel treatment through inhibiting the activation of nuclear factor-κB via the phosphoinositide 3-kinase/Akt pathway (46). *TP53INP1*, which is located on the chromosome 8q22, acts as a tumor suppressor involved in breast cancer cell proliferation and apoptotic activity, negatively regulated by miR-155 (47,48). Dysregulation on the 3'-UTR of this gene regulates competitive endogenous messenger RNA-mediated migration and invasion and is correlated with drug resistance (49,50). In line with these findings, the present study suggested that *MYD88*, the target gene of miR-155-3p, and T*P53INP1*, the target gene of miR-155-5p, were also crucial in promoting and suppressing the biological effects in PR breast tumor cells. Interference of *MYD88* or restoration of *TP53INP1* enhanced the effects more markedly compared with the single treatment group, which was mediated by NT21MP.

NT21MP, a synthetic 21-mer peptide antagonist of CXCR4, derived from vMIP-II, was previously identified to inhibit cancer growth and metastasis by competitively binding SDF-1α to CXCR4 (23-25,51). The present study verified that SDF-1α inhibited the expression of miR-155-3p and enhanced the level of miR-155-5p, whereas these effects were reversed with NT21MP in MCF-7 parental and PR cells. This indicated that NT21MP acted as a potential antagonist attenuating the effect of the SDF-1α/CXCR4 axis. The combined use of NT21MP with miR-155-3p mimics, miR-155-5p inhibitor, si-MYD88, and pc-TP53INP1 contributed to decreases of cell proliferation, migration, invasion, cell cycle, and apoptosis, compared with that in MCF-7 PR cells. These changes suggested that NT21MP may reverse breast cancer drug resistance and improve the efficacy of paclitaxel through regulating miR-155-3p/5p or *MYD88/TP53INP1*.

In conclusion, the present study elucidated that, not only miR-155-3p/5p, but also their target genes *MYD88/TP53INP1* are involved in the molecular regulation of PR breast cancer cells. Their combination with NT21MP significantly improved the sensitivity to paclitaxel, thus providing a novel clinical therapeutic strategy for the majority of women with breast cancer. However, the specific mechanism underlying how NT21MP regulates the potential miRNAs or the corresponding targets to reverse drug resistance clinically requires further investigation.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LYZ, CJC and QLY designed the research; YYW, LY, LYZ and YL organized, analyzed and interpreted the data; HNX, YYW, WRW, TTC, YL, HFW, SLC and LY performed the experiments. QLY, WRW, CJC and YYW drafted and revised the manuscript; WRW, CJC and QLY were responsible for supervision. 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.

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