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Original Article

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miR-1205/DNAJB1 reverses docetaxel chemoresistance in human triple negative breast carcinoma cells via regulation of mutp53/TAp63 signaling

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Abstract

Chemoresistance is the major cause of therapeutic failure in human triple negative breast carcinoma (TNBC). Docetaxel (DOC), a first-line therapeutic drug in TNBC treatment, is limited for long-term use due to the development of chemoresistance. Thus, overcoming chemoresistance of DOC remains an important challenge to improve patient's outcome of TNBC. In this study, we aimed to investigate the molecular mechanism behind DOC chemoresistance and the possible therapeutic effects of miRNAs. Utilizing qRT-PCR analysis, we discovered that miR-1205 is gradually downregulated in human triple negative breast carcinoma MDA-MB-231 and docetaxel-resistant MDA-MB-231 (MDA-MB-231/DOC) cells compared with Hs 578Bst normal human breast fibroblasts. Cell viability, cell cycle and apoptosis assays in MDA-MB-231/DOC cells indicated that miR-1205 overexpression enhances docetaxel sensitivity by reducing cell viability as well as inducing G2/M cell cycle arrest and cell apoptosis. Western blot analysis, dual-luciferase reporter assay, co-immunoprecipitation assay and chromatin immunoprecipitation assay revealed that miR-1205 overexpression disrupts the stable complex formation of DNAJB1, mutp53 and TAp63 by directly reducing DNAJB1 expression, which abates the sequestrating effect of mutp53 on TAp63, thereby leading to the enhanced DOC sensitivity in MDA-MB-231/DOC cells. Our findings demonstrate the role of the miR-1205/DNAJB1 axis in the docetaxel resistance of TNBC, which may offer a promising therapeutic approach to resolve docetaxel resistance in TNBC.

Key words triple negative breast carcinoma, docetaxel resistance, miR-1205/DNAJB1, mutp53/TAp63 signaling

Introduction

Triple negative breast cancer (TNBC), the leading cause of breast cancer-related death in females worldwide, accounts for about 15% of breast cancer cases, and is known for high relapse rate and poor overall survival (OS) [1–3]. Chemotherapy is still the first choice for the management of TNBC; however, the development of chemoresistance becomes a very serious problem and often results in the failure of anti-TNBC chemotherapy [4–7]. TNBC chemoresistance is complex, which is induced by the interaction and collaboration of numerous factors and signaling pathways. A complete understanding of this network is a significant challenge and important for the identification of new treatment targets [8]. Docetaxel (DOC) is recommended as the first-line therapeutic drug in the treatment of early or advanced stage TNBC, but the development of chemoresistance obviously reduces DOC efficacy [9]. To overcome DOC chemoresistance, many researchers have investigated its potential underlying mechanisms [10–12]. Docetaxel resistance is either intrinsic or acquired by adopting various mechanisms, including genetic alterations, increased efflux of drugs, and change of tumor microenvironment. Several combination therapies have been proposed to improve the therapeutic potential of docetaxel in cancers, and novel therapeutic strategies that may allow reversal of

docetaxel resistance include alterations of enzymes, improving drug uptake, and enhancement of apoptosis. However, these methods are still under investigation [13]. Thus, uncovering the mechanisms responsible for DOC resistance is critical for overcoming DOC resistance and then improving therapeutic outcomes for patients with chemo-resistant TNBC.

MicroRNAs (miRNAs) are short, non-coding oligonucleotides involved in a variety of physiological and pathological processes [14]. In the development of cancers, miRNAs can function as both oncogenes and tumor suppressors by regulating the expressions of target genes [15, 16]. Accumulating evidence indicates that miRNAs modify the effects of chemo-therapeutic agents, and are associated with chemoresistance in several types of cancers [17]. Dai *et al.* [18] reported that miR-222 promotes doxorubicin resistance in breast cancer by regulating Bim pathway. Han *et al.* [19] reported that miR-181c reduces chemoresistance in breast cancer by down-regulating osteopontin. miR-1205, a member of the PVT1 region, has been identified as a tumor suppressor in several lines of cancers, and high miR-1205 predicts a better prognosis for these cancers [20, 21]. In non-small cell lung cancer, miR-1205 suppresses tumorigenesis by disconnecting the synergy between KRAS and MDM4/E2F1 [22]. In laryngeal squamous cell carcinoma, miR-1205 and the E2F1 loop contribute to cancer progression [23]. However, the role of miR-1205 in the pathological progress of TNBC has never been explored.

In this study, we identified that miR-1205 is down-regulated in tissues from TNBC patients. We also found that miR-1205 expression is significantly down-regulated in the MDA-MB-231/DOC cells compared with that of the parental cells. We further explored the role of miR-1205 in DOC resistance in MDA-MB-231/DOC cells and investigated the therapeutic effect of miR-1205.

Materials and Methods

Chemicals and reagents

Docetaxel (DOC) was purchased from MCE (Beijing, China). miR-1205 mimic was purchased from Ribobio (Guangzhou, China). Chemicals were purchased from Sigma-Aldrich (St Louis, USA). Antibodies were purchased from Santa Cruz Biotechnology (Dallas, USA) and Abcam (Cambridge, USA). The antibodies used in this study were as follows: DNAJB1 (sc-398766, 1:1000 for western blotting, 1:100 for Co-IP; Santa Cruz), p53 (ab32389, 1:1000 for western blotting, 1:200 for Co-IP; Abcam), p63 (1:1000 for western blotting, 1:200 for Co-IP) (ab124762; Abcam), ATM (ab199726, 1:1000; Abcam), Bax (ab32503, 1:1000; Abcam), GAPDH (sc-365062, 1:500; Santa Cruz). Other chemicals and reagents were purchased from Beyotime (Nantong, China) and Sangon (Shanghai, China).

Cell culture and treatment

Human triple negative breast carcinoma MDA-MB-231 cells were obtained from American Type Culture Collection (ATCC, Manassas, USA) and cultured in complete Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100 μg/mL streptomycin. Human mammary gland cell line Hs 578Bst were purchased from the Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM-H Medium (Gibco) with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. HEK293T cells were obtained from American Type Culture Collection (ATCC) and cultured in RPMI-1640 medium (Gibco) with 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin. The docetaxel-resistant MDA-MB-231 cells (MDA-MB-231/DOC) were established as previously reported [24]. Initially, cells were treated with 20 nM DOC for 48 h and then kept in fresh DMEM medium for 72 h. Live cells were treated again with 40 nM DOC for 48 h and then kept in fresh DMEM for 72 h. In this way, the dose of DOC was increased up to 1000 nM. At the end of one year, cells became DOC resistant (MDA-MB-231/DOC) and identified by cell viability assay. The IC_{50} of DOC in MDA-MB-231/DOC cells was about 10 folds higher than in MDA-MB-231 cells. MDA-MB-231/DOC cells were cultured in DMEM containing 10% FBS, 100 U/mL penicillin and 100 μg/mL streptomycin and maintained at 37°C in a humidified atmosphere containing 5% CO_2.

Cell transfection

miR-1205 mimic (5′-UCUGCAGGGUUUGCUUUGAG-3′), miR-NC (5′-UUCUCCGAACGUGUCACGUTT-3′), plasmids (pcDNA3.1-DNAJB1, pcDNA3.1-p53, pcDNA3.1-p63, pcDNA3.1-vector), and siRNAs (p53-siRNA sense: 5′-CACUACAACUACAUGUGUA-3′, and antisense: 5′-UACACAUGUAGUUGUAGUG-3′; scrambled control sense: 5′CCUACGCCACCAAUUUCGU-3′, and antisense: 5′-ACG AAAUUGGUGGCGUAGG-3′) were obtained from GenePharma (Shanghai, China). The transfections of miRNA mimics, plasmids or siRNAs were conducted using Lipofectamine 2000 (Invitrogen, Waltham, USA) according to the manufacturer's instructions. Forty-eight hours post-transfection, cells were harvested for gene expression analysis and further experiments.

qRT-PCR analysis

Total RNA was isolated using Trizol Reagent (TaKaRa, Dalian, China) and reverse-transcribed with M-MLV reverse transcriptase (Promega, Madison, USA). qRT-PCR was performed using the one-step RNA PCR kit (TaKaRa). The expression level of miR-1205 was assessed with gene-specific primers (F: 5′-CACGCATCTGCAGG GTTT-3′, and R: 5′-CCAGTGCAGGGTCCGAGGTA-3′) as described. The PCR profile was as follows: 1 cycle, 95°C for 5 min; 40 cycles, 95°C for 15 s, 55°C for 30 s, and 70°C for 30 s. *U6* (F: 5′-CTCGCTTCGGCAGCACA-3′, and R: 5′-AACGCTTCACGAATTT GCGT-3′) was validated as an internal control, and the $2^{-\Delta\Delta CT}$ equation was used to calculate the relative expression.

Western blot analysis

Total protein was extracted from cells using RIPA lysis buffer (Beyotime) and the concentration of each sample was measured using BCA protein assay kit (Beyotime). The proteins were separated on 12% SDS-PAGE and blotted onto PVDF membranes (Sangon). The membranes were blocked by BSA solution and incubated with primary antibody at 4°C overnight, followed by incubation with HRP-conjugated secondary antibody at 37°C for 2 h. The protein bands were visualized using the ECL assay kit (Beyotime). The density of each band was normalized to the expression of GAPDH.

Cell viability assay

Following treatment, MTT solution (Beyotime) was added into each well of the 96-well pates and incubated for 2 h at 37°C. The culture medium was removed and 100 μL of DMSO was added to dissolve

the formazan crystals. The absorbance of each well was measured at 570 nm using a microplate reader (Bio-Rad, Hercules, USA).

Cell cycle assay

MDA-MB-231 or MDA-MB-231/DOC cells were cultured in serum-free DMEM for 24 h to synchronize into the G0 phase and incubated with indicated treatments such as miR-1205 mimic transfection. After treatment, the cells were collected, washed with PBS, fixed in ice-cold 70% ethanol, and incubated with propidium iodide (PI; 50 μg/mL) in the dark for 30 min. The distribution of the cell cycle was assessed with a FACSaria-I flow cytometer (Becton-Dickinson, Franklin Lakes, USA).

Cell apoptosis assay

The cells were harvested and fixed in 70% ethanol on ice for 30 min, and digested with 100 μg/mL ribonuclease A at 37°C for 20 min. The cells were further stained with Annexin V-FITC and PI on ice for 20 min, and then subject to flow cytometric analysis on the FACSaria-I flow cytometer to assess the percentage of apoptotic cells.

Dual-luciferase reporter assay

TargetScan (http://www.targetscan.org/), miRDB (http://mirdb.org/) and miRanda (http://www.microrna.org) were used to predict the target of miR-1205. For the measurement of miR-1205 function, dual-luciferase report assay was conducted. HEK293T or MDA-MB-231 cells were plated in 6-well plates, and co-transfected with 100 pM of either miR-1205 mimic or control, 40 ng of either pGL3-DNAJB1-3′UTR-WT or pGL3-DNAJB1-3′UTR-MUT, and 4 ng of pRL-TK (Promega) using Lipofectamine 2000. Forty-eight hours after transfection, HEK293T or MDA-MB-231 cells were collected and analyzed using the Firefly/Renilla Dual-luciferase Reporter Assay System (Promega). For the measurement of transcriptional activity of TAp63, dual-luciferase report assay was conducted [25]. The human gene (ATM or Bax) promoter was sub-cloned into pGL3 vector (Promega) in HEK293T cells. Cells were co-transfected with gene promoter vector, pcDNA3.1-TAp63 and internal control plasmid, and luciferase assay was performed 48 hours after transfection using the Firefly/Renilla Dual Luciferase Reporter Assay System (Promega).

Co-immunoprecipitation assay

The cells were grown to 90% confluence in 6-well or 60-mm plates and transfected with plasmids/miRNAs. Subsequent steps of the co-immunoprecipitation (Co-IP) assay were performed as previously described [26]. Cells of different groups were homogenized in IP lysis/wash buffer containing proteasome inhibitor MG-132 (10 mM; Sigma-Aldrich). The supernatants were collected after centrifugation at 14,000 *g* and added to anti-target antibody-cross-linked Protein A/G Plus Agarose (100 μL : 1 mL supernatant sample; Sangon) for incubation at 4°C overnight as previously reported [27]. Following incubation, nonspecific binding was eliminated by repeated washing with IP lysis/wash buffer (Sangon). Eluted IP products were used for western blot analysis using indicated antibodies.

Chromatin immunoprecipitation assay (ChIP)

Chromatin immunoprecipitation (ChIP) assay was performed using Pierce Magnetic ChIP kit (ThermoFisher Scientific, Waltham, USA) according to the previous report [28]. Cells were cross-linked by addition of formaldehyde (1% final concentration) to attached cells, rocked in 50 mM HEPES buffer (pH 7.5) containing 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40, 0.25% Triton X-100, and protease inhibitors (Beyotime), and then lysed in 10 mM Tris buffer (pH 8.0) containing 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, and protease inhibitors (Beyotime). Chromatin was collected, dialyzed against TE buffer and pre-cleared with a mixture of protein A and protein G Sepharose. Pre-cleared chromatin was incubated with 2 μg of anti-p63 antibody overnight at 4°C. Immunoprecipitates were washed and pellets were resuspended in 100 μL of TE and incubated at 55°C for 3 h. Cross-links were reversed by incubating samples at 65°C overnight, and samples were then extracted with phenol:chloroform and precipitated with ethanol. Pellets were re-suspended in 100 μL of H_2O and subject to PCR analysis. Negative control IgG was also used in ChIP reaction.

Statistical analysis

Statistical analysis was conducted using SPSS 20.0. All data were presented as the mean ± SD with three independent experiments, and triplicate repeats were included in each experiment. Differences were determined using Student's *t*-test or one-way ANOVA followed by Tukey's post hoc test. The difference was statistically significant at $P < 0.05$.

Results

Downregulation of miR-1205 correlates with DOC chemoresistance of MDA-MB-231 cells

To explore the role of miR-1205 in DOC chemoresistance of TNBC, *in vitro* experiments were conducted. First, miR-1205 level was evaluated in Hs 578Bst cells, MDA-MB-231 cells and MDA-MB-231/DOC cells, and the results showed that miR-1205 was gradually down-regulated in MDA-MB-231 cells and MDA-MB-231/DOC cells, compared with Hs 578Bst cells (Figure 1A). Then, miR-1205 overexpression by miR-1205 mimic transfection significantly induced cytotoxicity of MDA-MB-231 cells (Figure 1B) and enhanced docetaxel sensitivity of MDA-MB-231/DOC cells (Figure 1C). These data indicated that downregulation of miR-1205 correlated with DOC chemoresistance of MDA-MB-231 cells.

miR-1205 overexpression enhances docetaxel sensitivity via inducing G2/M cell cycle arrest and cell apoptosis

To explore the function of miR-1205 on DOC sensitivity in TNBC cells, the cell cycle and cell apoptosis of MDA-MB-231 and MDA-MB-231/DOC cells with indicated treatments were analyzed. First, MDA-MB-231 cells with miR-1205 overexpression showed G2/M cell cycle arrest (from 6.45% ± 1.45% to 12.53% ± 2.44%) and enhanced cell apoptosis (from 1.32% ± 1.10% to 19.98% ± 1.96%) (Figure 2). Next, MDA-MB-231/DOC cells with miR-1205 overexpression were treated with docetaxel (2 μg/mL). The results showed that docetaxel sensitivity was enhanced by strengthening G2/M cell cycle arrest ($P < 0.01$) and cell apoptosis ($P < 0.01$) in miR-1205-overexpressing MDA-MB-231/DOC cells compared with MDA-MB-231/DOC cells without miR-1205 overexpression (Figure 3). These data indicated that miR-1205 overexpression could reverse DOC chemoresistance of MDA-MB-231/DOC cells.

miR-1205 overexpression enhances docetaxel sensitivity via directly targeting DNAJB1

To investigate the mechanism by which miR-1205 enhances

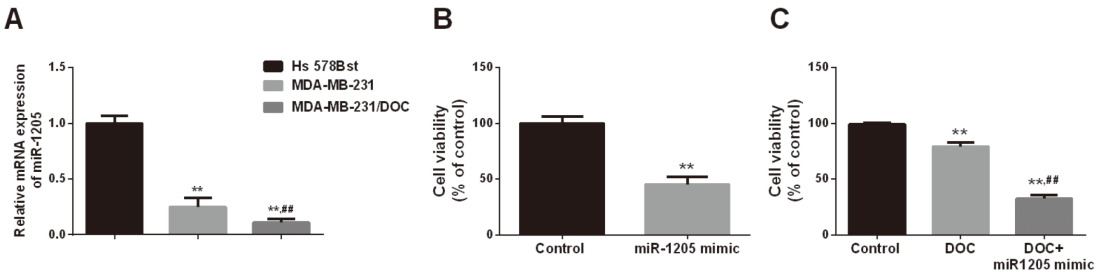

Figure 1. The effect of miR-1205 overexpression on docetaxel sensitivity in TNBC cells (A) The expression levels of miR-1205 were analyzed by qRT-PCR in Hs 578Bst, MDA-MB-231 and MDA-MB-231/DOC cells. $^{**}P<0.01$ vs Hs 578Bst, $^{\#\#}P<0.01$ vs MDA-MB-231. (B) The effect of miR-1205 overexpression on cell viability of MDA-MB-231 cells. Cells were transfected with miR-1205 mimic for 48 h and cell viability was assessed by MTT assay. $^{**}P<0.01$ vs Control. (C) The effect of miR-1205 overexpression on DOC sensitivity of MDA-MB-231/DOC cells. Cells with or without miR-1205 mimic transfection were treated with DOC (2 μg/mL) for 48 h and cell viability was assessed by MTT assay. $^{**}P<0.01$ vs Control; $^{\#\#}P<0.01$ vs DOC. All data were expressed as the mean ± SD of three experiments and each experiment included triplicate repeats.

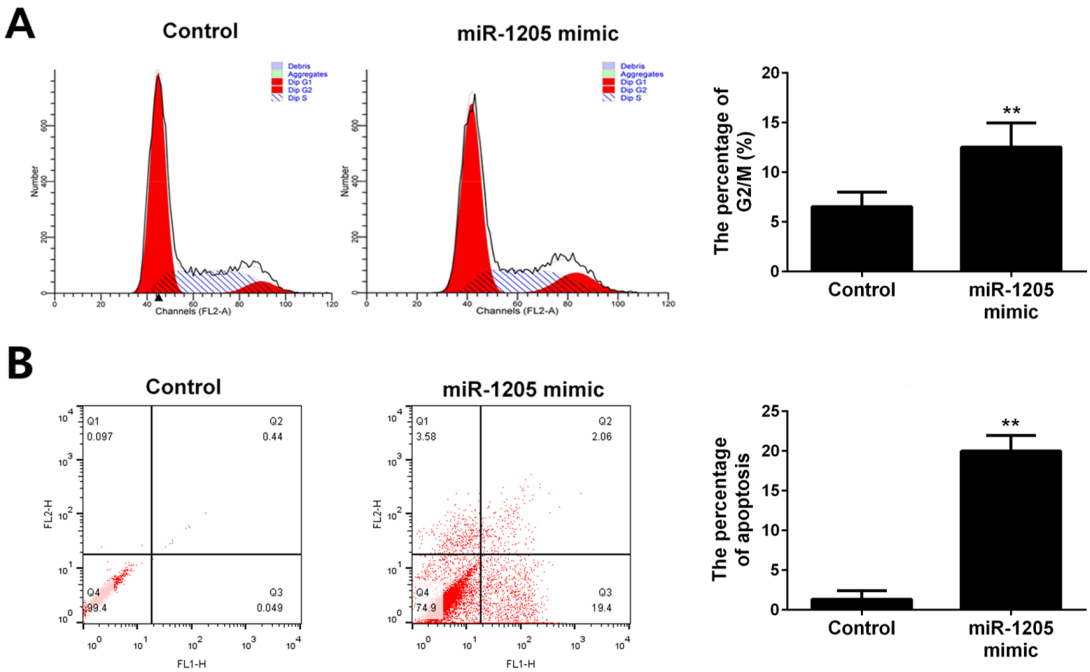

Figure 2. The effect of miR-1205 overexpression on cell cycle and cell apoptosis of MDA-MB-231 cells (A) Cells were transfected with miR-1205 mimic and cell cycle was assessed by PI staining. $^{**}P<0.01$ vs Control. (B) Cells were transfected with miR-1205 mimic and cell apoptosis was assessed by Annexin V-FITC and PI staining. $^{**}P<0.01$ vs Control. All data were expressed as the mean ± SD of three experiments and each experiment included triplicate repeats. Control: MDA-MB-231 cells without treatment.

docetaxel sensitivity, the target of miR-1205 in MDA-MB-231 cells was analyzed. First, using bioinformatics and dual-luciferase reporter assay, *DNAJB1* was predicted to be one of the target genes of miR-1205 in MDA-MB-231 cells (Figure 4A,B). Then, western blot analysis confirmed that DNAJB1 was significantly up-regulated in MDA-MB-231/DOC cells compared with that in MDA-MB-231 cells and Hs 578Bst cells (Figure 4C). Moreover, miR-1205 overexpression significantly inhibited DNAJB1 expression in MDA-MB-231/DOC cells (Figure 4D). Finally, rescue experiments confirmed that restoration of DNAJB1 expression by pcDNA3.1-DNAJB1 plasmid transfection partially reversed the enhanced effect of miR-1205 on docetaxel sensitivity in MDA-MB-231/DOC cells (Figure 5), which mainly affected cell viability, cell cycle and cell apoptosis. These data indicated that miR-1205 exerted its effect by directly down-regulating DNAJB1 in MDA-MB-231/DOC cells.

miR-1205/DNAJB1 affects docetaxel sensitivity by regulating mutp53/TAp63 signaling

The downstream effectors of miR-1205/DNAJB1 in MDA-MB-231 cells were further investigated. First, using co-immunoprecipitation assay, both mutp53 and TAp63 were found to be the targets of DNAJB1 (Figure 6A). Then, western blot analysis confirmed that mutp53 and TAp63 levels were significantly up-regulated in MDA-MB-231/DOC cells compared with those in MDA-MB-231 cells, and the expressions of mutp53 and TAp63 were significantly down-regulated by miR-1205 mimic transfection in MDA-MB-231/DOC cells (Figure 6B). Finally, miR-1205 disrupted the stable complex formation of mutp53 and TAp63 and abated the sequestrating effect of mutp53 on TAp63, leading to enhanced effect of docetaxel on G2/

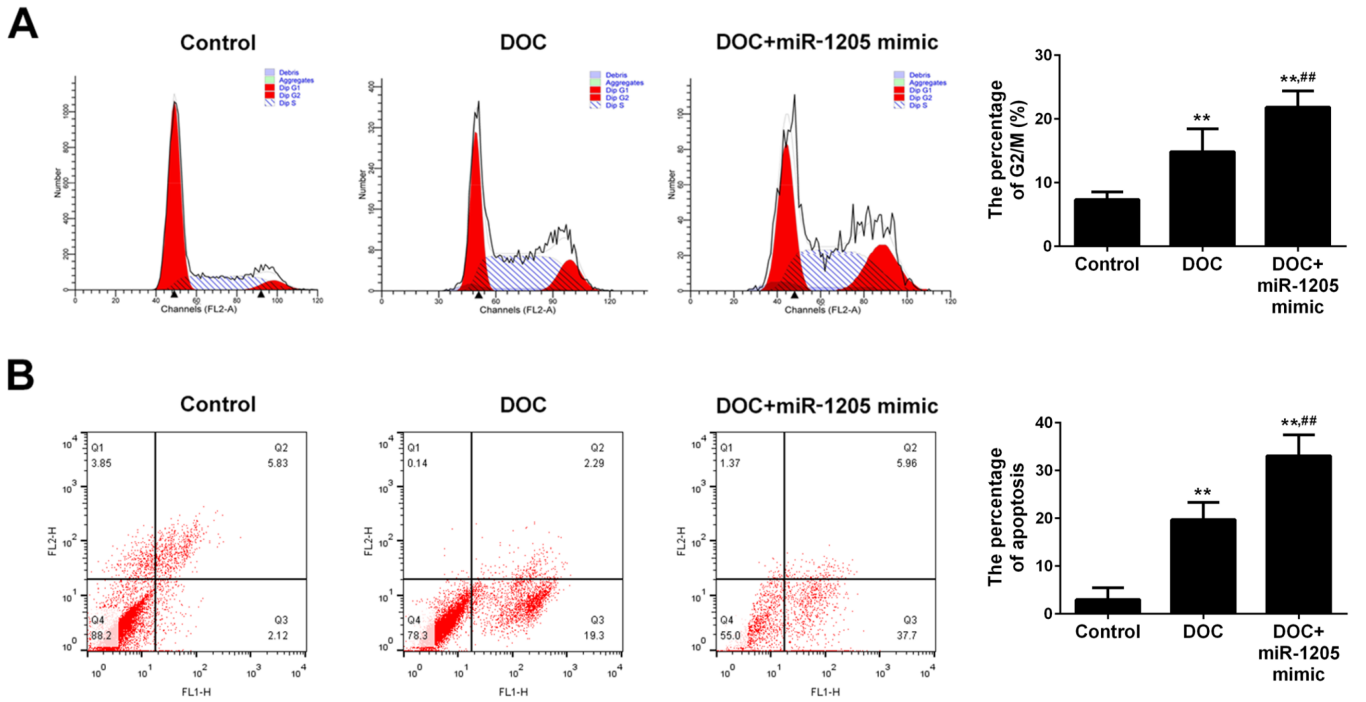

Figure 3. The combined effect of miR-1205 overexpression and docetaxel and on cell cycle and cell apoptosis of MDA-MB-231/DOC cells (A) Cells with or without miR-1205 overexpression were treated with DOC (2 μg/mL) for 24 h and cell cycle was assessed by PI staining. ***P*<0.01 vs Control; $^{\#\#}$*P*<0.01 vs DOC. (B) Cells with or without miR-1205 overexpression were treated with DOC (2 μg/mL) for 24 h and cell apoptosis was assessed by Annexin V-FITC and PI staining. ***P*<0.01 vs Control; $^{\#\#}$*P*<0.01 vs DOC. All data were expressed as the mean ± SD of three experiments and each experiment included triplicate repeats. Control: MDA-MB-231/DOC cells without treatment.

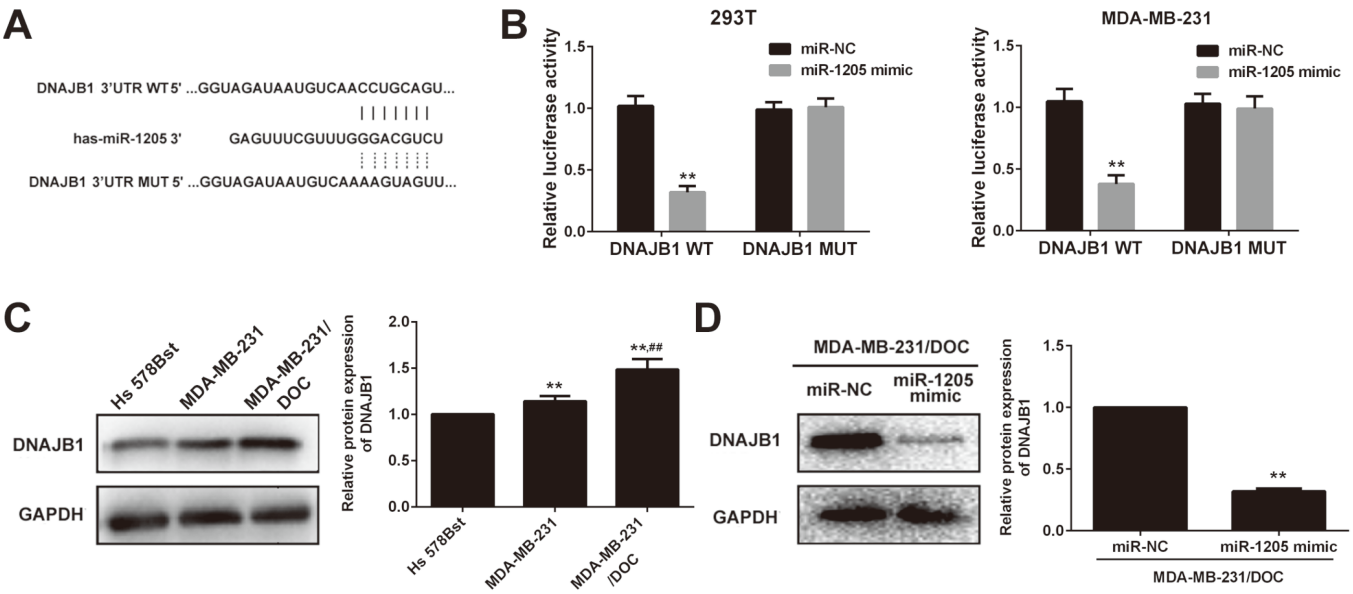

Figure 4. The effect of miR-1205 overexpression on DNAJB1 expression in MDA-MB-231 and MDA-MB-231/DOC cells (A) The predicted binding site for miR-1205 in DNAJB1 3′-UTR. (B) The luciferase reporter vector containing WT DNAJB1 3′-UTR or MUT DNAJB1 3′-UTR and miR-NC or miR-1205 mimic were co-transfected into HEK293 and MDA-MB-231 cells, and luciferase activity was determined and normalized to Renilla luciferase. ***P*<0.01 vs miR-NC. (C) The protein expression of DNAJB1 in Hs 578Bst, MDA-MB-231 and MDA-MB-231/DOC cells. **P*<0.05, ***P*<0.01 vs Hs 578Bst, $^{\#\#}$*P*<0.01 vs MDA-MB-231 cells. (D) The effect of miR-1205 overexpression on DNAJB1 expression in MDA-MB-231/DOC cells. Cells were transfected with miR-NC or miR-1205 mimic and DNAJB1 expression was assessed by western blot analysis. ***P*<0.01 vs miR-NC. All data were expressed as the mean ± SD of three experiments and each experiment included triplicate repeats.

M cell cycle arrest and cell apoptosis in MDA-MB-231/DOC cells (Figure 6C). Meanwhile, the transcription activity of TAp63 on its downstream target p21 was assessed using dual-luciferase reporter assay, and miR-1205 had no direct effect on the transcription ac-

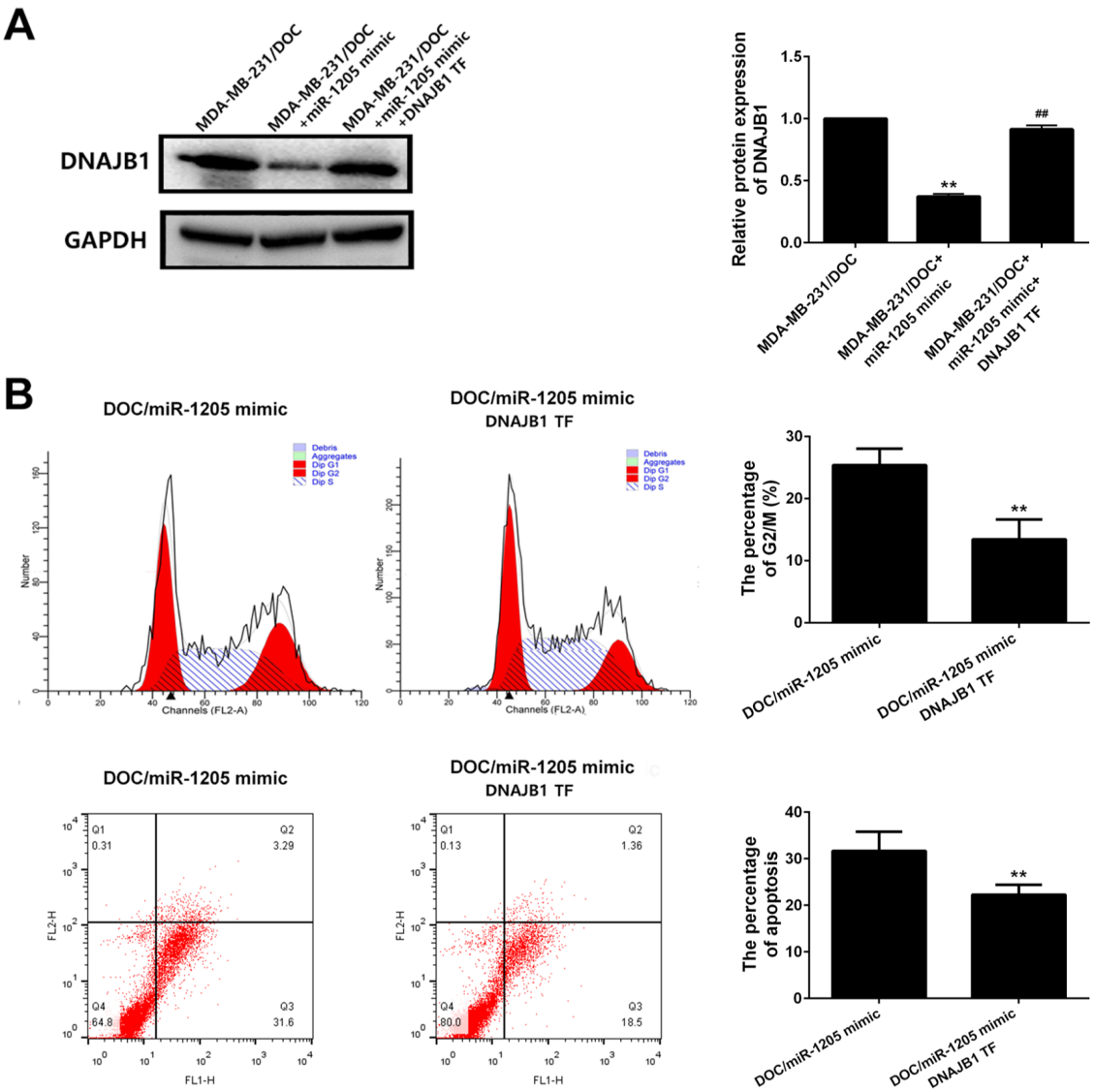

Figure 5. Restoration of DNAJB1 expression reverses miR-1205's enhancing effect on docetaxel sensitivity in MDA-MB-231/DOC cells (A) The protein expression of DNAJB1 in MDA-MB-231/DOC cells. Cells with miR-1205 overexpression were transfected with pcDNA3.1-DNAJB1 plasmid and DNAJB1 expression was assessed by western blot analysis. $^{**}P<0.01$ vs MDA-MB-231/DOC; $^{\#\#}P<0.01$ vs MDA-MB-231/DOC+miR-1205 mimic. (B) The effect of DNAJB1 overexpression on cell cycle and cell apoptosis of MDA-MB-231/DOC cells with DOC/miR-1205 mimic co-treatment. TF: DNAJB1 transfection. MDA-MB-231/DOC cells with DOC/miR-1205 mimic co-treatment were presented as control. $^{**}P<0.01$ vs DOC/miR-1205 mimic. All data were expressed as the mean ± SD of three experiments and each experiment included triplicate repeats.

tivity of TAp63 (Figure 6D). These data indicated that miR-1205/DNAJB1 exerted the effect on docetaxel sensitivity by regulating mutp53/TAp63 signaling.

DNAJB1-dependent mutp53/TAp63 signaling contributes to miR-1205's effect on docetaxel sensitivity via regulating ATM and Bax

The detailed mechanism underlying the effect of miR-1205/DNAJB1 on mutp53/TAp63 signaling was investigated. First, DNAJB1 overexpression resulted in stable complex formation of mutp53 and TAp63 and up-regulated expression of mutp53 but not TAp63 (Figure 7A). Then, p53 knockdown restored miR-1205's enhancing effect on docetaxel sensitivity in MDA-MB-231/DOC cells over-expressing DNAJB1, which might eliminate the sequestrating effect of mutp53 on TAp63 by reducing the binding of mutp53 and TAp63 (Figure 7B,C). Next, dual-luciferase reporter assay and ChIP assay showed that ATM and Bax were the direct transcriptional targets of TAp63, and miR-1205 overexpression enhanced the expressions of both ATM and Bax by indirectly regulating the expression of TAp63 in a DNAJB1-dependent manner. Meanwhile, DNAJB1 over-expression partially reversed the effect of miR-1205 on ATM and Bax expressions (Figure 8). These data indicated that miR-1205/DNAJB1 exerted their effect on docetaxel sensitivity by indirectly regulating ATM and Bax.

Discussion

miRNAs have been established as potential diagnostic and prognostic markers for various cancers [29]. Dysregulation of miRNA profile has been associated with disease development, including metastasis and chemoresistance. Several groups of researchers

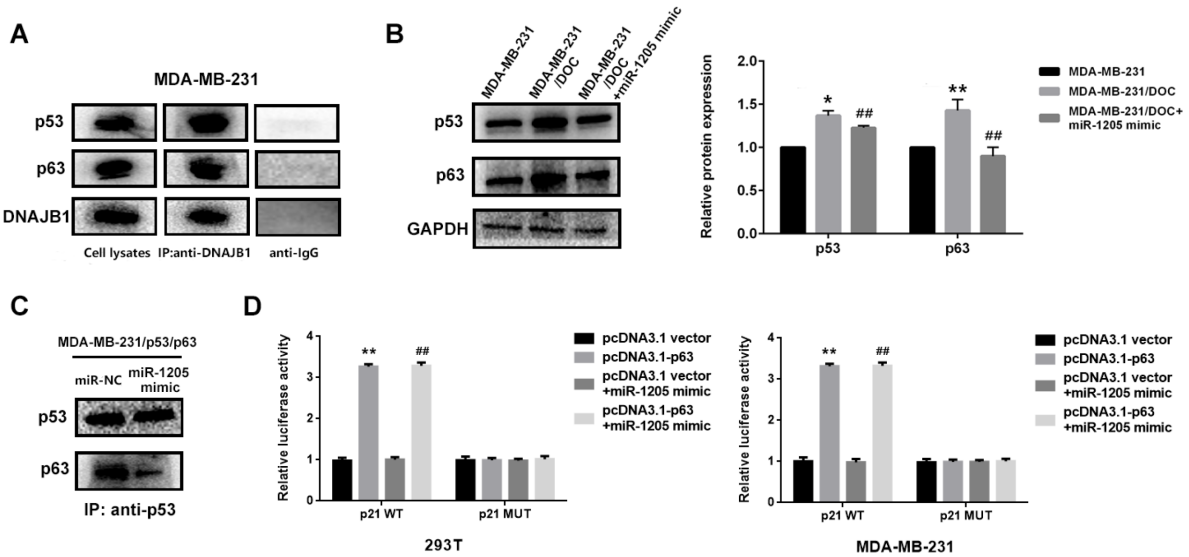

Figure 6. The effect of miR-1205 overexpression on expression and interaction of DNAJB1, mutp53 and TAp63 in MDA-MB-231 and MDA-MB-231/DOC cells (A) The expression and interaction of three proteins in MDA-MB-231 cells. First, the expressions of p53, p63 and DNAJB1 in cell lysates were assessed by western blot analysis. Then, the interaction of p53, p63 and DNAJB1 in MDA-MB-231 cells was assessed by Co-IP. **P* <0.05, ***P*<0.01 vs MDA-MB-231; $^{\#\#}$*P*<0.01 vs MDA-MB-231/DOC. (B) The effect of miR-1205 overexpression on expressions of mutp53 and TAp63 in MDA-MB-231/DOC cells. Cells were transfected with or without miR-1205 mimic and the protein expressions of p53 and p63 were assessed by western blot analysis. (C) The effect of miR-1205 overexpression on interaction of mutp53 and TAp63 in MDA-MB-231 cells with p53 and p63 overexpression. Cells with p53 and p63 overexpression were transfected with miR-NC or miR-1205 mimic and the interaction of p53 and p63 was assessed by Co-IP. (D) The effect of miR-1205 on the transcription activity of p63. The luciferase reporter vector containing WT p21 3′-UTR or MUT p21 3′-UTR, miR-NC or miR-1205 mimic and pcDNA3.1-vector or pcDNA3.1-p63 were co-transfected into HEK293 and MDA-MB-231 cells, and luciferase activity was determined and normalized to Renilla luciferase. ***P*<0.01 vs pcDNA3.1-vector; $^{\#\#}$*P*<0.01 vs pcDNA3.1-vector+miR-1205 mimic. All data were expressed as the mean ± SD of three experiments and each experiment included triplicate repeats.

have revealed that docetaxel and paclitaxel sensitivity could be altered by miRNAs in cancer cells and found that the development of chemoresistance is attributed to alterations at the level of miRNAs [30]. Other researchers have reported that aggressive behavior of cancer cells resulted from EMT-mediated metastasis can lead to docetaxel and paclitaxel resistance, and upstream mediators of EMT such as ZEB1/2, TGF-β and microRNAs are involved in regulating the response of cancer cells to docetaxel and paclitaxel [31]. However, the role of miR-1205 in chemotherapy-resistant TNBC has never been reported. The data from Wang *et al.* [21] revealed that copy numbers and expression of miR-1205 are increased in both castration-resistant prostate cancer cell lines and in primary tumors, indicating that miR-1205 plays an important role in cancer drug resistance. In our study, we focused on the correlation between miR-1205 and DOC resistance using an *in vitro* model of TNBC. By using the docetaxel-resistant MDA-MB-231 cells, we found that miR-1205 was down-regulated in MDA-MB-231/DOC cells compared with that in MDA-MB-231 cells. Additionally, functional experiments showed that miR-1205 overexpression attenuated the DOC resistance of MDA-MB-231/DOC cells by reducing cell viability as well as inducing G2/M cell cycle arrest and cell apoptosis, suggesting that miR-1205 might function as a tumor inhibitor in TNBC.

Molecular chaperones and co-chaperones are well known to be the cornerstones of dynamic multi-protein complexes that affect protein maturation and protein degradation [32]. Increasing evidence confirms that most chaperones and co-chaperones appear to be highly significant in cancers [33]. Elevated levels of HSP expression in cancers usually portend a poor prognosis and increased resistance to therapies. Elevated expression of HSPs in transformed cells contributes to the suppression of apoptosis, which is an important characteristic role of HSPs in aiding tumor progression and resistance to treatment [34]. HSP40 belongs to the DNAJ family of large, under-studied proteins that contain a J-domain, through which they interact and help HSP70 chaperonage, and hence earn the status of HSP70 co-chaperones [35]. These proteins are further subcategorized into three subclasses: DNAJA, DNAJB, and DNAJC. Many of the HSP40 family members are overexpressed in many human cancer types, including gastric, colorectal, cervical and lung cancers [36–38]. DNAJB1 is a member of DNAJ family that regulates the ATP hydrolysis activity of HSP70; however, it has recently been found to function as an independent chaperone, particularly in various types of cancers [39]. Park *et al.* [39] reported that DNJAB1 knockdown enhances the sensitivity of lung cancer cells to gefitinib. Moses *et al.* [40] reported that targeting the HSP40/HSP70 chaperone axis is a novel strategy to treat castration-resistant prostate cancer. In this study, we found that DNAJB1 is one of the target genes of miR-1205 in MDA-MB-231 cells, and rescue experiments confirmed that restoration of DNAJB1 expression partially reversed miR-1205's enhancing effect on docetaxel sensitivity in MDA-MB-231/DOC cells. These data revealed that miR-1205 exerts its reversal effect on docetaxel resistance of TNBC cells via direct regulation of DNAJB1 expression. Previous study has reported that DNAJB1/HSP40, HSPA1A/HSP70 and HSP90A/HSP90 can form a multi-protein complex, promote the binding of mutp53/TAp73α, and then result in increased cell viability in the presence of chemotherapeutic agents. Multiprotein complex of molecular chaperones and co-chaperones can abate the sequestrating effect of mutp53 on TAp73, leading to the enhanced effect of chemo-agents on cell cycle arrest and cell apoptosis. In this study, we found that suppression of

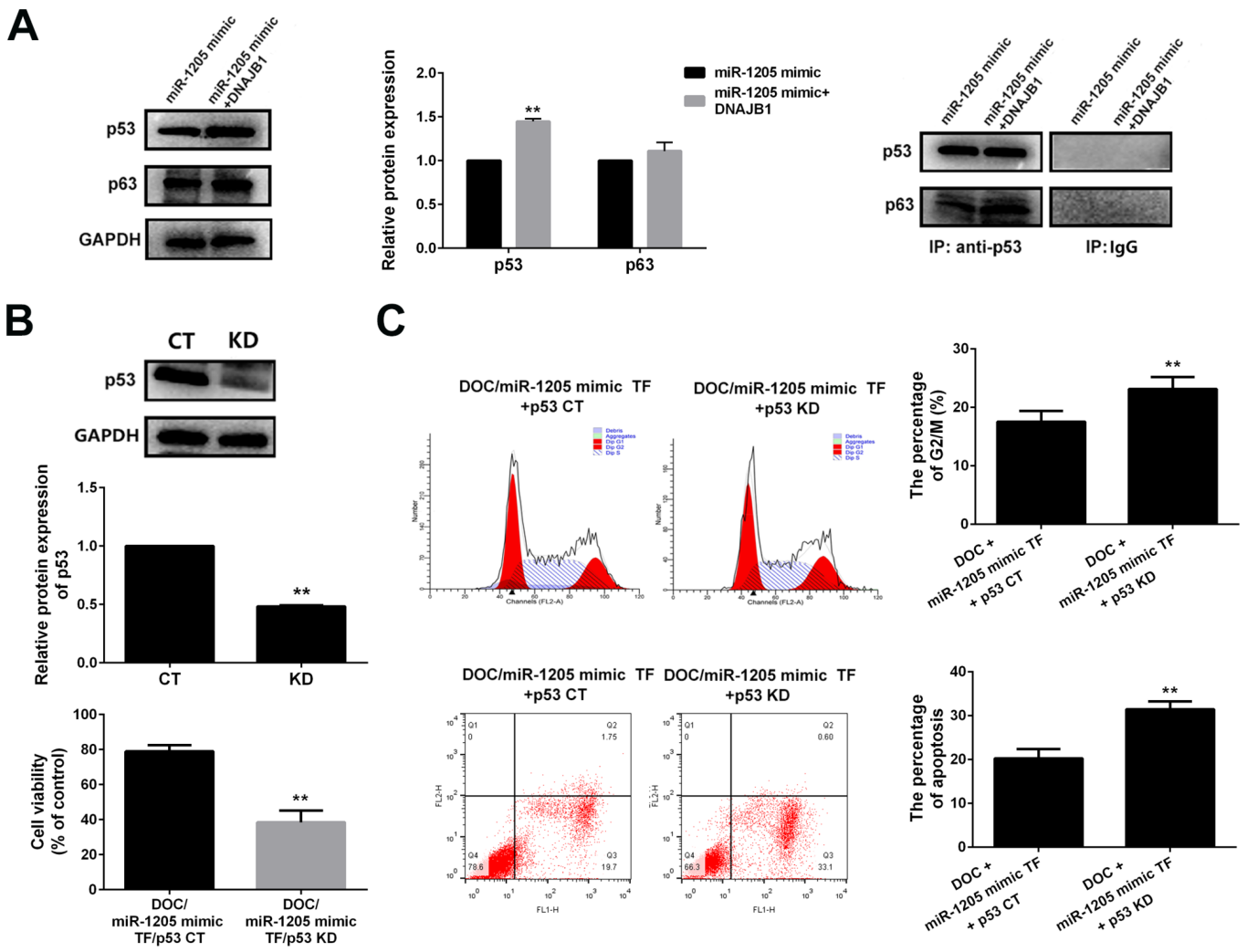

Figure 7. Restoration of mutp53/TAp63 signaling reverses miR-1205-DNAJB1/'s effect in MDA-MB-231/DOC cells (A) The expressions and interaction of mutp53 and TAp63 were analyzed. Cells with miR-1205 overexpression were transfected with or without pcDNA3.1-DNAJB1 plasmid, then protein expressions of p53 and p63 were assessed by western blot analysis and the interaction of p53 and p63 was assessed by Co-IP. ***P*<0.01 vs miR-1205 mimic. (B) The effect of p53 knockdown on miR-1205-DNAJB1's effect in MDA-MB-231/DOC cells. First, p53 was knocked down by siRNA transfection and p53 expression was assessed by western blot analysis. ***P*<0.01 vs CT. Then, cells with p53 knockdown and DNAJB1 overexpression were co-treated with DOC/miR-1205 mimic, and cell viability was assessed by MTT assay. ***P*<0.01 vs DOC/miR-1205 mimic TF/p53 CT. (C) Cells with p53 knockdown and DNAJB1 overexpression were co-treated with DOC/miR-1205 mimic, cell cycle was assessed by PI staining and cell apoptosis was assessed by Annexin V-FITC and PI staining. TF: DNAJB1 transfection. MDA-MB-231/DOC cells with DNAJB1 overexpression and DOC/miR-1205 mimic co-treatment were presented as control. ***P*<0.01 vs DOC/miR-1205 mimic TF/p53 CT. All data were expressed as the mean ± SD of three experiments and each experiment included triplicate repeats.

DNAJB1 by miR-1205 disrupted the stable complex formation of mutp53 and TAp63, but not TAp73, in MDA-MB-231/DOC cells, which enhanced docetaxel sensitivity in MDA-MB-231/DOC cells. However, miR-1205 had no direct effect on the transcription activity of p53 and p63, which exerted their effects by down-regulating DNAJB1 expression. In addition, p53 knockdown restored miR-1205's effect in DNAJB1-overexpressing MDA-MB-231/DOC cells, which might eliminate the sequestrating effect of mutp53 on TAp63. Further dual-luciferase reporter assay and ChIP assay showed that cell cycle-related ATM kinase and cell apoptosis-related Bax protein are the direct transcriptional targets of TAp63, and miR-1205 exerts its effect by enhancing the expressions of ATM and Bax in a DNAJB1-dependent manner (Figure 9). This study revealed that miR-1205 could reverse docetaxel resistance in MDA-MB-231/DOC cells and its target might be DNAJB1; however, there might be additional miR-1205 targets which warrant further investigation.

In conclusion, we found that stable interaction of DNAJB1 with mutp53 and TAp63 occurred in MDA-MB-231/DOC cells, resulting in docetaxel resistance. However, miR-1205 overexpression disrupted this stable complex formation by directly reducing DNAJB1 expression, and led to G2/M cell cycle arrest and cell apoptosis. Collectively, our data reveal a new molecular mechanism involved in docetaxel resistance in TNBC and provide a promising therapeutic approach to resolve the drug resistance of TNBC.

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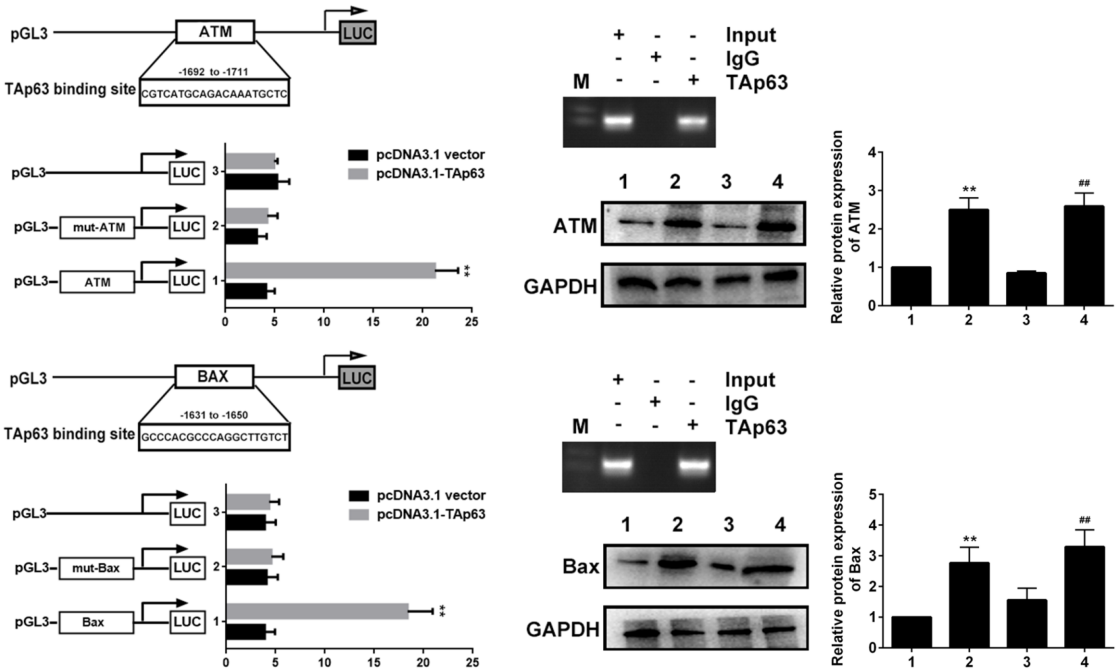

Figure 8. The effect of miR-1205-DNAJB1/mutp53/TAp63 on ATM and Bax in MDA-MB-231/DOC cells First, the luciferase reporter vector containing WT ATM or BAX 3′-UTR or MUT ATM or BAX 3′-UTR and pcDNA3.1-vector or pcDNA3.1-p63 were co-transfected into HEK293 cells, and luciferase activity was determined and normalized to Renilla luciferase. $^{**}P<0.01$ vs pcDNA3.1-vector. Then, PCR gel showed that p63 was enriched in the binding site of ATM or BAX promoter by ChIP assay. Finally, the effect of miR-1205 overexpression on the protein expression of ATM or BAX was assessed in MDA-MB-231/DOC cells. 1, MDA-MB-231/DOC cells; 2, miR-1205 mimic on MDA-MB-231/DOC cells; 3, miR-1205 mimic on MDA-MB-231/DOC cells with DNAJB1 overexpression; and 4, miR-1205 mimic on MDA-MB-231/DOC cells with DNAJB1 overexpression and p53 knockdown. $^{**}P<0.01$ vs MDA-MB-231/DOC cells; $^{\#\#}P<0.01$ vs MDA-MB-231/DOC cells with DNAJB1 overexpression. All data were expressed as the mean ± SD of three experiments and each experiment included triplicate repeats.

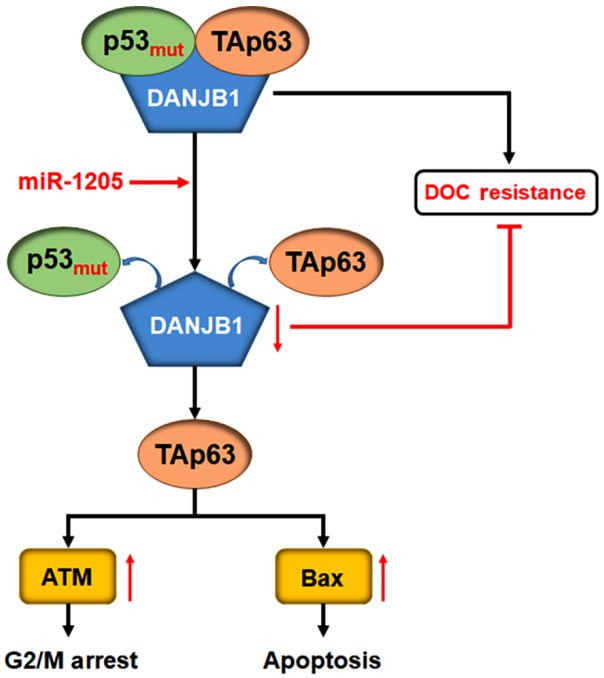

Figure 9. Diagram of the proposed mechanism of miR-1205 on docetaxel response in MDA-MB-231/DOC cells miR-1205 reverses docetaxel resistance in MDA-MB-231/DOC cells by disrupting the stable complex formation of DNAJB1, mutp53 and TAp63.

Health Bureau (No. MS201947).

Conflict of Interest

The authors declared that they have no conflict of interest.

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