

# miRNA-885-3p inhibits docetaxel chemoresistance in lung adenocarcinoma by downregulating Aurora A

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**Abstract.** Aurora A is a member of the mitotic serine/threonine kinase family. It is involved in key processes during mitosis and meiosis, and Aurora A upregulation is implicated in malignant transformation. In the present study, we revealed that Aurora A expression was significantly higher in docetaxel-resistant lung adenocarcinoma (LAD) cells than in parental cells. Higher levels of Aurora A expression were significantly correlated with higher chemoresistance and proliferation in LAD cells, while silencing Aurora A promoted caspase-3-dependent cell apoptosis by downregulating NF- $\kappa$ B and Bcl-2 and upregulating Bax expression. In addition, an increased proportion of cells in the G2/M phase and a decreased proportion of cells in the S phase were observed due to the suppression of Aurora A. Furthermore, we identified that microRNA-885-3p (miR-885-3p) could target Aurora A directly. There was significantly lower miR-885-3p expression in docetaxel-resistant LAD cells than in parental LAD cells. miR-885-3p could modulate the docetaxel response, cell proliferation and apoptosis in LAD cells *in vitro*. Moreover, we found that Aurora A overexpression or miR-885-3p inhibition was associated with more aggressive behaviour in LAD cells. Thus, miR-885-3p/Aurora A may be involved in the chemoresistance of LAD cells, and assessing miR-885-3p/Aurora A expression may be a potential method for indicating chemosensitivity to docetaxel-based chemotherapy.

## Introduction

For the past few years, lung cancer has gradually become one of the most common solid and malignant tumours that threaten the health of human beings. There are 1.8 million new patients each year that suffer from lung cancer (1). Approximately 80% of lung cancer cases are non-small cell lung cancer (NSCLC), which consists of lung adenocarcinoma (LAD), squamous cell carcinoma, large cell lung cancer and adenosquamous cell carcinoma (2). Since early symptoms are not obvious, early diagnosis is more difficult. Most lung cancer patients have lost their opportunity to undergo surgery by the time they are diagnosed, making chemotherapy the main treatment for NSCLC (3). Docetaxel is a semi-synthetic taxane antitumour agent that is utilized extensively in the clinical therapy of advanced NSCLC. Mechanistically, it induces abnormalities in microtubule dynamics by promoting microtubule polymerization. Abnormal microtubule dynamics activate spindle assembly checkpoint proteins to cause cell cycle arrest (4). In addition, taxanes can affect mitochondrial membrane permeability, resulting in the production and release of pro-apoptotic substances and the induction of apoptosis (5). Nonetheless, chemoresistance is the most important reason for the failure of docetaxel treatment (6).

The development of docetaxel resistance in tumour cells is a multi-step, multi-factor process involving the genomic and epigenetic abnormalities of key genes related to drug efflux, signal transduction, DNA damage repair, the cell cycle and apoptosis (7). Based on data from previous studies, our laboratory has confirmed that Aurora A promotes the resistance of primary hepatocarcinoma cells by regulating the NF- $\kappa$ B/miRNA-21/PTEN signalling pathway (8). Aurora A is a member of the mitotic serine/threonine kinase family that is encoded by the AURKA gene. Aurora A plays important roles in mitosis, which is associated with central body maturation and separation, spindle assembly and stability, and chromosome pairing (9). The expression and activation peak of Aurora A appears in the late stage of cell mitosis during the G2 to M phase transition, and its localization changes with cell cycle evolution (10). In NSCLC, Aurora A expression is upregulated, causing cell aneuploidy formation and promoting malignant transformation (11). Aurora A gene expression abnormalities are also very common in the formation of

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drug-resistant phenotypes, but the role Aurora A plays remains unclear in the process of docetaxel resistance in human lung adenocarcinoma.

MicroRNAs (miRNAs) are small non-coding RNA molecules that can be found in plants, animals and some viruses. miRNAs contain approximately 22 non-coding nucleotides that bind to the 3'-untranslated region (3'-UTR) of the target gene mRNA and inhibit its expression. miRNAs play an important role in epigenetic regulation. miRNAs have a small number of bases that bind to the site of its target genes, and complete pairing is not required; thus, one miRNA can target multiple genes, and the target genes also have multiple miRNA binding sites that result in a complex regulatory network. miRNAs play an important role in cell proliferation and apoptosis, blood cell differentiation, insulin secretion, late embryonic development, tumour development and many other important physiological and pathological processes. Current studies have demonstrated that miRNA expression abnormalities are very common in the development of drug-resistance in tumour cells, and the possible mechanisms include the abnormal modulation of cell proliferation, apoptosis, the cell cycle and signalling pathways. miRNA-mediated chemotherapy resistance is one of the key research areas of chemotherapy resistance.

According to high throughput miRNA chip data from our previous research, miRNA-885-3p (miR-885-3p) was identified as one of the most downregulated miRNAs in docetaxel-resistant SPC-A1/DTX cells (human lung adenocarcinoma) compared with those in parental SPC-A1 cells (12). Since miR-885-3p is involved in cell apoptosis in human cancer cells (13,14), we hypothesized that miR-885-3p downregulation may be related to chemoresistance in SPC-A1/DTX cells. In the present study, we explored the function of miR-885-3p and Aurora A in chemoresistance in human LAD cells. Our results indicated that miR-885-3p could act as a chemosensitizer to docetaxel in human LAD cells by targeting Aurora A.

## Materials and methods

**Cell culture.** Human lung adenocarcinoma cell lines (SPC-A1 and NCI-H1299) were purchased from the Tumor Cell Bank of the Chinese Academy of Medical Sciences (Shanghai, China). The docetaxel-resistant SPC-A1 and NCI-H1299 cell lines (SPC-A1/DTX and H1299/DTX) were established and maintained in our laboratory. All the cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and ampicillin (100 U/ml)-streptomycin (100 µg/ml) at 37°C and 5% CO<sub>2</sub>.

**Plasmids, shRNA, miRNA mimics and miRNA inhibitors.** miR-885-3p mimics and inhibitors (anti-miR-885-3p) were synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China), as well as the negative controls (miR-NC mimics or anti-miR-NC). Short hairpin RNAs (shRNAs) specifically targeting the human Aurora A gene (GenBank no. NM\_003600) were designed to knock down Aurora A expression. The shRNA sequences targeting Aurora A were as follows: Aurora A-shRNA sense, 5'-GATCCATGCCCTGCTTAACTGTCAATTCAAGAGATGACAGTAAGACAGGGC ATAGA-3'; negative control (NC) shRNA sense, 5'-GATCCA AGCTGAAGTACAACCTTCTTCAAGAGAGAAGGTTG

TACTTCAGCTTAGA-3'. The aforementioned sequences were inserted into the pSilencer4.1-CMVneo vector (between the *Bgl*III(A-GATCT) and *Hind*III(A-AGCTT) enzyme sites). The constructed plasmids were named pSil/shAuro and pSil/NC, respectively. All newly constructed plasmids were confirmed by DNA sequencing. A plasmid vector (pMD18/Auro) expressing Aurora A was purchased from Sino Biological, Inc. (Beijing, China). Lipofectamine PLUS (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used for cell transfection. Ampicillin-streptomycin-free medium was used for transfection. All the procedures were carried out according to the manufacturer's protocols. Stably transfected cell lines were selected with G418 (400 µg/ml) 48 h after transfection, and individual clones were isolated and maintained in a medium containing G418 (100 µg/ml).

**RNA extraction and real-time quantitative RT-PCR (RT-qPCR) assay.** Total RNA was extracted from cultured cells using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. miRNA expression was determined by stem-loop reverse-transcription (RT) and real-time quantitative PCR. The primers were designed as follows: miR-885-3p, 5'-GTCGTATCCAGTGCAGGGTCC GAGGTATTCGCACTGGATACGACTATCCA-3'; and U6, 5'-CGCTTCACGAATTTGCGTGTCA-3'. RT was performed using a PrimeScript RT reagent kit (Takara Bui Inc., Otsu, Japan) according to the manufacturer's instructions. Reverse transcriptase reactions were performed in a Mastercycler Thermocycler (Eppendorf AG, Hamburg, Germany) at 16 °C for 30 min, 42 °C for 30 min and 85 °C for 5 min. The qRT-PCR primers were designed as follows: Aurora A sense, 5'-AAT GCCCTGTCTTACTGTCATTC-3' and antisense, 5'-TCCAGA GATCCACCTTC-TCATC-3'; GAPDH sense, 5'-GACTCA TGACCACAGTCCATGC-3' and antisense, 5'-AGAGGCAGG GATGATGTTCTG-3'; miR-885-3p sense, 5'-CGTTAGGCA GCGGGGTGTAG-3', and antisense, 5'-ATCCAGTGCAGG GTCCGAGG-3'; U6 sense, 5'-GCTTCGGCAGCACATATA CTTAAAT-3' and antisense, 5'-CGCTTCACGAATTTGCGT GTCAT-3'. Real-time PCR was performed using a MicroRNA assay kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. The reaction conditions were 50°C for 2 min, 95°C for 10 min, 40 cycles of 95°C for 15 sec and 60°C for 1 min. Reactions were performed in triplicate using an ABI StepOne RT-PCR system.

**In vitro chemosensitivity assay.** Single-cell suspensions were prepared and seeded in 96-well plates (2x10<sup>3</sup> cells/well) and cultured overnight. After incubation for 48 h with freshly prepared docetaxel, a 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) solution (0.5 mg/ml) was added. After incubation for 4 h, the culture medium was removed and replaced with 100 µl of DMSO in each well. The absorbance at 490 nm was measured using a microplate reader (model 680; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The concentration gradient was designed to cover the killing efficiency of the docetaxel against SPC-A1 or H1299 cells from 0 to 100%: 1, 2, 4, 8, 16, 32, 64 and 128 µg/l for the parental strains. For the drug-resistant strains, docetaxel was designed to have a concentration gradient of 4, 8, 16, 32,

64, 128, 256 and 512  $\mu\text{g/l}$ . The cell survival curve was plotted using GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) and the  $\text{IC}_{50}$  values were calculated. The relative survival rate was calculated as: Relative survival rate = (OD experiment group - OD blank control group)/(OD negative control group - OD blank control group)  $\times$  100%. The blank control group referred to the control well containing the culture medium only, and the negative control group contained the cells and the culture solution without the addition of docetaxel. All assays were performed in sextuplicate and repeated at least 3 times.

**Colony formation assay.** Cells were trypsinized into single cell suspensions and added to 6-well plates at 500 cells/well. Following 14 days of culture, the RPMI-1640 medium was removed, and the colonies were fixed with methanol. All the cell colonies were then stained with a 0.1% crystal violet solution and counted manually. Each experiment was performed in triplicate.

**Apoptosis analysis.** An Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (KeyGen Biotech, Co., Ltd., Nanjing, China) was used to detect apoptosis in cancer cells according to the manufacturer's instructions. All the samples were assayed in triplicate.

**Cell cycle analysis.** Cells were collected and washed with ice-cold phosphate-buffered saline (PBS), and then fixed in 70% ethanol overnight at  $-20^{\circ}\text{C}$ . The fixed cells were rehydrated using PBS for 10 min and subjected to PI/RNase staining, followed by flow cytometric analysis using a FACScan flow cytometer and CellQuest software (both from BD Biosciences, San Jose, CA, USA).

**Western blotting assay.** Cells were harvested by suspension in lysis buffer (1 mM dithiothreitol, 0.125 mM EDTA, 5% glycerol, 1.0 mM phenylmethylsulfonylfluoride, 1.0 mg/ml leupeptin, 1.0 mg/ml pepstatin, 1.0 mg/ml aprotinin, 1% Triton X-100 in 12.5 mM TRIS-HCl buffer, pH 7.0) on ice. The protein concentration was measured using Pierce™ BCA Protein Assay kit (Thermo Fisher Scientific, Inc., Rockford, IL, USA). Then, equal weights (50  $\mu\text{g}$ ) of cell protein lysates were separated on 10% SDS polyacrylamide gels, followed by being electrophoretically transferred onto polyvinylidene difluoride membranes (PVDF) (Roche Diagnostics, Basel, Switzerland). Next, the membranes were blocked in PBS containing 5% non-fat milk and 0.2% Tween-20 for 1 h at room temperature and incubated overnight at  $4^{\circ}\text{C}$  rabbit anti-Aurora A (dilution 1:100; cat. no. 4718T), p-Aurora A (dilution 1:100; cat. no. 3079T), cleaved caspase-3 (dilution 1:200; cat. no. 9654S), NF- $\kappa\text{B}$  (dilution 1:200; cat. no. 4764T), Bcl-2 (dilution 1:50; cat. no. 4223T), Bax (dilution 1:50; cat. no. 5023T), E-cadherin (dilution 1:150; cat. no. 3195T) and vimentin (dilution 1:150; cat. no. 5741T) proteins in PBS containing 0.1% Tween-20 followed by incubation with horseradish peroxidase-conjugated anti-mouse IgG (dilution 1:1,000; cat. no. 58802S) at  $37^{\circ}\text{C}$  for 1 h. An anti-GAPDH monoclonal antibody (dilution 1:500; cat. no. 97166T) was used as a control. ECL detection reagents (EMD Millipore, Billerica, MA, USA) were added on the membranes for 1 min and were immediately exposed to X-ray

film (Kodak, Rochester, NY, USA). ImageJ analysis software (<https://imagej.nih.gov/ij/>) was used to quantify the band intensities. All antibodies were purchased from Univ-bio Inc (Shanghai, China).

**Luciferase activity.** Human docetaxel-resistant LAD cells (SPC-A1/DTX) grown in a 48-well plate were co-transfected with miRNA mimics/inhibitors and pLUC firefly luciferase vectors containing empty, wild-type or mutant Aurora A 3'-UTR sequences using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). The cells were collected and lysed at 48 h post-transfection to assess the luciferase activities using a dual-luciferase assay kit (Promega Corporation, Madison, WI, USA). The relative luciferase activities were calculated by normalizing the ratio of Firefly/Renilla luciferase to that of the negative control-transfected cells.

**Wound healing assay.** Wound healing assays were used to assess cell migration. In brief, cells were seeded in 12-well plates and cultured to confluence. Wounds of 1.0-mm width were created with a plastic scribe, and the cells were washed with PBS and cultured in serum-free medium. At 24 h after wounding, cell migration was observed under a light microscope. The distance of cell migration under five randomly chosen areas was measured to determine the wound area.

**Transwell assay.** Cells in serum-free medium were seeded into inserts (800 cells/each insert, pore size 8  $\mu\text{m}$ ; Corning Inc., Corning, NY, USA), which were then transferred into wells with medium containing 10% FBS and cultured for an additional 24 h. Thereafter, the non-invading cells on the top of the membrane were scraped, and the invaded cells on the bottom of the membrane were fixed in methanol and stained with a 0.05% crystal violet solution. The number of invaded cells on the membrane was then counted manually under an inverted microscope. Each experiment was performed in triplicate.

**Bioinformatics and statistical analyses.** Online miRNA databases (TargetScan, miRBase, and PicTarget) were used to predict the target genes of miR-885-3p. Statistical analyses were conducted using SPSS software v16.0 (SPSS, Inc., Chicago, IL, USA). The experimental data were expressed as the mean  $\pm$  SEM. For the comparison of means between two groups, a two-tailed t-test was conducted, and for the comparison of means among three or more groups, one-way ANOVA and LSD were used. Differences were considered significant when  $P < 0.05$ .

## Results

**Aurora A is significantly upregulated in docetaxel-resistant LAD cells.** To detect the role of Aurora A in lung adenocarcinoma resistance, we first analysed the expression of Aurora A in docetaxel-resistant LAD cells by qRT-PCR ( $P < 0.05$ , Fig. 1A). Compared with that in the parental human lung adenocarcinoma SPC-A1 and NCI-H1299 cell lines, Aurora A was significantly upregulated by  $(2.78 \pm 0.16)$ -fold and  $(2.43 \pm 0.12)$ -fold in the docetaxel-resistant LAD cell lines SPC-A1/DTX and H1299/DTX. Additionally, Aurora A protein levels were significantly higher in SPC-A1/DTX

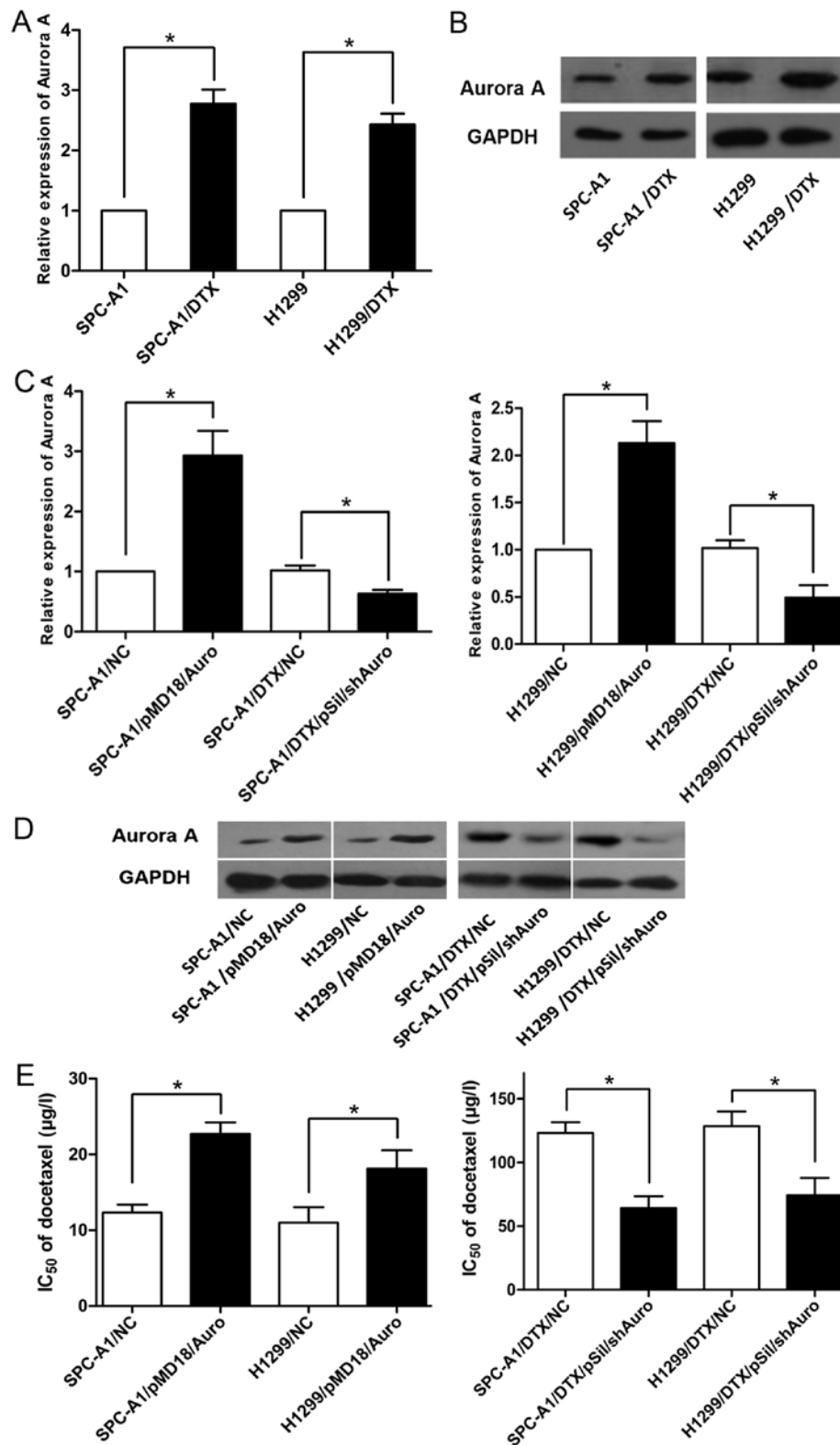


Figure 1. Association between Aurora A expression and the responses of LAD cells to docetaxel. (A) The relative expression levels of Aurora A were examined in LAD cells and docetaxel-resistant LAD cells by qRT-PCR. (B) The protein levels of Aurora A were assessed in LAD cells and docetaxel-resistant LAD cells by Western blotting. (C and D) SPC-A1 and H1299 cells were transfected with NC or pMD18/Auro; SPC-A1/DTX and H1299/DTX cells were transfected with NC or pSil/shAuro. qRT-PCR and western blotting were used to verify the transfection efficiency. (E) MTT assays were used to assess docetaxel sensitivity, and the IC<sub>50</sub> values of docetaxel were determined. Each experiment was performed at least in triplicate. \*P<0.05. LAD, lung adenocarcinoma.

and H1299/DTX cells than in parental SPC-A1 and H1299 cells (P<0.05, Fig. 1B). Therefore, these data indicated that the upregulation of Aurora A may play a role in the development of docetaxel resistance in LAD cells.

*Expression of Aurora A is positively correlated with the resistance of LAD cells to docetaxel.* After confirming that Aurora A was amplified in the drug-resistant cells, we regulated Aurora A expression in parental and resistant SPC-A1

and H1299 cells by using the plasmid vectors pMD18/Auro and pSil/shAuro to explore the relationship between Aurora A expression and the degree of docetaxel resistance. Transfection efficiency was validated by RT-qPCR and western blotting ( $P < 0.05$ , Fig. 1C and D), and the Aurora A expression levels were increased in the pMD/Auro-transfected SPC-A1 and H1299 cells and decreased in the pSil/shAuro-transfected SPC-A1/DTX and H1299/DTX cells. MTT assays were used to calculate the chemosensitivity ( $IC_{50}$  of docetaxel) of various cell lines (SPC-A1, H1299, SPC-A1/DTX and H1299/DTX cells) with different expression levels of Aurora A. Higher expression of Aurora A promoted docetaxel resistance in SPC-A1 or H1299 cells, and the chemosensitivity of SPC-A1/DTX and H1299/DTX cells transfected with pSil/shAuro was significantly lower than that of the control cells ( $P < 0.05$ , Fig. 1E). Similarly, the colony formation viability of SPC-A1 and H1299 cells transfected with pMD/Auro was enhanced, and pSil/shAuro transfection decreased the colony formation rate of SPC-A1/DTX and H1299/DTX cells ( $P < 0.05$ , Fig. 2A). The MTT and colony formation assay results demonstrated that Aurora A may be a key induction factor for docetaxel resistance in LAD cells.

*Silencing Aurora A affects chemotherapy-induced apoptosis and cell cycle distribution in SPC-A1/DTX cells.* Next, we aimed to determine the relationship between Aurora A and chemotherapy-induced apoptosis and cell cycle distribution. pSil/shAuro was transfected into SPC-A1/DTX cells, and the effects of Aurora A inhibition on SPC-A1/DTX cell apoptosis were determined. The apoptosis rate was significantly higher in SPC-A1/DTX/pSilAuro cells than in SPC-A1/DTX/NC cells treated with the same docetaxel concentration (half of the  $IC_{50}$  concentration for 48 h) ( $P < 0.05$ , Fig. 2B). To analyse the mechanisms by which Aurora A expression affect cell proliferation, flow cytometric analyses of the cell cycle were performed. As shown in Fig. 2C, Aurora A inhibition resulted in an increased proportion of cells in the G2/M phase and a decreased proportion of cells in the S phase ( $P < 0.05$ , Fig. 2C).

*Silencing Aurora A affects apoptosis by regulating NF- $\kappa$ B and Bcl-2/Bax.* According to the function of Aurora A, we analysed the expression changes of molecules related to cell apoptosis and proliferation. Western blot assays revealed that pSil/shAuro decreased NF- $\kappa$ B protein levels in SPC-A1/DTX and H1299/DTX cells ( $P < 0.05$ , Fig. 2D). Moreover, the expression levels of cleaved-caspase-3 were increased when SPC-A1/DTX and H1299/DTX cells were transfected with pSil/shAuro ( $P < 0.05$ , Fig. 2D). Additionally, Bcl-2 downregulation and Bax upregulation were observed in pSil/shAuro-transfected SPC-A1/DTX and H1299/DTX cells, which reflected a decrease in the anti-apoptosis ability of the cells (Bcl-2/Bax ratio).

*miR-885-3p targets Aurora A directly.* By using open access databases (PicTarget and miRBase) and studying our previous microRNA profiles of docetaxel-resistant human LADs, we found miR-885-3p to be a preferred upstream candidate miRNA for controlling Aurora A due to the putative binding site within its 3'-UTR (Fig. 3B); miR-885-3p was significantly downregulated in SPC-A1/DTX and H1299/DTX

cells ( $P < 0.05$ , Fig. 3A). Then, we designed mimics and an inhibitor of miR-885-3p and validated their transfection efficiency ( $P < 0.05$ , Fig. 3C). To determine whether Aurora A is a direct downstream target of miR-885-3p, luciferase reporter assays were performed. A fragment of the Aurora A 3'-UTR containing the putative miR-885-3p binding site was cloned into a luciferase reporter vector. As shown in Fig. 3D, the luciferase reporter assays indicated that the luciferase activities of LAD cells transfected with the Aurora A-wt construct were significantly suppressed after the transfection of miR-885-3p mimics and were significantly increased after the transfection of the miR-885-3p inhibitor; however, there was no significant difference found in the cells transfected with the Aurora A-mut construct. Then, we determined the effect of miR-885-3p on Aurora A protein expression and found that miR-885-3p mimics decreased Aurora A protein expression in SPC-A1/DTX cells, while the miR-885-3p inhibitor increased Aurora A protein expression (Fig. 3E). Due to these results, we concluded that miR-885-3p downregulated Aurora A expression by targeting its 3'-UTR directly.

*miR-885-3p is involved in docetaxel resistance in lung adenocarcinoma.* To gain more insight into how miR-885-3p is involved in Aurora A-induced chemoresistance, we used gain-of-function and loss-of-function experiments. First, SPC-A1 and H1299 cells were transfected with a miR-885-3p inhibitor or negative control, and SPC-A1/DTX and H1299/DTX cells were transfected with miR-885-3p mimics or a negative control. MTT assays revealed that the  $IC_{50}$  values of docetaxel in SPC-A1 and H1299 cells were significantly increased, and the  $IC_{50}$  values of docetaxel in SPC-A1/DTX and H1299/DTX cells were decreased ( $P < 0.05$ , Fig. 4A). Then, the growth rates of SPC-A1 and H1299 cells transfected with the miR-885-3p inhibitor were increased, but the colony formation efficiency of SPC-A1/DTX and H1299/DTX cells was decreased by miR-885-3p mimics ( $P < 0.05$ , Fig. 4B).

*miR-885-3p affects chemotherapy-induced apoptosis by regulating NF- $\kappa$ B and Bcl-2/Bax.* To further examine whether miR-885-3p participated in cell apoptosis and the molecular mechanisms of miR-885-3p in apoptotic regulation, flow cytometric analyses of SPC-A1/DTX apoptosis were performed. As expected, miR-885-3p overexpression significantly increased apoptosis in SPC-A1/DTX cells exposed to docetaxel ( $P < 0.05$ , Fig. 4C). Western blots indicated that the expression of NF- $\kappa$ B was significantly lower in SPC-A1/DTX and H1299/DTX cells than in cells transfected with the negative control. Additionally, cleaved-caspase-3 levels were increased in SPC-A1/DTX and H1299/DTX cells with miR-885-3p overexpression ( $P < 0.05$ , Fig. 4D). Bcl-2 downregulation and Bax upregulation were also detected in miR-885-3p mimic-transfected SPC-A1/DTX and H1299/DTX cells ( $P < 0.05$ , Fig. 4D). These data revealed that miR-885-3p may regulate the response to docetaxel by promoting cell apoptosis.

*High miR-885-3p expression in lung adenocarcinoma is associated with decreased Aurora A expression and chemotherapeutic resistance.* To further explore whether Aurora A was involved in the function of miR-885-3p in response to docetaxel, we then performed rescue experiments and evalu-

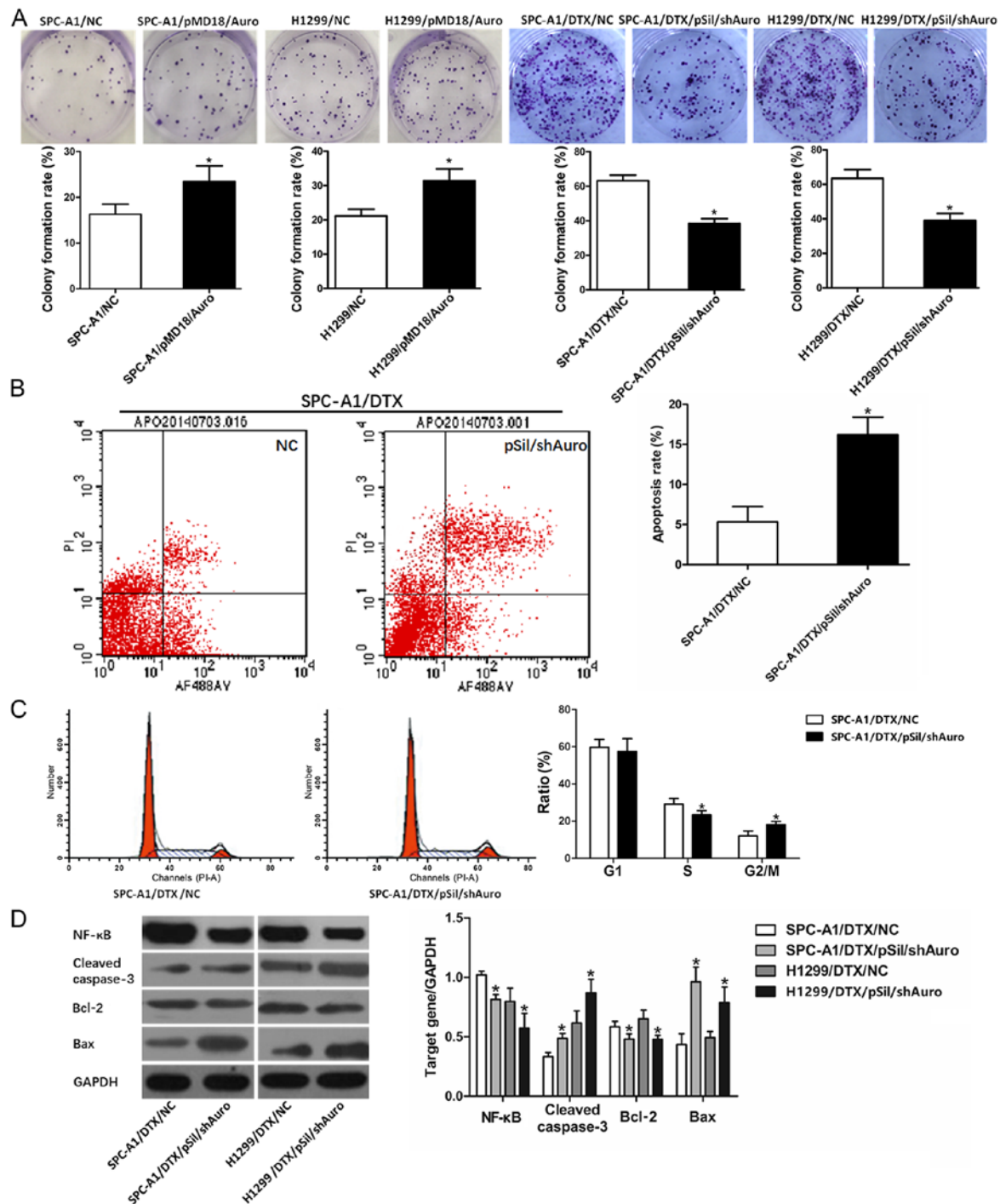


Figure 2. Effect of pMD18/Auro or pSil/shAuro on cell proliferation, cell cycle distribution and apoptosis-related proteins in LAD cells and docetaxel-resistant LAD cells. (A) Colony formation assays of SPC-A1 and H1299 cells and SPC-A1/DTX and H1299/DTX cells transfected with pMD18/Auro or pSil/shAuro. (B) Flow cytometric analysis of apoptosis in pSil/shAuro-NC and pSil/shAuro-transfected SPC-A1/DTX cells. (C) Flow cytometric results for cell cycle distribution in pSil/shAuro-transfected SPC-A1/DTX cells. (D) Western blotting for apoptosis-related proteins (NF-κB, cleaved caspase-3, Bcl-2 and Bax) in SPC-A1/DTX/pSil/shAuro (and SPC-A1/DTX /NC) cells and H1299/DTX/pSil/shAuro (and H1299/DTX/NC) cells. Equal loading was confirmed by the equal GAPDH levels. The results represent the average of three independent experiments (mean ± SEM). \*P<0.05. LAD, lung adenocarcinoma.

ated LAD cell proliferation and apoptosis. After transfection with pMD18/Auro, SPC/DTX cells were co-transfected with miR-885-3p mimics, which could partially rescue the Aurora A expression upregulation and docetaxel IC<sub>50</sub> increase in SPC-A1/DTX cells (P<0.05, Fig. 5A and B). Co-transfection of pSil/shAuro and a miR-885-3p inhibitor could partially rescue the Aurora A expression downregulation and the colony formation arrest in SPC-A1 cells (P<0.05,

Fig. 5C and D). Furthermore, co-transfection could partially rescue the decreased expression of cleaved caspase-3 protein in SPC-A1/DTX and H1299/DTX cells induced by the upregulation of Aurora A (Fig. 5E). As expected, co-transfection could partially rescue the Bcl-2 protein overexpression and decreased Bax protein expression in SPC-A1/DTX and H1299/DTX cells induced by miR-885-3p inhibition. NF-κB protein analyses revealed similar results. Collectively, these data indicated that

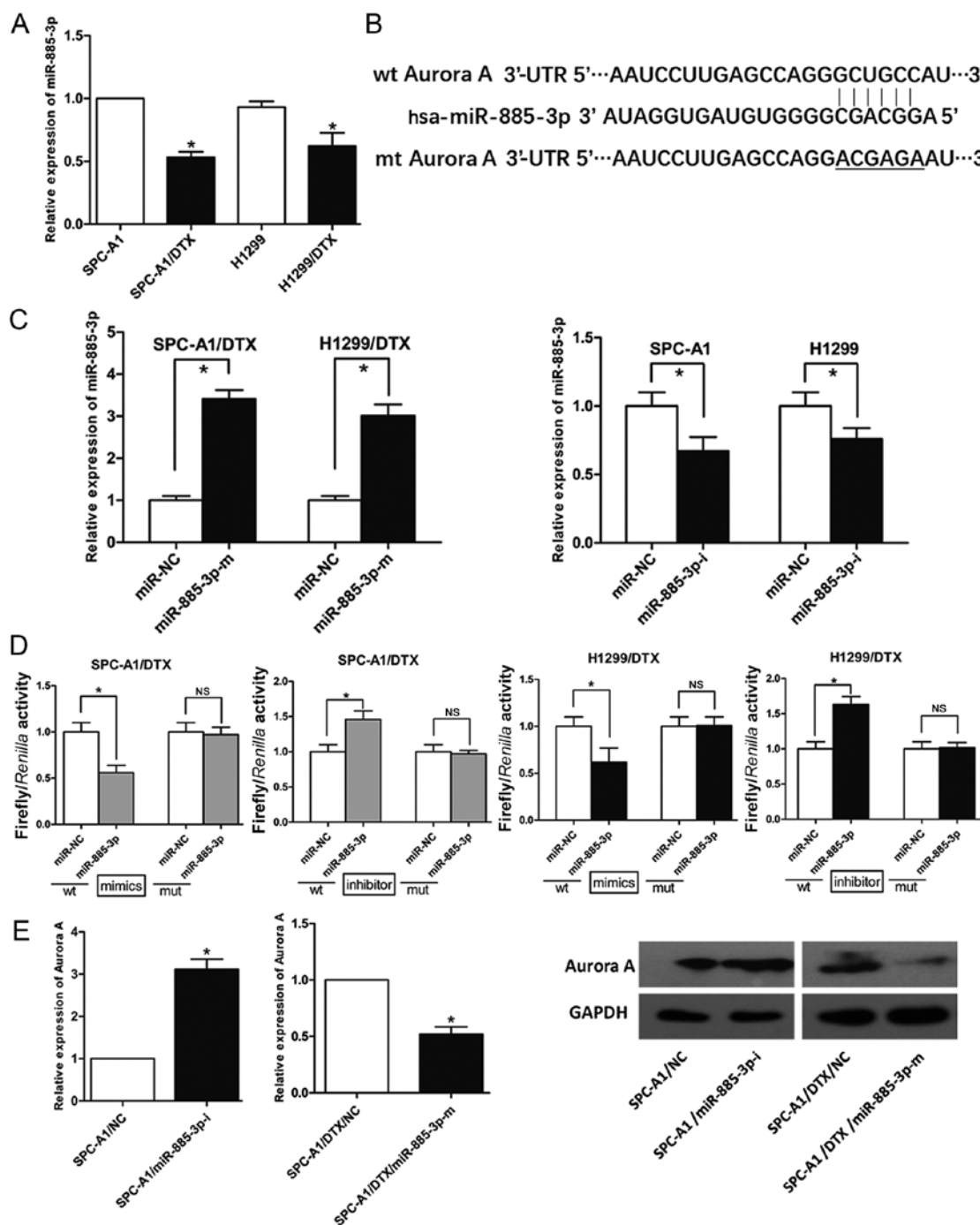


Figure 3. Aurora A is a direct target of miR-885-3p. (A) qRT-PCR analysis of miR-885-3p expression in docetaxel-resistant and parental LAD cells. (B) Sequence of the miR-885-3p binding site in the Aurora A 3'-UTR as predicted by TargetScan and miRbase; the 3'-UTR region of Aurora A mRNA is partially complementary to miR-885-3p. (C) At 48 h after SPC-A1/DTX and H1299/DTX cells were transfected with miR-885-3p mimics (or miR-mimics/NC), SPC-A1 and H1299 cells were transfected with miR-885-3p inhibitor (or miR-inhibitor/NC), and qRT-PCR assays were performed to determine the expression of miR-885-3p. (D) SPC-A1/DTX and H1299/DTX cells were co-transfected with miR-885-3p mimics or inhibitor and the pLUC vector with the Aurora A 3'-UTR-wt or mut. After 24 h, the luciferase activity was measured. The values are presented as the relative luciferase activity after normalization to the *Renilla* luciferase activity. (E) qRT-PCR and western blot analyses of Aurora A protein expression in SPC-A1 and SPC-A1/DTX cells transfected with miR-885-3p inhibitor (or miR inhibitor/NC) or mimics (or miR mimics/NC). The data are expressed as the mean value  $\pm$  SEM of the results obtained from three independent experiments. \* $P < 0.05$ . LAD, lung adenocarcinoma; miR-885-3p-m, miR-885-3p mimics; miR-885-3p-i, miR-885-3p inhibitor; NS, not significant.

miR-885-3p may regulate the chemosensitivity of LAD cells in part by targeting Aurora A.

*miR-885-3p/Aurora A is involved in epithelial-mesenchymal transition (EMT) in LAD cells.* To determine the association between miR-885-3p/Aurora A expression and

EMT in LAD cells, SPC-A1/DTX cells were transfected with miR-885-3p mimics and pSil/shAuro. Cell wound scratch assays and Transwell invasion assays revealed significant inhibition of the migration and invasion abilities of SPC-A1/DTX cells ( $P < 0.05$ , Fig. 6A and B). SPC-A1/DTX cells were transfected with pSil/shAuro or



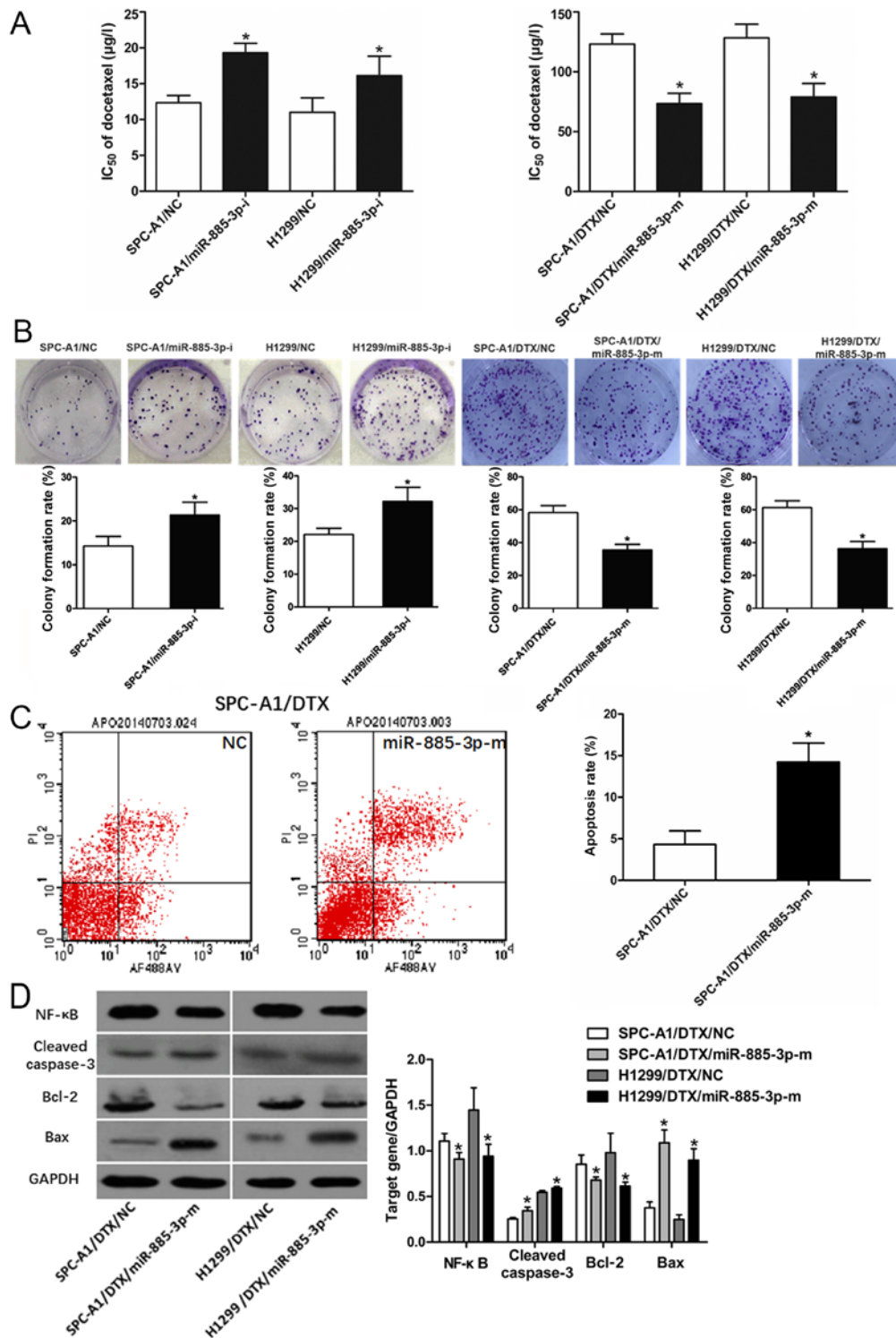


Figure 4. miR-885-3p regulates the response of lung adenocarcinoma cells to docetaxel. (A) Analysis of the IC<sub>50</sub> values of docetaxel in SPC-A1 and H1299 cells transfected with miR-885-3p inhibitor or miR inhibitor-NC and SPC-A1/DTX and H1299/DTX cells transfected with miR-885-3p mimics or miR mimics-NC. (B) Colony formation assays of SPC-A1 and H1299 cells transfected with miR-885-3p inhibitor or miR mimics/NC and SPC-A1/DTX and H1299/DTX cells transfected with miR-885-3p mimics or miR mimics/NC. (C) Flow cytometric analysis of apoptosis in miR-885-3p mimics/NC or miR-885-3p mimics-transfected SPC-A1/DTX cells. (D) Western blotting detection of apoptosis-related proteins (NF-κB, cleaved caspase-3, Bcl-2 and Bax) in SPC-A1/DTX/miR-885-3p-m (and SPC-A1/DTX/NC) and H1299/DTX/miR-885-3p-m (and H1299/DTX/NC) cells. Equal loading was confirmed by the equal GAPDH levels. The results represent the average of three independent experiments (mean ± SEM). \*P<0.05. miR-885-3p-m, miR-885-3p mimics; miR-885-3p-i, miR-885-3p inhibitor.

miR-885-3p mimics. As shown in Fig. 6C, pSil/shAuro and miR-885-3p mimics could increase E-cadherin and downregulate vimentin expression in SPC-A1/DTX cells. After transfection with the miR-885-3p inhibitor, SPC/DTX

cells were co-transfected with pSil/shAuro. The co-transfection partially rescued the cell migration and invasion and E-cadherin/vimentin changes induced by the miR-885-3p inhibitor (Fig. 6D-F).



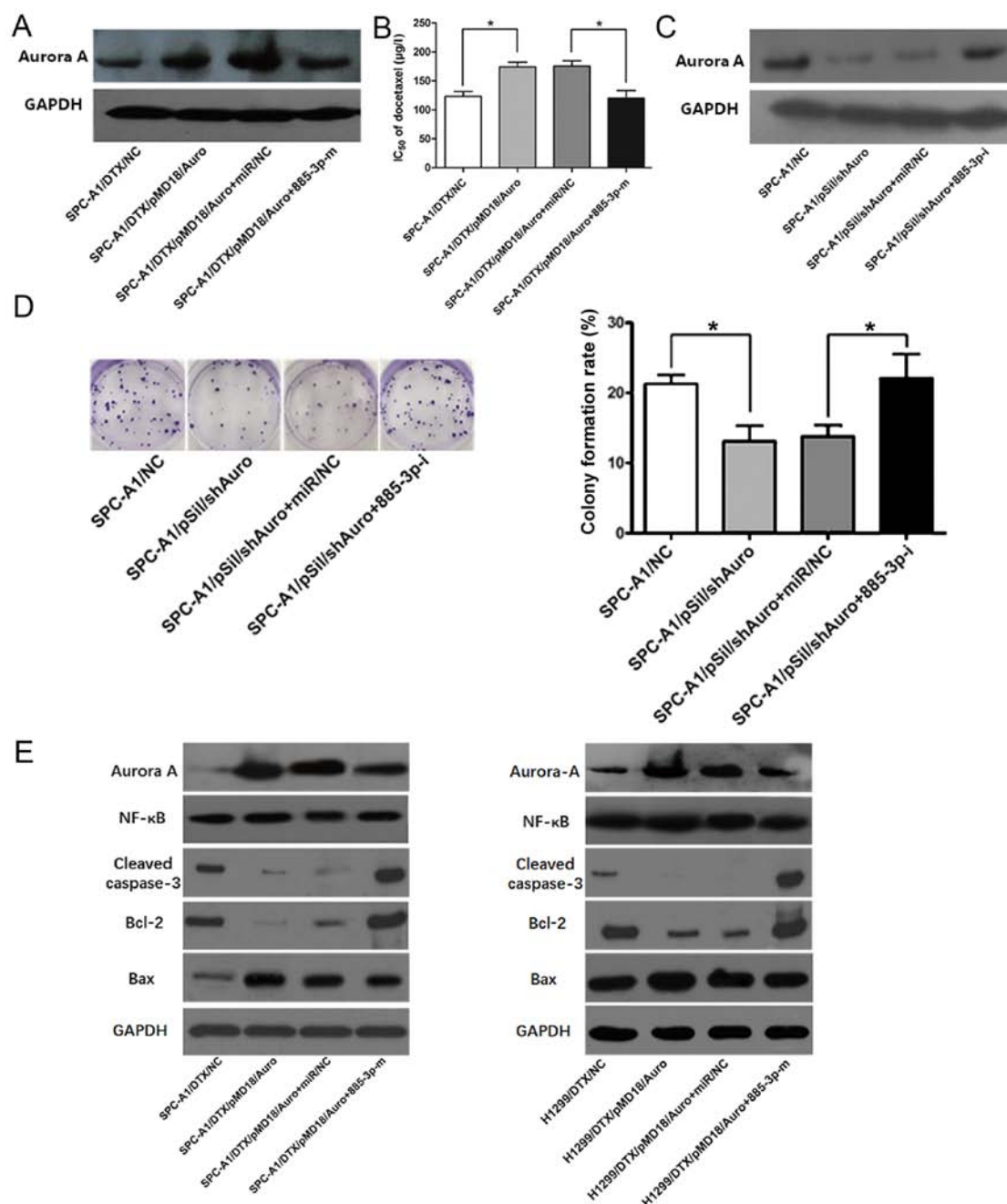


Figure 5. miR-885-3p mimics or inhibitor partially rescue the effects of pMD18/Auro and pSil/shAuro on the sensitivity of SPC-A1/DTX and SPC-A1 cells to docetaxel. (A) Western blot analysis of Aurora A protein expression in pMD18/NC and pMD18/Auro-transfected SPC-A1/DTX cells and SPC-A1/DTX cells co-transfected with pMD18/Auro and miR-885-3p mimics/NC or miR-885-3p mimics. (B) Analysis of the IC<sub>50</sub> values of docetaxel in pMD18/NC and pMD18/Auro-transfected SPC-A1/DTX cells and SPC-A1/DTX cells co-transfected with pMD18/Auro and miR-885-3p mimics/NC or miR-885-3p mimics. (C) Western blot analysis of Aurora A protein expression in pSil/shAuro/NC and pSil/shAuro-transfected SPC-A1 cells and SPC-A1 cells co-transfected with pSil/shAuro and miR-885-3p inhibitor/NC or miR-885-3p inhibitor. (D) Colony formation assay of pSil/shAuro/NC and pSil/shAuro-transfected SPC-A1 cells and SPC-A1 cells co-transfected with pSil/shAuro and miR-885-3p inhibitor/NC or miR-885-3p inhibitor. (E) Western blot analysis of Aurora A, NF-κB, cleaved caspase-3, Bcl-2 and Bax protein expression in pMD18/NC and pMD18/Auro-transfected SPC-A1/DTX and H1299/DTX cells and SPC-A1/DTX and H1299/DTX cells co-transfected with pMD18/Auro and miR-885-3p mimics/NC or miR-885-3p mimics. The data were expressed as the mean value ± SEM of the results obtained from three independent experiments. \*P<0.05. miR-885-3p-m, miR-885-3p mimics; miR-885-3p-i, miR-885-3p inhibitor.

## Discussion

Aurora A is a member of a new serine/threonine kinase family that plays an important role in mitosis (15). In the process of forming the spindle, Aurora A inhibition can cause the formation of the single-pole spindle and influence the stability of the spindle. In addition, Aurora A also participates in the

regulation of the G2/M phase cell cycle checkpoint; Aurora A overexpression can counter the checkpoint activation induced by DNA damage in the G2/M phase, resulting in genome instability and tumour formation. Increasing evidence has revealed that aberrant Aurora A expression was found in lung adenocarcinoma, liver, colon and breast cancer, and many other malignant tumours (16,17). The overexpression of

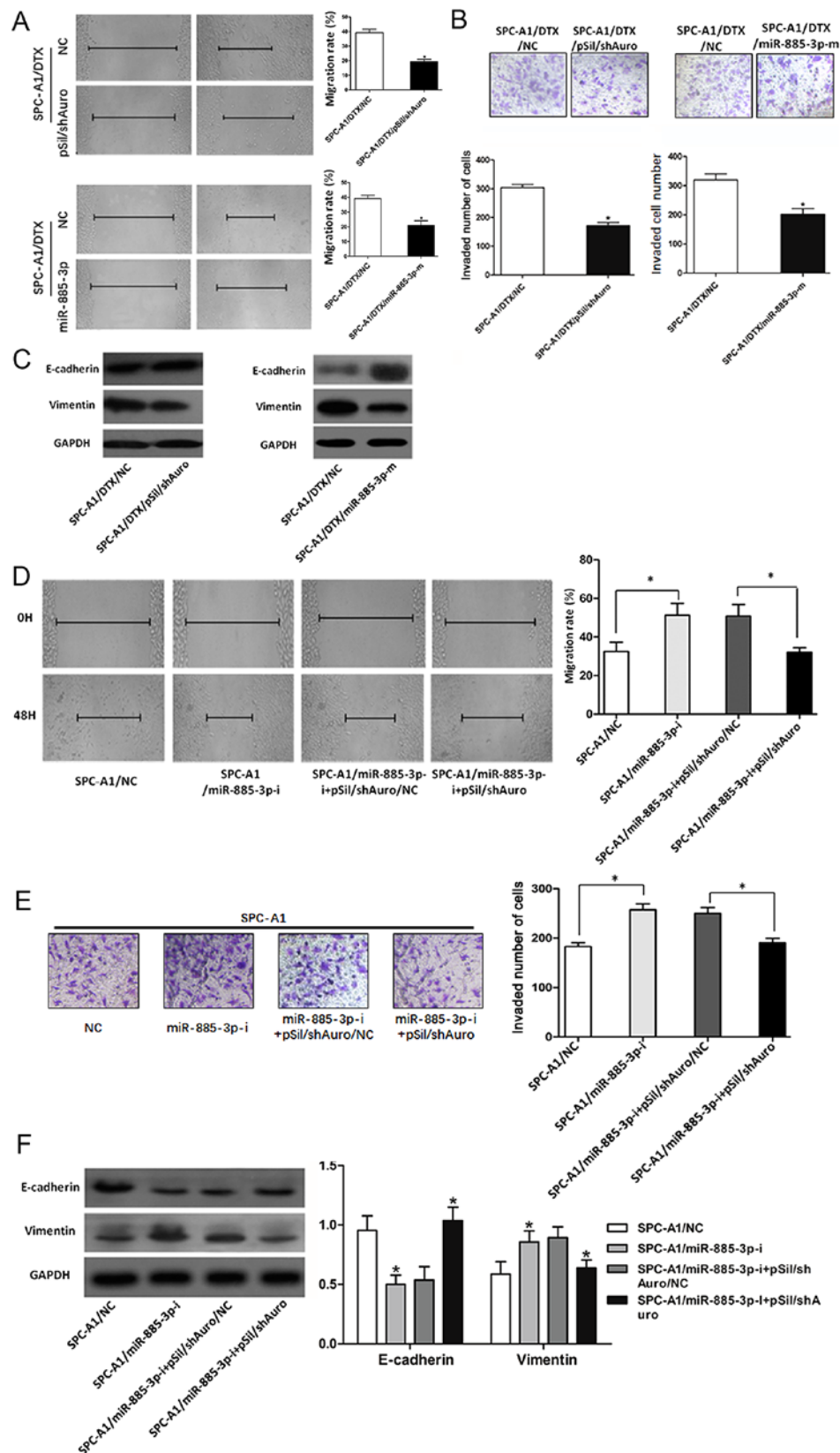


Figure 6. miR-885-3p/Aurora A is involved in epithelial-mesenchymal transition. (A) Wound healing assay of pSil/shAuro/NC and pSil/shAuro-transfected SPC-A1/DTX cells and miR-885-3p mimics/NC or miR-885-3p mimic-transfected SPC-A1 and SPC-A1/DTX cells. (B) Transwell invasion assays were used to analyse the cell invasion ability of pSil/shAuro/NC and pSil/shAuro-transfected and miR-885-3p mimics/NC or miR-885-3p mimic-transfected SPC-A1/DTX cells. (C) SPC-A1/DTX cells were transfected with pSil/shAuro (or pSil/shAuro/NC) or miR-885-3p mimics (or mimics/NC), and E-cadherin and vimentin protein levels were detected by western blot analysis. GAPDH was used as an internal control. (D) Wound healing assay of miR-885-3p inhibitor/NC or miR-885-3p inhibitor-transfected SPC-A1 cells and SPC-A1 cells co-transfected with pSil/shAuro/NC or pSil/shAuro. (E) Transwell invasion assay of miR-885-3p inhibitor/NC and miR-885-3p inhibitor-transfected SPC-A1 cells and SPC-A1 cells co-transfected with pSil/shAuro/NC or pSil/shAuro. (F) Western blot analysis of E-cadherin and vimentin protein levels in miR-885-3p inhibitor/NC or miR-885-3p inhibitor-transfected SPC-A1 cells and SPC-A1 cells co-transfected with pSil/shAuro/NC or pSil/shAuro. GAPDH was used as an internal control. The data are expressed as the means of three separate experiments  $\pm$  SEM; \* $P < 0.05$ . miR-885-3p-m, miR-885-3p mimics; miR-885-3p-i, miR-885-3p inhibitor.

Aurora A in cells can cause aneuploidy formation and promote cell malignant transformation. It has been reported that the overexpression of Aurora A could promote growth and inhibit apoptosis in tumour cells (18). For instance, it was found that Aurora A overexpression promoted human embryonic kidney HEK293T cell proliferation and cell migration via the activation of cyclin E/CDK2 and cyclin B1 (19). In ovarian cancer, the combination of the Aurora A kinase inhibitor alisertib and a CHEK1 inhibitor triggered apoptosis, reduced the population of stem cells and increased the effect of taxanes and platinum compounds (20). Aurora A is also a good therapeutic target for inhibiting cancer cell growth in gastric carcinoma, glioblastoma and small cell lung cancer (21-23). Furthermore, the relationship between Aurora A expression and tumour invasion and metastasis has been recently revealed. Kozyreva *et al* revealed that the combination of an Aurora A inhibitor with eribulin could lead to a synergistic increase in apoptosis in mammary tumours, as well as cytotoxic autophagy in metastases (24). Maimaiti *et al* reported that the simultaneous overexpression of Aurora A and CFL-1 associated with lymph node metastasis in thyroid cancer (25). The inhibition of Aurora A suppressed thyroid cancer cell migration *in vitro* and decreased lymph node metastasis in nude mice (25). Notably, the roles of Aurora A overexpression in drug-resistance in tumour cells remain unclear. Sun *et al* reported that the inhibition of Aurora A promotes chemosensitivity via induction of cell cycle arrest and apoptosis in cervical cancer cells (26). De Bacco *et al* revealed that Aurora A inhibitors can radio-sensitize tumours and convert GSC-positive selection (27). These data indicated that Aurora A may play an important role in chemo- or radio-resistance in human cancers. In our previous study, Aurora A promoted the phosphorylation of the nuclear I $\kappa$ B $\alpha$  protein and increased NF- $\kappa$ B (NF $\kappa$ B) activity, thus promoting chemoresistance in HCC cells (8). However, the association of Aurora A expression with chemoresistance in LAD cells remains unclear. To investigate this relationship, the associations between Aurora A protein expression levels and docetaxel sensitivity in SPC-A1 and H1299 cells were analysed in the present study. It was found that the expression level of Aurora A was negatively associated with chemosensitivity in LAD cells. Additionally, Aurora A overexpression increased the IC<sub>50</sub> values of docetaxel and cell growth in both the SPC-A1 and H1299 cell lines. Then, we further investigated the effect of Aurora A expression on chemosensitivity in LAD cells. At the same concentration of docetaxel, silencing Aurora A increased apoptosis compared to that in the control cells by enhancing caspase-3-dependent apoptosis. In addition, Aurora A inhibition increased the proportion of cells in the G2/M phase and decreased the proportion of cells in the S phase. Moreover, western blot assays revealed that pSil/shAuro decreased NF- $\kappa$ B, Bcl-2 levels, as well as Bax protein levels, in SPC-A1/DTX cells. Therefore, the overexpression of Aurora A promoted the formation of chemoresistant LAD cells.

Chemosensitivity is an important reason for the therapeutic failure of lung adenocarcinoma. The mechanism of chemotherapy resistance is very complicated and includes ATP binding box transporters, drug metabolism enzymes, hyperfunction-induced drug metabolism changes, drug target changes, apoptotic protein level changes (such as Bcl-2 and

Bax) and DNA damage repair abnormalities (28). Increasing evidence has shown that miRNAs may play a cursory role in the development of drug resistance (29). miRNAs can reduce target gene expression at the transcriptional level, affect multiple molecular signalling pathways concurrently, and regulate key pathways in cell apoptosis, the cell cycle and drug metabolism (30). In the present study, it was concluded that the 3'-UTR of Aurora A is a direct target of miR-885-3p. This conclusion was confirmed by four levels of evidence. First, bioinformation prediction was conducted using the Pictarget and miRBase platforms; second, there was a negative association between the expression of miR-885-3p and Aurora A in drug-resistant cells; third, luciferase reporter assays confirmed that Aurora A-wt can block the effects of miR-885-3p on the expression of Aurora A in drug-resistant cells; and fourth, subsequent rescue experiments revealed that miR-885-3p-regulated Aurora A expression was associated with docetaxel resistance development. Recently, miR-885-3p was found to have binding sites in the 3'-UTR regions of the Mdm4, Akt1 and Bcl-2 genes; these binding sites affect protein expression and apoptosis-related proteins. In a study of epithelial cell carcinoma, miR-885-3p acted as a negative regulator of the AKT1 gene to affect cell metabolism and chemotherapy drug sensitization (31). To reveal the relationship between miR-885-3p and docetaxel resistance development, SPC-A1 and H1299 cells were transfected with a miR-885-3p inhibitor, and SPC-A1/DTX and H1299/DTX cells were transfected with miR-885-3p mimics. The IC<sub>50</sub> values of docetaxel and cell proliferation rates in SPC-A1 and H1299 cells were significantly increased, while the IC<sub>50</sub> values of docetaxel and cell proliferation rates in SPC-A1/DTX and H1299/DTX cells were decreased. Then, we further investigated the effect of miR-885-3p on chemosensitivity in LAD cells. SPC-A1/DTX and H1299/DTX cells were stably transfected with miR-885-3p mimics, and it was observed that miR-885-3p overexpression increased apoptosis by enhancing caspase-3-dependent apoptosis. Additionally, western blot assays revealed that miR-885-3p mimics decreased NF- $\kappa$ B and Bcl-2 and upregulated Bax protein levels in SPC-A1/DTX and H1299/DTX cells. Moreover, the effects of Aurora A inhibition on chemoresistance in docetaxel-resistant LAD cells were similar to the effects of the miR-885-3p mimics. The miR-885-3p inhibitor could partially rescue the effects of Aurora A silencing on chemosensitivity in docetaxel-resistant LAD cells. In conclusion, miR-885-3p may confer docetaxel chemoresistance in LAD cells partially by targeting Aurora A and then regulating NF- $\kappa$ B and Bcl-2/Bax expression.

Epithelial-mesenchymal transition (EMT) includes mainly the process of cell polarity loss; the surrounding cells and matrix gradually disintegrate, and the abilities of migration and movement become abnormal (32). Research have shown that EMT is also closely connected with chemoresistance in tumour cells (33). In a study on miR-885-3p, increased miR-885-3p levels could block the BMP/Smad/Id1 signalling pathway and regulate the function of TGF- $\beta$ , which induced epithelial-mesenchymal transition (13). Additionally, D'Assoro *et al* found that Aurora A kinase could activate the EMT pathway, which is responsible for the development of distant metastases in breast cancer cells (34). In our previous study, it was revealed that the methylation-associated silencing

of miR-129-3p promoted the epithelial-mesenchymal transition, invasion and metastasis of hepatocellular cancer by targeting Aurora A (35). To determine the connection between miR-885-3p/Aurora A expression and EMT in LAD cells, SPC-A1/DTX cells were transfected with miR-885-3p mimics and pSil/shAuro. The migration and invasion abilities of SPC-A1/DTX cells were significantly suppressed. Additionally, increased E-cadherin and decreased vimentin expression levels in SPC-A1/DTX cells were observed after transfection with miR-885-3p mimics or pSil/shAuro. These data indicated that Aurora A overexpression or miR-885-3p inhibition is associated with higher aggressive tumour behaviour; thus, we further established that the miR-885-3p-Aurora Axis may be partially responsible for EMT in human LAD cells.

In conclusion, the present study revealed that Aurora A expression was significantly upregulated in docetaxel-resistant LAD cells. Silencing Aurora A expression could significantly increase chemosensitivity in LAD cells. Moreover, miR-885-3p could affect the chemosensitivity of LAD cells to docetaxel by targeting Aurora A and then regulating NF- $\kappa$ B and Bcl-2/Bax expression. Further investigations using animal experiments and human LAD tissue samples are required to confirm the correlation between the function of miR-885-3p and its target Aurora A and the responses to docetaxel-based chemotherapy in LAD patients.

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### Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

### Authors' contributions

JC, JG, XC and RW contributed to the design of the experiments, the acquisition, analysis and the interpretation of the data. GH and LC conceived the study and contributed to the interpretation of the data. JC and GH wrote the manuscript. All authors read and approved the manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work are appropriately investigated and resolved.

### Ethics approval and consent to participate

All experiments were performed according to the guidelines of the Ethics Committee of Jingling Hospital.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

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