COPB2 promotes metastasis and inhibits apoptosis of lung adenocarcinoma cells through functioning as a target of miR-216a-3p

Mingxue Wang, Renjie Yu, Xuefeng Ling, Wa Cao, Yunyun Liu, Lei Fang, Jianlin Tong

Background: Lung adenocarcinoma is a non-small cell lung cancer with a high mortality. There is little published data on the role of coatomer protein complex subunit β (COPB2) in lung adenocarcinoma. The current study aimed to explore the effects of COPB2 on lung adenocarcinoma cells.

Methods: The differential expression of COPB2 in normal cells and lung adenocarcinoma cells was detected by quantitative real time-polymerase chain reaction (qRT-PCR) and Western blotting. Then, cell viability assay, flow cytometry and Transwell experiments were performed to study the effects of COPB2 on cell growth, apoptosis, migration and invasion. MiRNA targeting COPB2 was predicted by TargetScan and validated by luciferase assay, qRT-PCR and Western blotting. The effects of miRNA inhibitor on siCOPB2 were analyzed by rescue experiments. Finally, apoptosis and metastatic marker proteins were detected by Western blotting.

Results: COPB2 was high-expressed in lung adenocarcinoma cells. Silencing COPB2 inhibited cell viability and cell metastasis, and significantly increased apoptosis. MiR-216a-3p was predicted to be able to target COPB2. Rescue experiment showed that miR-216a-3p inhibitor promoted cell viability, migration and invasion, and inhibited apoptosis of lung adenocarcinoma cells, partly reversing the effects of siCOPB2. Moreover, Western blotting showed that siCOPB2 up-regulated expressions of cleaved Caspase-3, Caspase-3, BCL2 associated X (Bax), and E-Cadherin, and down-regulated expressions of BCL2 apoptosis regulator (Bel-2), N-Cadherin, and Vimentin, and the above effects were also partly reversed by miR-216a-3p inhibitor.

Conclusions: High-expressed COPB2 promotes metastasis and inhibits apoptosis of lung adenocarcinoma cells through functioning as a target of miR-216a-3p.

Keywords: Lung adenocarcinoma cells; coatomer protein complex subunit β; miR-216a-3p; metastasis, apoptosis

Introduction

According to the statistics released by International Agency for Research on Cancer in 2018, lung cancer has the highest global incidence (11.6%) and mortality rate (18.4%) compared with other malignant tumors (1). Lung cancer is classified by non-small cell lung cancer and small cell lung cancer, and lung adenocarcinoma is the most common type of non-small cell lung cancer (2). Lung adenocarcinoma originates from bronchial mucosa epithelium and bronchial mucosa. Due to the difficulty of early diagnosis and rapid
spread of the cancer cells, lung adenocarcinoma has a high mortality (2). Therefore, it is of significance to study the potential markers of lung adenocarcinoma and to inhibit the proliferation and migration of cancer cells.

Seven subunits, including Coatomer protein complex subunit β (COPB2), are contained in coatomer protein complex I (COPI). The molecular weight of COPB2 is 102 kD, and encoded β'-COP contains 906 amino acid residues of nuclear protein (3). Research (4-6) proved that COPB2 is overexpressed in various cancer cells such as colon cancer, prostate cancer, cervical cancer, gastric cancer, and is closely related to the occurrence and development of tumors.

MicroRNAs (miRNAs) are a type of non-coding small RNAs with approximately 18-25 nucleotides in length and are encoded by endogenous genes that regulate mRNA degradation or block mRNA translation through binding to the 3’ untranslated region (UTR) of the target mRNA (7,8). miRNAs are involved in many biological processes such as cell differentiation, reproduction, metabolism, and apoptosis, and they are potential targets for tumor intervention treatment. Xu et al. (9) demonstrated that miR-502 promotes tumor overgrowth in esophageal cancer by promoting phosphorylation of serine/threonine kinase (AKT) pathway; Cagle et al. (10) showed that miR-214 can down-regulate protein tyrosine kinase 6 (PTK6) and inhibit the development of prostate cancer cells. Researchers also found that miR-203a-3p targeting ATM gene inhibits the proliferation of ovarian cancer cells and promotes apoptosis of the cancer cells (11).

There is little published data on COPB2 in lung cancer and lung adenocarcinoma. Our previous experiments found that COPB2 was overexpressed in lung adenocarcinoma cells. The current study investigated the mechanism of COPB2 in lung adenocarcinoma cells, aiming to provide a new basis for the treatment of lung adenocarcinoma.

**Methods**

**Cell culture**

Normal lung epithelial cells BEAS-2B (95102433-RNA-5UG, SIGMA, USA) and lung adenocarcinoma cell lines H1299 (CRL-5803, ATCC, USA), A549 (KG007, KeyGEN BioTECH, China), SK-MES-1 (KG153, KeyGEN BioTECH, China), NCI-H23 (KG153, KeyGEN BioTECH, China), NCi-H23 (CM-0397, Procell, China), H1975 (CX0373, Boster, China) were purchased from the Chinese Academy of Sciences Cell Bank Type Library (Shanghai, China). The cells were separately added into Roswell Park Memorial Institute-1640 (RPMI-1640, PM150110, Procell, China) medium containing 10% fetal calf serum (FBS), and then cultured in a cell incubator containing 5% CO₂ at 37 °C. The medium were pre-added with 100 U/mL penicillin and 100 μg/mL streptomycin.

**Small interfering RNA and cell transfection**

Plasmid for COPB2 gene (CAT#: SC117165), a small interfering RNA (siRNA) to COPB2, and a negative control siRNA (CAT#: SR30004) were purchased from OriGene (USA). siRNA sequences targeting COBP2 were designed according to one previous research (12). One day before transfection, the cells were trypsinized and seeded in antibiotic-free RPMI-1640 medium containing 10% FBS. When 50% confluence was reached, the cells were washed by PBS and placed in OPTI-MEM medium (31985062, Invitrogen, USA). Thirty pmol miR-216a-3p mimic or inhibitor and 1 μg DNA were separately diluted by 50 μL OPTI-MEM medium. According to the instructions of Lipofectamine 2000 Transfection Reagent (Cat. No. 11668-027, Invitrogen, USA), 1 μL Lipofectamine 2000 solution was diluted by 50 μL OPTI-MEM medium, and mixed together. The medium was changed after incubation at 37 °C for 5 hours (h). After 24 h, the transfection efficiency was measured by qRT-PCR.

**Total RNA extraction and quantitative real time-polymerase chain reaction (qRT-PCR)**

Total RNAs in each cell line were extracted by Trizol reagent. After detecting the concentration of RNA by a UV Spectrophotometer (NanoDrop2000, Thermo Scientific, USA), total RNAs were then synthesized using Revert Aid First Strand cDNA Synthesis Kit (Thermo Scientific, K1622, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as an internal reference. The primer sequences used in qRT-PCR were as follows: COPB2-F, 5’-CTTCCTGTGCTCAGCTGCAAAG-3’, and COPB2-R, 5’-CACCCTATCTGCTGCTACCCG-3’; GAPDH-R, 5’-GGCTGGATGTCATACATTTCTCATT-3’, and GAPDH-F, 5’-GGAGCGAGATCCCTCCAAAAT-3’. According to the manufacturer’s instructions, miR-216a-3p was detected using miRNA reverse transcription kit (QP013, GeneCopoeia, USA) and TaqMan® Universal PCR Master Mix (4304437, ABI, USA). U6 served as an internal control. The primer sequences were as follows: miR-216a-3p-F, 5’-GCTGGCAACTGTGAGTCGTA-3’, and miR-
216a-3p-R, 5'-CTCAGCCGTTAACGTGACCT-3'; U6-F, 5'-AAAGCAATCATCGGACGACC-3', and U6-R, 5'-GTACAACACATTGTTTCTCGGA-3'. Ten μL FastStart Universal SYBR Green Master (04913914001, Roche, Switzerland), 6 μL diethyl pyrocarbonate water (DEPC water, R1600, Solarbio, China), 2 μL diluted cDNA, and 1 μL of each diluted upstream and downstream primers were added to a 96-well plate and mixed together. The reaction system was conducted on a PCR machine (C1000 Thermal Cycler, BIO RAD, USA) under the following amplification conditions: pre-denaturation at 95℃ for 10 minutes (min), denaturation at 95℃ for 15 seconds (sec), annealing at 60℃ for 1 min, for a total of 40 cycles. The gene expression level was calculated by 2^ΔΔCT.

**Western blotting**

The cells were washed by PBS once, digested by 0.025% trypsin (P822466-100g, Macklin, China), and then rinsed in PBS and collected into a centrifugal tube. One hundred μL RIPA lysate buffer (P0013C, Beyotime, China) was added to the cells and blown evenly. The cells were centrifuged (1-15PK, Sigma, USA) at 4℃, 16,000 ×g, and placed in a centrifugal tube. The protein concentration was determined by bicinchoninic acid (BCA) method, 100 μg protein was separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane (MILLIPORE, IPVH00010, USA). The membrane was washed by the Tris Buffered Saline and Tween 20 (TBST) 3 times (50 mM Tris, 150 mM NaCl and 2% Tween-20, pH 7.5) at room temperature for 10 min. Cleaved Caspase-3 (ab2302, 1:1,000, abcam, UK), Caspase-3 (ab32351, 1:5,000, abcam, UK), BCL2 apoptosis regulator (Bcl-2, ab59348, 1:1,000, abcam, UK), BCL2 associated X (Bax, ab32503, 1:5,000 abcam, UK), E-Cadherin (ab40772, 1:10,000, abcam, UK), N-Cadherin (ab18203, 1:1,000, abcam, UK), Vimentin (ab92547, 1:2,000, abcam, UK), GAPDH (ab8245, 1:20,000, abcam, UK) antibodies and corresponding bands were incubated overnight at 4℃. On the next day, the PVDF membrane was washed 3 times by TBST at room temperature for 10 min and then incubated with corresponding secondary antibodies (Anti-mouse IgG, #7076, 1:2,000, Cell Signaling Technology; Anti-rabbit IgG, #7074, 1:2,000, Cell Signaling Technology) for 1 h at room temperature. The PVDF membrane was washed by TBST 3 times. The protein bands were detected by ECL (RPN2232, GE Amersham, USA) in Gel imager and imaging system (Universal Hood II, BIO RAD, USA). Finally, the images were analyzed using Image J software (1.48, National Institutes of Health, USA). GAPDH served as an endogenous control.

**Cell viability**

Cell suspension (100 μL) was placed in a 96-well plate and cultured in an incubator for 24 h at 37℃ in 5% CO₂. Each group of cells was then seeded into a culture plate at 5×10³ per well. After incubating for 0, 24, 48, and 72 h, 10 μL Cell Couting Kit-8 (CCK-8, YZ-CK04-500T, Solarbio, China) solution was added. After incubating for 1 h in the incubator, cell absorbance was measured at 450 nm by a microplate reader (SpectraMax i3x, Molecular Devices, USA). Finally, cell viability was determined using GraphPad Prism software version 6.0.

**Flow cytometry**

The effects of changes of COPB2 expression on apoptosis were examined by flow cytometry. Cells at the log phase were seeded in a medium at a density of 5×10⁵ cells per well, and incubated in a 6-well plate for 24 h. The cells were then washed in phosphate buffered saline (PBS), centrifuged at 1,000 r/min for 5 min. Five μL Annexin V-FITC (130-093-060, Miltenyi, Germany) was added to the cells, gently mixed and incubated for 15 min, the supernatant was discarded, and the cells were added with 10 μL propidium iodide (PI, JBS-LI-001, ENZO, USA). Finally, the apoptosis of cells were analyzed by flow cytometry (FCM, 322457, Bio-Rad, USA).

**Cell migration and invasion**

Cell migration and invasion were detected by Transwell assay. The cells were fasted for 24 h and then trypsinized in 0.1% FBS supplementation medium. Transwell chambers (8.0 μm pore size, 353097-1, FALCON, USA) were placed in the plates, and 10% FBS medium was added to the plates to provide chemoattractants. The upper and lower chambers were separated by a polycarbonate membrane (8.0 μm pore size, 353097-1, FALCON, USA). For detection of cell invasion, cells (1×10⁶ cells per well) were seeded onto the upper chamber pre-coated with Matrigel on the bottom. After 48 h, Matrigel in the Transwell chamber was removed using cotton swab. The cells on the basal side of the membrane were first fixed by

© Translational Cancer Research. All rights reserved.  Transl Cancer Res 2020;9(4):2648-2659 | http://dx.doi.org/10.21037/tcr.2020.02.65
4% paraformaldehyde for 10 min and then stained by 0.1% crystal violet stain (G1063-10, Solarbio, China) for 20 min at room temperature. Finally, the cells were observed under a fluorescent inverted microscope (IX71, OLYMPUS, Japan) and counted.

**TargetScan forecast**

TargetScan database (http://www.targetscan.org) was used to predict target genes. To explore the relationship between miR-216a-3p and COBP2 in lung adenocarcinoma cells was predicted by TargetScan database.

**Luciferase assay**

A luciferase reporter plasmid (AP-MN-P-50, Axygen, USA) containing the COPB2-3’-UTR sequence and the mutant COPB2-3’-UTR (COPB2-3’-UTR-mut) sequence were respectively constructed. MiR-216a-3p mimics (CMH1914, Cohesion Bio, UK) could enhance endogenous miR-216a-3p and was used in the following experiments. The cells were seeded at a density of 50% in a 12-well plate containing DMEM medium (C1199500BT, Invitrogen, USA). After incubation for 16 h, the COPB2-3’-UTR or COPB2-3’-UTR-mut plasmid was co-transfected with miR-216a-3p mimic or control mimic, respectively. The medium was changed 6 h after the transfection. After cell transfection for 24 h at 37°C, the luciferase assay was detected by dual luciferase kit (Invitrogen, 1168-019, USA). 5× lysate (RIPA) was mixed with sterile water at a ratio of 1:4. After washing the cells by PBS, the cells were lysed using the prepared lysate and then incubated for 15 min at room temperature. Twenty μL lysed cells were added with 100 μL LAR II reagent to detect the firefly fluorescence value by GloMax 2020 (YQ163114524, Promega, USA). Then, 100 μL Stop &Glo reagent was then added to the cells to detect Renilla fluorescence (Renilla was an internal reference). Finally, the ratio of firefly/Renilla was calculated.

**Statistical analysis**

Statistical analysis was performed by SPSS 20.0 (SPSS Inc., Chicago, IL, USA) and the data were expressed as the mean ± standard deviation (SD). Comparisons among groups were performed using ANOVA. Differences were determined by the Student two-tailed t-test between two groups. P<0.05 was considered to be statistically significant.

**Results**

**COPB2 was high-expressed in lung adenocarcinoma cells**

COPB2 expression was analyzed by qRT-PCR and Western blotting. The results showed that COPB2 was high-expressed in various lung adenocarcinoma cells (P<0.001, Figure 1A,B,C), and its expression was the highest in SK-MES-1 cells. Therefore, SK-MES-1 cells were used in the following experiments.

**siCOPB2 promoted tumor cell apoptosis and inhibited cell viability, migration and invasion**

SiCOPB2 cells were obtained by transfection technique, and then the transfection was examined by Western blotting and qRT-PCR. The results showed that the expression of COPB2 was significantly reduced in the siCOPB2 cells (P<0.001, Figure 2A,B,C). The results of CCK-8 showed that the cell viability of the siCOPB2 group was lower than that of the control group (P<0.001, Figure 2D). Flow cytometry results showed that apoptosis of the cells in siCOBP2 group was greatly increased, which was statistically different from the control group (P<0.001, Figure 2E,F). By comparing the cell migration and invasion ability, it was found that cell number in the siCOPB2 group in the visual field was significantly reduced, but lower than that of the control group (P<0.001, Figure 2G,H,I,J). The transfected siCOPB2 cells inhibited the expression of COPB2 gene in tumor cells. By detecting cell viability, migration, invasion and apoptosis, we found that siCOPB2 promoted apoptosis and inhibited cell migration and invasion. The above results indicate that COPB2 can promote the proliferation and growth of tumor cells and inhibit apoptosis.

**MiR-216a-3p could target COBP2**

TargetScan database predicted that the COPB2 gene has a miR-216a-3p binding site at its 3’UTR (Figure 3A), suggesting that COPB2 expression could be regulated by miR-216a-3p. The luciferase assay showed that the expression of luciferase in the experimental group was lower than that in the control group (P<0.001, Figure 3B). QRT-PCR was performed to detect the mRNA expression level of miR-216a-3p (Figure 3C). No significant difference was observed between siCOPB2 group and negative control group, indicating that COPB2 has no regulatory effect
COPB2 is high-expressed in tumor cells. (A) The expression of COPB2 was determined by quantitative real time-polymerase chain reaction (qRT-PCR). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as an internal reference (**, P<0.001 vs. BEAS-2B). (B,C) The expression of COPB2 was analyzed by Western blotting. GAPDH served as an internal reference (**, P<0.001 vs. BEAS-2B). All experiments were repeated in triplicate. COPB2, coatomer protein complex subunit β.

on miR-216a-3p. Furthermore, qRT-PCR and Western blotting were performed to verify whether miR-216a-3p regulated the expression of mRNA and protein levels of COPB2 genes. The results showed that the expression of COPB2 was up-regulated in miR-216a-3p inhibitor group (compared with the siCOPB2 group, **P<0.001, Figure 3D,E,F), suggesting that miR-216a-3p could down-regulated the expression of COPB2. The above experimental results indicated that miR-216a-3p could down-regulating the expression of COPB2 gene in tumor cells.

The effect of SiCOPB2 on promoting apoptosis of lung adenocarcinoma cells and inhibiting cell metastasis could be partially reversed by miR-216a-3p inhibitor

The results of CCK8 assay showed that the cell viability of miR-216a-3p inhibitor was significantly enhanced compared with the siCOPB2 group (**P<0.001, Figure 4A), and flow cytometry results showed that miR-216a-3p inhibitor reduced apoptosis of cancer cells induced by siCOPB2 (**P<0.001, Figure 4B,C). The results of cell migration and invasion experiments revealed that compared with the siCOPB2 group, the migration and invasion abilities of miR-216a-3p inhibitor group were improved, and the number of cells in the experiment was significantly increased (P<0.001, Figure 4D,E,F,G). The above results suggest that miR-216a-3p inhibitor can promote cell migration and invasion, inhibit the apoptosis induced by siCOPB2. The results further confirmed that miR-216a-3p can promote the apoptosis of lung adenocarcinoma cells and inhibit the proliferation and migration of cancer cells through down-regulating the expression of COPB2 gene in tumor cells.

Effects of COPB2 on apoptosis and migration of the cancer cells

As shown in Figure 5, silencing COBB2 significantly up-regulated the expressions of cleaved Caspase-3, Caspase-3, Bax and E-Cadherin protein levels (P<0.001), while the expressions of Bcl-2, N-Cadherin and Vimentin was inhibited (P<0.001, Figure 5A,B), indicating that silencing
Figure 2 SiCOPB2 promoted tumor cell apoptosis and inhibited cell reproduction, migration and invasion. (A,B) The transfection efficiency of sicopb2 cells was detected by the expression of COPB2 through Western blotting. GAPDH served as an internal reference (**, P<0.001 vs. control; ##, P<0.001 vs. negative control). (C) The transfection efficiency of siCOPB2 cells was detected by the expression of COPB2 through qRT-PCR. GAPDH served as an internal reference (**, P<0.001 vs. control; ##, P<0.001 vs. negative control). (D) Cell counting Kit-8 (CCK-8) assay was performed to detect cell viability (**, P<0.001 vs. control; ##, P<0.001 vs. negative control). (E,F) The apoptosis progression in each group was evaluated by flow cytometry (**, P<0.001 vs. control; ##, P<0.001 vs. negative control). (G,H) The migration ability of each group was detected by Transwell assay (**, P<0.001 vs. control; ##, P<0.001 vs. negative control). (I,J) The invasive ability of each group was observed by Transwell assay (**, P<0.001 vs. control; ##, P<0.001 vs. negative control). All experiments were conducted in triplicate.
Figure 3 MiR-216a-3p could target COBP2. (A) TargetScan predicted the targeting of COBP2 for miRNA. (B) Expression of miR-216a-3p was detected by luciferase assay (**, \( P<0.001 \) vs. control + COBP2-3’-UTR; ##, \( P<0.001 \) vs. miR-216a-3p + COBP2-3’-UTR-mut). (C) The expression of miR-216a-3p in each group was detected by qRT-PCR. U6 served as an internal reference (&&, \( P<0.001 \) vs. siCOPB2). (D,E) The expression of COBP2 in each group was detected by Western blotting. GAPDH served as an internal reference (^^, \( P<0.001 \) vs. control; ##, \( P<0.001 \) vs. negative control; ^^^, \( P<0.001 \) vs. siCOPB2; ^^, \( P<0.001 \) vs. siCOPB2 + inhibitor). (F) The expression of COBP2 in each group was detected by qRT-PCR. GAPDH served as an internal reference (^^, \( P<0.001 \) vs. control; &&, \( P<0.001 \) vs. negative control; ^^^, \( P<0.001 \) vs. siCOPB2; ^^^, \( P<0.001 \) vs. siCOPB2 inhibitor). All experiments were repeated in triplicate.
Figure 4 The effect of SiCOPB2 on promoting apoptosis of lung adenocarcinoma cells and inhibiting cell metastasis could be partially reversed by miR-216a-3p inhibitor. (A) Cell viability was detected by cell counting Kit-8 (CCK-8) at 0, 24, 48, and 72 h. (B,C) Flow cytometry detected the apoptosis of the cells in each group. (D,E) The migration ability of each group was detected by Transwell assay. (F,G) The invasive ability of each group was observed by Transwell assay. All experiments were repeated in triplicate. **, P<0.001 vs. control; ##, P<0.001 vs. negative control; &&, P<0.001 vs. siCOPB2; ^, P<0.05 vs. siCOPB2 + inhibitor; ^~, P<0.001 vs. siCOPB2 + inhibitor.
COPB2 promoted apoptosis and inhibited metastasis of cancer cells, however, the miR-216a-3p inhibitor reversed such effects. In cells transfected with siCOPB2 and miR-216a-3p inhibitor, cleaved Caspase-3, Caspase-3, Bcl-2, Bax, E-Cadherin, N-Cadherin and Vimentin did not change significantly. The results showed that miR-216a-3p inhibitor can partially reverse siCOPB2 to promote tumor cell apoptosis and inhibit migration.

**Discussion**

The current study revealed the targeted relationship between COPB2 and miR-216a-3p, and their important roles in the development of lung adenocarcinoma. COPB2 was high-expressed in lung adenocarcinoma cells, and after silencing COBB2, proliferation and invasion of lung adenocarcinoma cells were significantly inhibited, by contrast, the apoptosis of cancer cells was greatly increased. COPB2 (3,13) plays an important role in the process of transporting intracellular protein and endoplasmic reticulum Golgi bubble-mediated. Scientists found that down-regulating COPB2 expression can promote cancer cell apoptosis (14-16), which confirmed our experimental results. In addition, miR-216a-3p was predicted as target gene of COPB2, and its inhibitor partially reversed the intervention of siCOPB2 on lung adenocarcinoma cells. To the best of our knowledge, we were the first to prove that miR-216a-3p can down-regulate COPB2, thereby inhibiting the migration and promoting apoptosis of cancer cells.

More than 20,000 miRNAs have been discovered since its discovery in 2000 (17). MiRNAs account for about 1–3% of human genes and regulate transcription of at least half of human genes, moreover, they can not only regulate target genes at the transcriptional level, but also at translation levels. As endogenous coding RNAs, miRNAs can also regulate genes involved in development of tumor, and they enter tumor cells more easily than siRNAs (18-20). The synthesis of miRNAs can regulate cell homeostasis and control the expressions of cancer-related enzymes. MiRNAs in body fluids can promote cell-to-cell connections. Some
miRNAs with distinctive characteristics can be used for the diagnosis of different diseases. In addition, single-stranded miRNAs can interfere with multiple targets in a pathway. Studies confirmed that miRNAs and cancer cells are related to cell cycle, apoptosis and metastasis, suggesting a regulatory role in the development of cancer (21-23). The current study was the first to demonstrate that silencing COBP2 could inhibit metastasis and invasion of lung adenocarcinoma cells and promote apoptosis. Then, mir-216a-3p targeting COBP2 was verified, and found to have similar effects to siCOPB2, indicating that mir-216a-3p can replace siCOPB2 and directly down-regulate COBP2 expression in vivo. Moreover, mir-216a-3p avoids the physiological barrier that siCOPB2 encounters during its propagation in the body, preventing itself from entering the tumor cells (24,25).

Lung adenocarcinoma occurs in bronchioles and alveolar epithelial cells (26). Finding regulatory genes for lung adenocarcinoma, inhibiting cancer cell metastasis, and accelerating cancer cell apoptosis are the key to the treatment of the disease (27). E-Cadherin and N-Cadherin are important components of cadherin and an important structure for adhesion between cells (28,29). Studies showed that (30), E-Cadherin and N-Cadherin play important roles in tumor metastasis and invasion. The conversion of E-Cadherin to N-Cadherin is one of the important mechanisms of epithelial-mesenchymal transition (EMT) and a potential mechanism for tumor development and metastasis. Vimentin is an intermediate fibrin in mesenchymal cells and skeleton protein that maintains cell integrity (31), more importantly, it is involved in cell adhesion, migration and apoptosis, and is high-expressed in a variety of tumors (32). We confirmed by Western blotting that silencing COBP2 can significantly up-regulate E-Cadherin and inhibit the expressions of N-Cadherin and Vimentin, thereby inhibiting the migration and invasion of lung adenocarcinoma cells. The mir-216a-3p inhibitor can only inhibit the anti-metastatic effect of siCOPB2 by down-regulating E-Cadherin.

Through apoptosis, cells actively maintain cell life in order to maintain homeostasis, eliminate useless or cancerous cells (33), therefore, apoptosis of cancer cells is seen to be able to protect normal tissues and treat cancers through inhibiting cancer cell proliferation (34,35). Resveratrol (36), bleomycin (37), dexamethasone (38) and other chemotherapeutic drugs, which could induce apoptosis of cancer cells, are widely used to treat cancers, and Caspase-3, Bax and Bcl-2 are key proteins regulating apoptosis. Bax and Bcl-2 belong to the Bcl-2 family, but the difference is that Bcl-2 is an anti-apoptotic protein, while Bax is a pro-apoptotic protein (39). In addition to the Bcl-2 family, the Caspase family also plays a key role in the process of apoptosis. Caspase-3 is one of the important apoptosis-executing proteins (39). Caspase-3 is a downstream regulatory protein of Bax and Bcl-2 (39,40). Bcl-2 can prevent the release of cytochrome C, thereby blocking the activation of Caspase protease and ultimately inhibiting apoptosis. Bax help cytochrome C enter the mitochondria and further activate Caspase-3 to promote apoptosis. Our study showed that cleaved Caspase-3, Caspase-3 and Bax proteins were up-regulated and Bcl-2 expression was down-regulated in lung adenocarcinoma cells through silencing COBP2. However, the effects of mir-216a-3p inhibitor on siCOPB2 were reversed, pro-apoptotic protein expression was inhibited, and anti-apoptotic protein expression was up-regulated, moreover, these results were consistent with our previous observations on cell apoptosis of siCOPB2 cells.

Conclusions

In conclusion, the present study confirms that silencing COBP2 promotes apoptosis and inhibits cell metastasis of lung adenocarcinoma cells, and such effects can be enhanced by miR-216a-3p inhibitor. Moreover, the result demonstrates for the first time that the intervention mechanism of miR-216a-3p underlying lung adenocarcinoma is related to the up-regulation of cleaved Caspase-3, Caspase-3, Bax, E-Cadherin and inhibition of Bcl-2 expression. This study provides new intervention targets for miRNA treatment of lung adenocarcinoma.

Acknowledgments

Funding: None.

Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2020.02.65). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures
performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Open Access Statement: This is an Open Access article distributed in accordance with the Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License (CC BY-NC-ND 4.0), which permits the non-commercial replication and distribution of the article with the strict proviso that no changes or edits are made and the original work is properly cited (including links to both the formal publication through the relevant DOI and the license). See: https://creativecommons.org/licenses/by-nc-nd/4.0/.

References
