Coiled-coil domain-containing 68 promotes non-small cell lung cancer cell proliferation *in vitro*

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Abstract. Coiled-coil domain-containing 68 (CCDC68) is a novel secretory protein that acts as a tumor suppressor gene in several types of malignant tumors. However, the role of CCDC68 in the development of lung cancer has not been extensively studied. In the present study, to explore the biological functions of CCDC68 in NSCLC, we performed cell proliferation, viability and apoptosis assays on human lung cancer cell lines upon CCDC68 gene silencing with short hairpin RNA. The results demonstrated that following knockdown of CCDC68 expression, cell proliferation was decreased and the apoptotic rates were increased in A549 and H1299 cells. The role and mechanism of CCDC68 in malignant tumors, particularly in lung cancer, should be further explored, and CCDC68 may serve as a novel target for treatment of lung cancer.

Introduction

Worldwide, lung cancer is the most common cause of cancer-associated mortality, accounting for 11.6% of all new cancer cases and 18.4% of all cancer-associated mortalities in 2018 (1). The high mortality rate of this disease is attributable to the diagnosis of the majority of patients at an incurable stage (2). Non-small cell lung cancer (NSCLC) accounts for >80% of all diagnosed lung cancer cases (3). Platinum-based chemotherapy, one of the primary agents used for treatment of NSCLC for the last two decades, has only resulted in a modest improvement in overall survival (4). Despite the development of drugs that target the growth of tumors, the overall survival rate of patients with NSCLC has remained low (5). Thus, identifying novel prognostic factors may assist clinical decisions and patient prognosis.

Coiled-coil domain-containing (CCDC) proteins participate in a variety of regulatory functions associated with their highly versatile coiled-coil motif (6). A range of CCDC proteins have been demonstrated to be associated with the development and progression of various malignant tumors, such as papillary thyroid carcinoma, breast tumor, bladder cancer and esophageal squamous cell carcinoma (7-10). The tumor-suppressive role of CCDC68 in tumors was initially reported in colorectal adenocarcinoma (11), which was also subsequently demonstrated in pancreatic ductal adenocarcinoma (PDAC) (12). However, the role of CCDC68 in NSCLC has not been extensively studied. Thus, the present study aimed to assess the expression of CCDC68 in lung cancer cell lines and determine the effects of CCDC68 knockdown.

Materials and methods

Cell culture and treatment. All cell lines were purchased from The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences and cultured at 37°C with 5% CO_2 in a humidified incubator. A549 cells were maintained in DME/F12 medium (Sigma-Aldrich; Merck KGaA) supplemented with 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin (HyClone; Cytiva). NCI-H1299, NCI-H1975 and 95-D cells were all cultured in DMEM (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% FBS and 1% penicillin-streptomycin.

Tissue samples. A total of 15 paired NSCLC and normal lung tissues were collected after resection from Xi'an Chest Hospital between June 2018 to February 2019. The patients included 9 males and 6 females, with a mean age of 56 years (range, 38-71). The distance between normal lung tissue and lung cancer tissue was >3 cm. The tissues were frozen in -80°C. The patients who participated in this study did not undergo any treatment before surgery. Written informed consent was obtained from each participant, and the study was approved by the Ethics Committee of Xi'an Chest Hospital (no. 2019-S0014).

Lentiviral vector transfection. Short hairpin (sh)RNAs were constructed using a linearized GV115 lentiviral vector. *Escherichia coli* strain DH5α was used to amplify the lentiviral vector and auxiliary packaging vector plasmid (all purchased from Shanghai GeneChem Co., Ltd.). The plasmids

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were then transfected into 293T cells and cultured in DMEM medium containing 10% FBS. After 48-72 h in culture, the virus was harvested. The sequence of the CCDC68 shRNA and the shCtrl were 5'-GAAGCCCAGAATAAAGAACTA-3' and 5'-TTCTCCGAACGTGTCACGT-3', respectively. The shCtrl lentivirus was termed psc3741 and the CCDC-shRNA lentivirus was termed LVpGCSIL-004PSC50502-1. For lentiviral transfection, NSCLC cells were plated in 6-well plates ($1x10^5$ cell/well) and the shCtrl or the CCDC68-shRNA lentivirus was added according to the multiplicity of infection (MOI=5), using Lipofectamine® 2000 transfection reagent (cat. no. 11668019; Thermo Fisher Scientific, Inc.). Cultured cells were inspected at 72 h post-transfection with a fluorescence microscope (Olympus Corporation) at x100 magnification.

Tissue immunohistochemistry. Paraffin-embedded paired NSCLC and normal lung tissues were cut into 4-μm sections and dried at 60°C overnight. The sections were dewaxed in dimethylbenzene solution twice at room temperature for 10 min and rehydrated through graded ethanol to water, followed by antigen retrieval and penetration in 0.5% Triton X-100 in PBS at room temperature for 20 min. Endogenous peroxidase was blocked with 3% hydrogen peroxide at room temperature for 10 min, washed with PBS for 3 min three times, blocked at room temperature for 30 min with normal goat serum (Abcam) and incubated with the rabbit anti-CCDC68 primary antibody (1:400; cat. no. ab97815; Abcam) overnight at 4°C. Subsequently, Goat anti-rabbit IgG antibody (1:2,000; cat. no. 7074; CST Biological Reagents Co., Ltd.) was added, then the sections were incubated at room temperature for 1 h and washed with PBS. 3'3'-Diaminobenxidine (Beyotime Institute of Biotechnology) was used to develop the color for 3 min at room temperature. Slides were counterstained with hematoxylin for 5 min at room temperature, mounted after rinsing with water and observed using a light microscopy (Olympus Corporation).

RNA extraction and reverse transcription-quantitative (RT-qPCR). Total RNA of transfected A549 and NCI-H1299 cells was extracted using SuperfecTRI (Shanghai Pufei Biotechnology Co., Ltd.) according to the manufacturer's protocol. An ultraviolet spectrophotometer was used to measure the concentration of extracted RNA. A total of 2 μg RNA and 2 μl (0.5 μg/μl) reverse transcription primer (Bulge-Loop™ miRNA qPCR Primer Set; cat. no. MQP-0102; Guangzhou RiboBio Co., Ltd.) were reverse-transcribed using an M-MLV Reverse Transcriptase kit (cat. no. M1705; Promega Corporation) and dNTPs (cat. no. U1240; Promega Corporation) according to the manufacturer's protocol. SYBR® Master mix Real-Time PCR system (Takara Bio, Inc.) was used to perform qPCR in 12-μl reactions with the following reaction conditions: Pre-denaturation for 30 sec at 95°C; 40 cycles of 5 sec at 95°C and 30 sec at 60°C; and dissociation for 15 sec at 95°C, 30 sec at 60°C and 15 sec at 95°C. The sequences of the primers (Shanghai GeneChem Co., Ltd.) were as follows: CCDC68 forward, 5'-CCTTGT ATGAGTCTACGTCCGC-3' and reverse, 5'-ATCTGGGTC CTGATCTTTTGC-3'; GAPDH forward, 5'-TGACTTCAA CAGCGACACCCA-3' and reverse, 5'-CACCCTGTTGCT GTAGCCAAA-3'. GAPDH was used as the loading control.

The relative gene expression levels were measured and compared using the $2^{-\Delta\Delta Cq}$ method (13).

Cell proliferation assay. A549 and NCI-H1299 transfected cells were digested during the logarithmic growth phase using trypsin. The resuspended cells were seeded in a 96-well plate ($2x10^3$ cells/well) in 100 μl supplemented DME/F12 medium or DMEM and incubated overnight in 5% CO_2 incubator at 37°C. The number of cells was continuously counted using a Celigo Imaging Cytometer (Nexcelom Bioscience, Ltd.) for 5 days. To obtain the cell proliferation ratio curve, the cell count value of each group of cells at each time point was normalized to the first day of the respective cell group.

Cell viability assay. A549 and NCI-H1299 transfected cells were seeded into a 96-well culture plate at a density of 2,000 cells/well and allowed to grow to subconfluence. 3-(4,5)-dimethylthiahiazo(-z-y1)-3, 5-di-phenytetrazolium-romide (MTT; Genview PTY, Ltd.) incubation was performed according to the manufacturer's instructions. A total of 0.5 mg/ml MTT was added to each well and incubated for 4 h at 37°C, followed by the addition of 100 μl DMSO (Shanghai Shiyi Chemical Reagent Co., Ltd.). After 10 min of shaking, the optical density (OD) of the cells was measured at 490 nm using a microplate reader (Tecan Group, Ltd.). The mean count of three repetitions for 5 consecutive days in each group was used for analysis. The cell viability curve was obtained by normalizing the OD values of each time point to the first day of readings.

Apoptosis assay. A549 and NCI-H1299 transfected cells were passaged on day 3 post-transfection and harvested when confluence reached 70%. Cells were digested with trypsin and resuspended with DME/F12 medium or DMEM, centrifuged at 1,300 x g for 5 min at room temperature and washed twice in PBS. Cells were then washed in pre-cooled D-Hanks solution (pH, 7.2-7.4; Beijing Solarbio Science & Technology Co., Ltd.) at 4°C. The cell apoptosis kit (cat. no. V35113; Thermo Fisher Scientific, Inc.) was then used to perform the next steps, where 1X binding buffer was added to wash cells, following by centrifugation at 1,300 x g for 3 min at room temperature. Cells were then resuspended in 1X binding buffer and stained by adding 10 μl Annexin V-allophycocyanin (Thermo Fisher Scientific, Inc.) at room temperature in the dark for 15 min. Subsequently, 1X binding buffer was added based on the cell volume, and apoptosis was analyzed using a BD FACS Calibur flow cytometer (Accuri™ C6 Plus; BD Biosciences).

Western blotting. Transfected A549 and NCI-H1299 cells were lysed using a lysis buffer (100 mM Tris-HCl, pH 6.8; 100 mM DTT; 2% SDS; 10% glycerin; Beijing Solarbio Science & Technology Co., Ltd.), and the protein concentration was determined using a BCA Protein Assay kit (Beyotime Institute of Biotechnology). A total of 45 μg protein from the cell lysates were resolved on a 10% SDS gel using SDS-PAGE (Bio-Rad Laboratories, Inc.) and transferred to a PVDF membrane (EMD Millipore). The membrane was blocked in TBS + 0.05% Tween-20 solution containing 5% skimmed milk at 4°C overnight and incubated with the primary antibodies rabbit anti-CCDC68 (1:300;
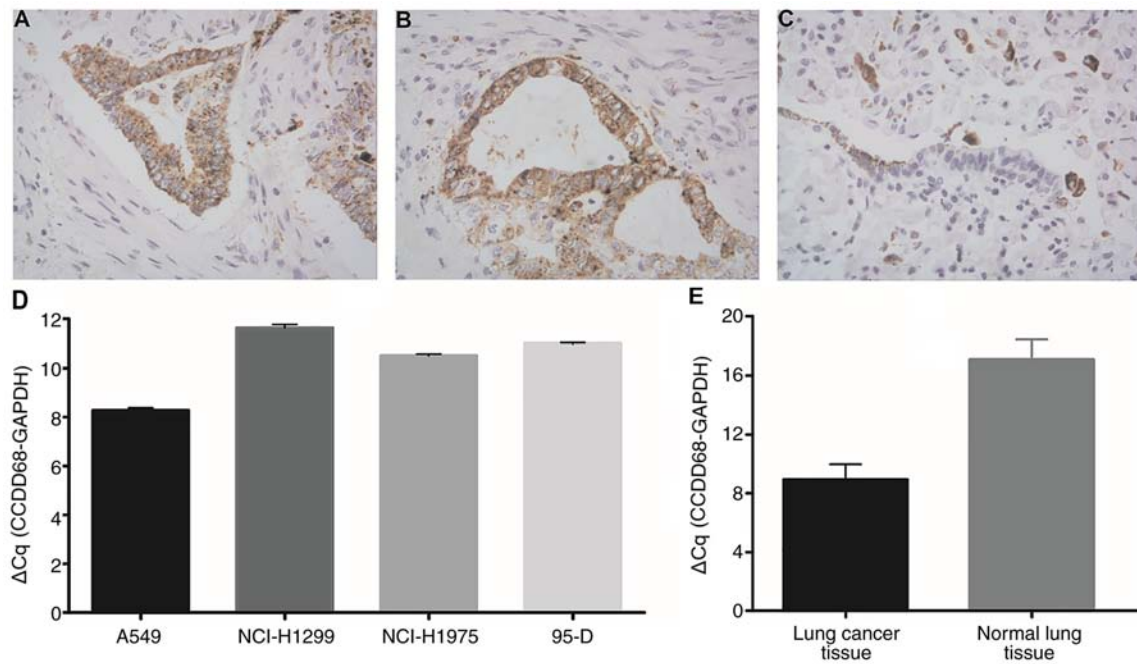
Figure 1. Expression of CCDC68 in lung cancer tissue and four non-small-cell lung cancer cell lines. (A and B) CCDC68 was strongly expressed in the cytoplasm in lung cancer tissues. (C) CCDC68 was weakly expressed in bronchial columnar epithelial and alveolar epithelial cells of normal lung tissues. (D) CCDC68 mRNA expression was high in four non-small cell lung cancer cell lines. (E) CCDC68 mRNA expression was high in lung cancer tissue. CCDC68, coiled-coil domain-containing 68.

cat. no. ab97815; Abcam) and mouse anti-GAPDH (1:2,000; cat. no. sc-32233; Santa Cruz Biotechnology, Inc.) primary antibodies overnight at 4°C. Subsequently, the membrane was incubated with goat anti-rabbit IgG (1:2,000; cat. no. 7074) or horse anti-mouse IgG (1:2,000; cat. no. 7076; both from CST Biological Reagents Co., Ltd.) secondary antibody for 1.5 h at room temperature. An ECL Western Blotting Substrate kit (Thermo Fisher Scientific, Inc.) was used to visualize the protein bands.

Statistical analysis. Data are presented as the mean ± standard deviation. Statistical analysis was performed using SPSS version 19.0 software (IBM Corp.). Differences between two groups were compared using a paired Student's t-test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

CCDC68 is highly expressed in NSCLC tissues and cell lines. Immunohistochemistry analysis of tissues from patients with NSCLC demonstrated that CCDC68 was strongly expressed in the cytoplasm in lung cancer tissues (Fig. 1A and B). In normal lung tissues, weak expression of CCDC68 was observed in bronchial columnar epithelial and alveolar epithelial cells (Fig. 1C). CCDC68 mRNA expression levels were determined in A549, H1299, H1975 and 95-D NSCLC cell lines, as well as in lung cancer and normal lung tissues using RT-qPCR. The results demonstrated that CCDC68 mRNA expression was high in all tested cell lines (Fig. 1D) and in lung cancer tissue (Fig. 1E). The four cell lines used are all human NSCLC cell lines. A549, NCI-H1975 and 95-D are derived from primary lung tissue, A549 is a commonly used and mature gene

knockout cell line. Unlike the other cell lines, NCI-H1299 cells are derived from a lymph node metastasis, and these cells partially lack p53 protein homogeneity (14). The simultaneous use of A549 and NCI-H1299 cell lines in the present study can increase the conviction of the experiments.

CCDC68 knockdown efficiency in non-small-cell lung cancer cell lines. The shCCDC68 lentivirus was transfected into A549 and H1299 cells to knock down the expression of CCDC68. Fluorescence microscopy was used to observe the transfection efficiency. The cells transfected with shCCDC68 and shCtrl were considered the experimental and the negative control group, respectively. The percentage of infected cells was >80%, and cell morphology was normal 3 days post-transfection in both groups (Fig. 2). To further verify the efficiency of CCDC68 knockdown, the protein and mRNA expression levels of CCDC68 were assessed in transfected cells. The results demonstrated that compared with that in the shCtrl group, CCDC68 expression was decreased in A549 and H1299 cells at the mRNA and protein level following transfection with shCCDC68 (Fig. 3)

Knockdown of CCDC68 reduces cell proliferation. The effects of CCDC68 on NSCLC cell proliferation was determined using a Celigo Imaging Cytometer and MTT assay. The results demonstrated that over 5 days, the proliferation of cells observed with a fluorescence microscope in the shCtrl group was higher compared with that of cells in the shCCDC68 group in A549 and H1299 cells (Fig. 4A and B), which was consistent with the results observed for the cell viability curve (Fig. 4C and E). The MTT viability assay demonstrated that the viability of A549 and H1299 cells in the shCCDC68 group was significantly lower compared
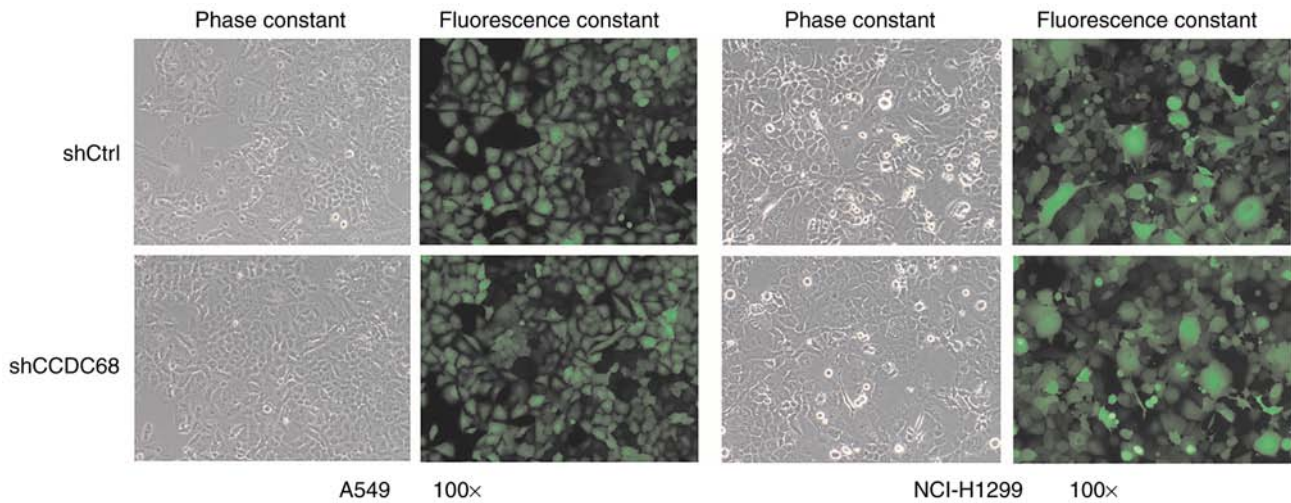
Figure 2. Observation of transfection using fluorescence microscopy. Magnification, x100. sh, short hairpin; CCDC68, coiled-coil domain-containing 68.
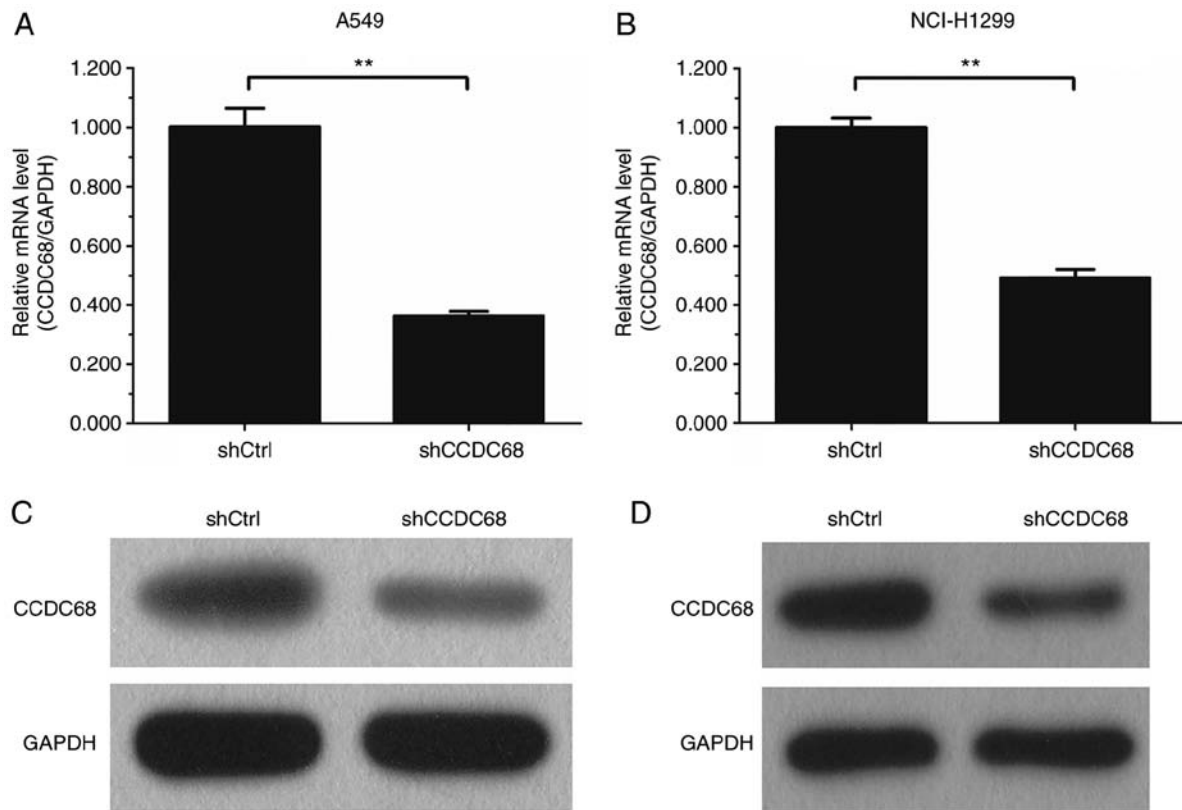

Figure 3. Expression of CCDC68 in shCCDC68-transfected A549 and H1299 cells. (A and B) Reverse transcription-quantitative PCR demonstrated that the mRNA expression levels of CCDC68 in (A) A549 and (B) H1299 cells were significantly lower in shCCDC68-transfected cells compared with those in shCtrl-transfected cells. (C and D) Western blots demonstrating the protein expression levels of CCDC68 in (C) A549 and (D) H1299 cells transfected with CCDC68 or shCtrl. **P<0.01. CCDC68, coiled-coil domain-containing 68; sh, short hairpin.

with that in the respective shCtrl groups (Fig. 4D and F). Therefore, CCDC68 may be associated with the proliferative capacity of NSCLC cells.

Effects of CCDC68 on apoptosis. Flow cytometry was used to analyze the effects of CCDC68 on apoptosis in NSCLC cells following CCDC68 knockdown. At 3 days post-shRNA lenti-viral infection, the proportion of apoptotic cells was higher in the CCDC68-knockdown A549 and H1299 cells compared

with that in the respective shCtrl groups (Fig. 5). These results demonstrated that CCDC68 knockdown induced apoptosis in NSCLC cells.

Discussion

During the past two decades, there has been a focus on iden-tifying potential biomarkers for the diagnosis and treatment of malignant tumors, such as DNA methylation (15), growth
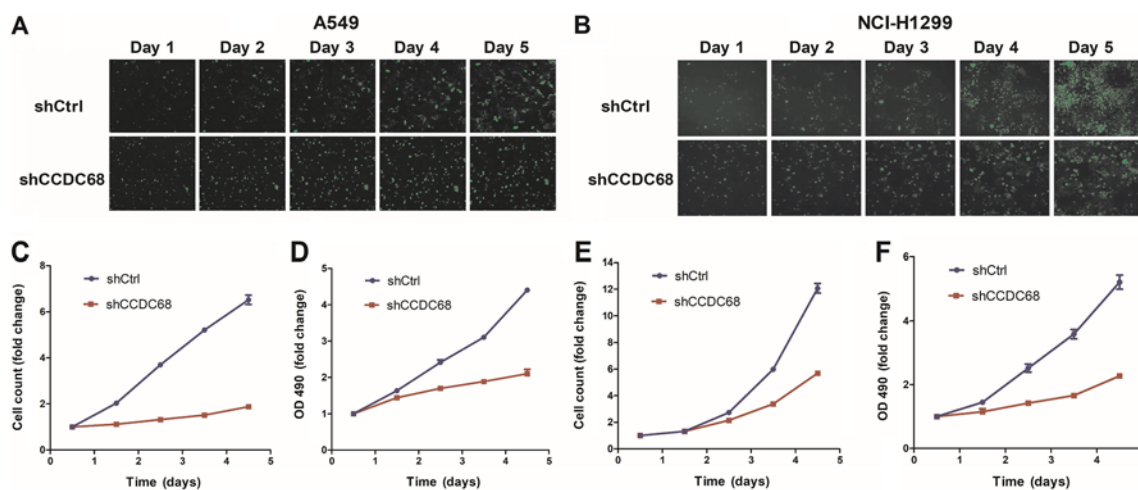
Figure 4. A549 and H1299 cell proliferative ability determined using Celigo and MTT assays. (A-F) The proliferation of (A and C) A549 and (B and E) H1299 cells was significantly reduced following knockdown of CCDC68 compared with the respective shCtrl groups. Proliferation of shCCDC68-transfected cells was lower compared with that in the shCtrl group as determined by MTT assay in (D) A549 and (F) H1299 cells. CCDC68, coiled-coil domain-containing 68; sh, short hairpin.

factors and protein kinases (16). Research on lung cancer is more prominent (17), and specific driver mutations, such as those in epidermic growth factor receptor, anaplastic lymphoma kinase and v-ros avian UR2 sarcoma virus oncogene homolog 1, can be used to identify patients that are sensitive to certain inhibitors, such as gefitinib, crizotinib, and others (18-20). Tyrosine kinase inhibitors (TKIs) have significantly improved overall response rates and progression-free survival compared with standard chemotherapeutic regimens for subgroups of patients carrying specific gene mutations; however, TKIs do not improve overall survival in all patients with NSCLC (21). Therefore, identifying novel candidate targets and exploring their underlying mechanisms may result in the identification of more suitable targets.

All proteins of the CCDC family contain coiled-coil structures and this motif exerts a series of biological functions, such as cell division, regulation of gene expression, membrane fusion and drug delivery or extrusion (22,23). Several studies have demonstrated that CCDC proteins are abnormally expressed in various types of tumors and are associated with tumor migration, invasion and metastasis (24,25); CCDC67 and CCDC8 may serve as novel potential therapeutic targets or biomarkers for the diagnosis and prognosis of specific malignant tumors (7,8), and CCDC34 can function as both, therapeutic targets and biomarkers (9,10). Previous studies have explored the role of CCDC proteins in lung cancer; for example, low levels of CCDC19 are associated with an unfavorable outcome in patients with lung squamous cell carcinoma (26) and with NSCLC pathogenesis (27). CCDC6 may represent a predictive biomarker of resistance to conventional single-mode therapy and yield insight on tumor sensitivity to poly(ADP-ribose) polymerase (PARP) inhibitors in NSCLC (28). CCDC106 is highly expressed in NSCLC tissues and cell lines, and its expression levels are associated with an advanced TNM stage, lymph node metastasis and poor overall survival in patients with NSCLC (29). As a member of the CCDC family proteins, CCDC68, also termed cutaneous T cell lymphoid antigen se57-1, has a molecular weight for

39 kDa and is located on chromosome 18q21.2 (12). Studies on CCDC68 in malignant tumors are rare, it has previously been demonstrated in colorectal adenocarcinoma and PDAC as a tumor-suppressive gene (11,12). However, the role of CCDC68 in lung cancer has not been extensively studied.

The present exploratory study found that CCDC68 was highly expressed in lung cancer tissues compared with normal lung tissues, which appeared to be inconsistent with the effects of CCDC68 in other malignant tumors. To clarify the role of CCDC68 in lung cancer, the present study preliminarily explored the biological functions of CCDC68 in NSCLC cell lines. The results of the *in vitro* experiments demonstrated that CCDC68 was expressed in NSCLC cell lines. The high expression levels of CCDC68 highlighted its potential involvement in the proliferation of NSCLC cells. RNAi technology is widely used for gene silencing to explore protein function, and for the treatment of malignant tumors in *in vitro* and *in vivo* models of various types of cancer (30,31). In the present study, the expression of CCDC68 was knocked down in A549 and H1299 cells, and the effects of CCDC68 knockdown on cell proliferation and apoptosis were assessed. The results demonstrated that CCDC68 knockdown reduced the proliferation and increased apoptosis in A549 and H1299 cells. These results suggested that CCDC68 increased tumorigenesis *in vitro* in NSCLC cells. This result contradicts previous studies, which suggested that CCDC68 may function as a tumor suppressor. For example, in patients with colorectal cancer, the CCDC68 copy number was decreased and its expression was downregulated compared with normal tissues (11). Additionally, ~60% of PDAC cases exhibited lost/reduced CCDC68 protein expression compared with normal pancreas specimens of healthy patients. CCDC68 was expressed significantly in normal duct epithelium and well-differentiated PDAC; thus, CCDC68 serves as a tumor suppressor in PDAC (12). Studies on the effects and mechanisms of CCDC68 in malignant tumors are relatively lacking, and these results should be further verified.
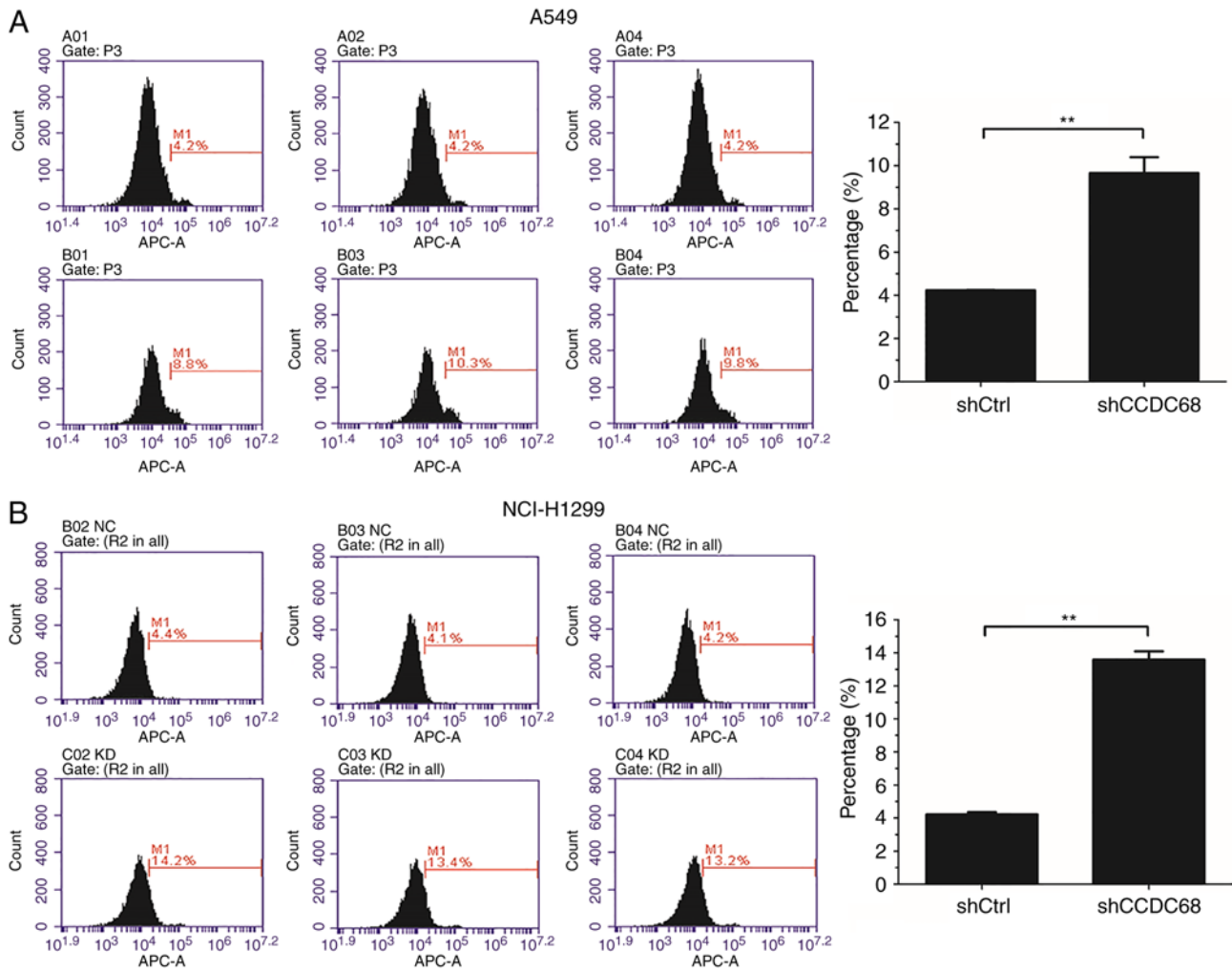
Figure 5. Effects of CCDC68 knockdown on apoptosis in A549 and H1299 cells. (A and B) The apoptotic rates of (A) A549 and (B) H1299 cells infected with the shCtrl and shCCDC68 lentiviruses were determined by flow cytometry. All experiments were performed independently at least three times. **P<0.01. CCDC68, coiled-coil domain-containing 68; sh, short hairpin.

CCDCs have been demonstrated to serve different roles in malignant tumors, and researchers have tried to explore their underlying mechanisms, including those in lung cancer. In lung cancer, CCDC106 enhances the expression of the cell cycle-regulating proteins cyclin A2 and cyclin B1 and promotes A549 and H1299 cell proliferation, which is dependent on the AKT signaling pathway (29). In breast and cervical cancer, protein kinase CK2-mediated CCDC106 phosphorylation is required for p53 degradation; the CK2/CCDC106/p53 signaling axis may be a novel therapeutic target (32). CCDC19 significantly reduces the proliferation and cell cycle progression of NSCLC cells *in vitro* via the PI3K/AKT/c-Jun signaling pathway (27), and serves as a potential tumor suppressor by modulating the cell cycle and the MAPK signaling pathway in nasopharyngeal carcinoma (33). CCDC6 acts as a novel biomarker for the clinical use of PARP1 inhibitors in malignant pleural mesothelioma (34) and high-grade urothelial bladder cancer (35) in addition to lung cancer (36). CCDC6 fuses with proto-oncogenes, which are associated with the occurrence and development of various types of malignant tumors, such as ovarian epithelial tumor, breast cancer, NSCLC and others (37). Therefore, CCDC6 may serve as a potential therapeutic target for patients with these

tumors (36). CCDC34 is upregulated in bladder cancer (9), esophageal squamous cell carcinoma (ESCC) (10) and cervical cancer (38) compared with adjacent normal tissues. Knockdown of CCDC34 expression significantly reduces the phosphorylation of the MAPK family proteins and the signal transduction factor AKT (9,39). Additionally, a positive association between CCDC34 with VEGF and MVD expression has been identified, highlighting the potential mechanism by which CCDC34 promotes tumorigenesis (10,38). Together, the present and previous studies suggest that the functions of various CCDCs in malignant tumors are based on the specific CCDC and the specific type of cancer, and the mechanisms of CCDCs in lung cancer appear to vary. CCDC68 is a novel centriole subdistal appendage (SDA) component required for hierarchical SDA assembly in human cells and is involved in centrosome microtubule anchorin. The functions of CCDC68 in microtubule anchoring are mainly mediated by CEP170, and loss of CCDC68 affects microtubule nucleation at the early stage of microtubule regrowth (40). The mechanism of CCDC68 in cancer has not yet been fully elucidated and since CCDC68 exhibits differing effects dependent on the type of cancer, the role and mechanism of CCDC68 in NSCLC requires further investigation.

In conclusion, the present study preliminarily determined the expression and explored the role of CCDC68 in lung cancer cell lines. The results demonstrated that CCDC68 was expressed in human NSCLC cell lines, and *in vitro* knockdown of CCDC68 expression reduced the proliferation and increased apoptosis in A549 and H1299 cells. Future studies should examine the expression of CCDC68 in lung cancer tissues and its effects in *in vivo* animal models of lung cancer. CCDC68 may serve as a potential candidate biomarker for detection of malignant transformation of lung cancer. Whether CCDC68 may also serve as a therapeutic target requires further study.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

JWL, TH and JD conceived and designed the study. JWL, JD and ZJL performed the experiments. YF and JLX analyzed data. JWL and TH wrote and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2018.
2. Balata H, Fong KM, Hendriks LE, Lam S, Ostroff JS, Peled N, Wu N and Aggarwal C: Prevention and early detection for NSCLC: Advances in Thoracic Oncology 2018. J Thorac Oncol 14: 1513-1527, 2019.
3. Rodriguez-Lara V, Hernandez-Martinez JM and Arrieta O: Influence of estrogen in non-small cell lung cancer and its clinical implications. J Thorac Dis 10: 482-497, 2018.
4. Schiller JH, Harrington D, Belani CP, Langer C, Sandler A, Krook J, Zhu J and Johnson DH; Eastern Cooperative Oncology Group: Comparison of four chemotherapy regimens for advanced non-small-cell lung cancer. N Engl J Med 346: 92-98, 2002.
5. Li HD and Liu SL: Molecular targeted therapy for non-small cell lung cancer: The reality in China and coping strategy. Prac J Med Pharm 35: 373-379, 2018.
6. Burkhard P and Stetefeld J: Coiled coils: A highly versatile protein folding motif. Trends Cell Biol 11: 82-88, 2001.
7. Yin DT, Xu J, Lei M, Li H, Wang Y, Liu Z, Zhou Y and Xing M: Characterization of the novel tumor-suppressor gene CCDC67 in papillary thyroid carcinoma. Oncotarget 7: 5830-5841, 2016.
8. Pangeni RP, Channathodiyil P, Huen DS, Eagles LW, Johal BK, Pasha D, Hadjistephanou N, Nevell O, Davies CL, Adewumi AI, et al: The GALNT9, BNC1 and CCDC8 genes are frequently epigenetically dysregulated in breast tumours that metastasise to the brain. Clin Epigenetics 7: 57, 2015.
9. Gong Y, Qiu W, Ning X, Yang X, Liu L, Wang Z, Lin J, Li X and Guo Y: CCDC34 is up-regulated in bladder cancer and regulates bladder cancer cell proliferation, apoptosis and migration. Oncotarget 6: 25856-25867, 2015.
10. Hu DD, Li PC, He YF, Jia W and Hu B: Overexpression of coiled-coil domain-containing protein 34 (CCDC34) and its correlation with angiogenesis in esophageal squamous cell carcinoma. Med Sci Monit 24: 698-705, 2018.
11. Sheffer M, Bacolod MD, Zuk O, Giardina SF, Pincas H, Barany F, Paty PB, Gerald WL, Notterman DA and Domany E: Association of survival and disease progression with chromosomal instability: A genomic exploration of colorectal cancer. Proc Natl Acad Sci USA 106: 7131-7136, 2009.
12. Radulovich N, Leung L, Ibrahimov E, Navab R, Sakashita S, Zhu CQ, Kaufman E, Lockwood WW, Thu KL, Fedyshyn Y, et al: Coiled-coil domain containing 68 (CCDC68) demonstrates a tumor-suppressive role in pancreatic ductal adenocarcinoma. Oncogene 34: 4238-4247, 2015.
13. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
14. Gazdar AF, Girard L, Lockwood WW, Lam WL and Minna JD: Lung cancer cell lines as tools for biomedical discovery and research. J Natl Cancer Inst 102: 1310-1321, 2010.
15. Pan Y, Liu G, Zhou F, Su B and Li Y: DNA methylation profiles in cancer diagnosis and therapeutics. Clin Exp Med 18: 1-14, 2018.
16. Suurmeijer AJH, Kao YC and Antonescu CR: New advances in the molecular classification of pediatric mesenchymal tumors. Genes Chromosomes Cancer 58: 100-110, 2019.
17. Kalemkerian GP, Narula N, Kennedy EB, Biermann WA, Donington J, Leighl NB, Lew M, Pantelas J, Ramalingam SS, Reck M, et al: Molecular testing guideline for the selection of patients with lung cancer for treatment with targeted tyrosine kinase inhibitors: American Society of Clinical Oncology endorsement of the College of American Pathologists/International Association for the Study of Lung Cancer/Association for Molecular Pathology clinical practice guideline update. J Clin Oncol 36: 911-919, 2018.
18. Roeper J and Griesinger F: Epidermal growth factor receptor tyrosine kinase inhibitors in advanced nonsmall cell lung cancer: What is the preferred first-line therapy? Curr Opin Oncol 31: 1-7, 2019.
19. Pirker R and Filipits M: From crizotinib to lorlatinib: Continuous improvement in precision treatment of ALK-positive non-small cell lung cancer. ESMO Open 4: e000548, 2019.
20. Liu S, Yang H, Jang Y, Zhang T, Yan R and Zhang J: Evolution strategy of ROS1 kinase inhibitors for use in cancer therapy. Future Med Chem 10: 1705-1720, 2018.
21. Rocco D, Della Gravara L and Battiloro C: The role of combination chemo-immunotherapy in advanced non-small cell lung cancer. Expert Rev Anticancer Ther 19: 561-568, 2019.
22. Mcfarlane AA, Orriss GL and Stetefeld J: The use of coiled-coil proteins in drug delivery systems. Eur J Pharmacol 625: 101-107, 2009.
23. Frezzo JA and Montclare JK: Exploring the potential of engineered coiled-coil protein microfibers in drug delivery. Ther Deliv 6: 643-646, 2015.
24. Zhong J, Zhao M, Luo Q, Ma Y, Liu J, Wang J, Yang M, Yuan X, Sang J and Huang C: CCDC134 is down-regulated in gastric cancer and its silencing promotes cell migration and invasion of GES-1 and AGS cells via the MAPK pathway. Mol Cell Biochem 372: 1-8, 2013.
25. Tsolakis AV, Grimelius L and Islam MS: Expression of the coiled coil domain containing protein 116 in the pancreatic islets and endocrine pancreatic tumors. Islets 4: 349-353, 2012.
26. Wang Y, Liu Z, Luo R and Xie Y: Decreased CCDC19 is correlated with unfavorable outcome in lung squamous cell carcinoma. Int J Clin Exp Pathol 11: 802-807, 2018.

27. Liu Z, Mai C, Yang H, Zhen Y, Yu X, Hua S, Wu Q, Jiang Q, Zhang Y, Song X and Fang W: Candidate tumour suppressor CCDC19 regulates miR-184 direct targeting of C-Myc thereby suppressing cell growth in non-small cell lung cancers. J Cell Mol Med 18: 1667-1679, 2014.

28. Cerrato A, Morra F, Di Domenico I and Celetti A: NSCLC mutated isoforms of CCDC6 affect the intracellular distribution of the wild type protein promoting cisplatinum resistance and PARP inhibitors sensitivity in lung cancer cells. Cancers (Basel) 12: 44, 2019.

29. Zhang X, Zheng Q, Wang C, Zhou H, Jiang G, Miao Y, Zhang Y, Liu Y, Li Q, Qiu X and Wang E: CCDC106 promotes non-small cell lung cancer cell proliferation. Oncotarget 8: 26662-26670, 2017.

30. Dana H, Chalbatani GM, Mahmoodzadeh H, Karimloo R, Rezaiean O, Moradzadeh A, Mehmandoost N, Moazzen F, Mazraeh A, Marmari V, *et al*: Molecular mechanisms and biological functions of siRNA. Int J Biomed Sci 13: 48-57, 2017.

31. Jain S, Pathak K and Vaidya A: Molecular therapy using siRNA: Recent trends and advances of multi target inhibition of cancer growth. Int J Biol Macromol 116: 880-892, 2018.

32. Ning Y, Wang C, Liu X, Du Y, Liu S, Liu K, Zhou J and Zhou C: CK2-mediated CCDC106 phosphorylation is required for p53 degradation in cancer progression. J Exp Clin Cancer Res 38: 131, 2019.

33. Liu Z, Li X, He X, Jiang Q, Xie S, Yu X, Zhen Y, Xiao G, Yao K and Fang W: Decreased expression of updated NESG1 in nasopharyngeal carcinoma: Its potential role and preliminarily functional mechanism. Int J Cancer 128: 2562-2571, 2011.

34. Morra F, Merolla F, D'Abbiero D, Ilardi G, Campione S, Monaco R, Guggino G, Ambrosio F, Staibano S, Cerrato A, *et al*: Analysis of CCDC6 as a novel biomarker for the clinical use of PARP1 inhibitors in malignant pleural mesothelioma. Lung Cancer 135: 56-65, 2019.

35. Morra F, Merolla F, Criscuolo D, Insabato L, Giannella R, Ilardi G, Cerrato A, Visconti R, Staibano S and Celetti A: CCDC6 and USP7 expression levels suggest novel treatment options in high-grade urothelial bladder cancer. J Exp Clin Cancer Res 8: 90, 2019.

36. Cerrato A, Visconti R and Celetti A: The rationale for drug-gability of CCDC6- tyrosine kinase fusions in lung cancer. Mol Cancer 17: 46, 2018.

37. Laxmi A Gupta P and Gupta J: CCDC6, a gene product in fusion with different protoncogenes, as a potential chemotherapeutic target. Cancer Biomark 24: 383-393, 2019.

38. Liu LB, Huang J, Zhong JP, Ye GL, Xue L, Zhou MH, Huang G and Li SJ: High expression of CCDC34 is associated with poor survival in cervical cancer patients. Med Sci Monit 24: 8383-8390, 2018.

39. Lei YY, Wang WJ, Mei JH and Wang CL: Mitogen-activated protein kinase signal transduction in solid tumors. Asian Pac J Cancer Prev 15: 8539-8548, 2014.

40. Huang N, Xia Y, Zhang D, Wang S, Bao Y, He R, Teng J and Chen J: Hierarchical assembly of centriole subdistal appendages via centrosome binding proteins CCDC120 and CCDC68. Nat Commun 8: 15057, 2017.