miR-23b suppresses lung carcinoma cell proliferation through CCNG1

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Abstract. Lung carcinoma with high incidence rate could be divided into four subtypes, including small cell carcinoma, squamous cell carcinoma, adenocarcinoma and large cell carcinoma. miR-23b has been reported to have a low expression and play major roles in abundant tumors, however there is little research in lung carcinoma and hence the purpose of this study was to explore the impact of miR-23b in lung carcinoma. The RNA level of miR-23b and cyclin G1 (CCNG1) was measured by reverse transcription quantitative PCR. Luciferase activity reporter assay was used to verify that CCNG1 is a target of miR-23b. MTT and Transwell assays were utilized to test the functional studies of miR-23b in lung cancer cells. In lung carcinoma and lung cancer cells miR-23b expression is low compared with that in paracancerous tissues and normal lung cells. Low miR-23b expression inhibited lung cancer cell proliferation measured by MTT assay. We applied luciferase reporter to determine whether CCNG1 is a target of miR-23b and there was a negative correlation between them. Moreover, interference with CCNG1 reduced the cell proliferation ability, which partially reversed function of miR-23b. miR-23b inhibited cell proliferation of lung cancer by directly targeting CCNG1. It is suggested that miR-23b/CCNG1 axis may present a new target for the treatment of lung cancer.

Introduction

Lung carcinoma has the highest incidence and mortality rates worldwide (1,2). Lung carcinoma could be divided into several subtypes, including small cell carcinoma, squamous cell carcinoma, adenocarcinoma and large cell carcinoma

and the last three types are collectively called non-small cell lung cancer (NSCLC), accounting for 80-85% of all the lung carcinomas (3). There are several incidence factors in the pathogenesis of lung carcinoma, including air pollution, smoking, occupation and eating habits (4,5). Therefore, to clarify the molecular mechanisms of the tumorigenesis and progression of lung carcinoma is necessary.

MicroRNAs (miRNAs) are a cohort of small non-coding RNAs, which are ~22-28 oligonucleotides in length (6). miRNAs affect the cell process regulation by binding to the 3'UTR of the mRNA of their target gene at post-transcriptional level (7-14). miR-23b, has been reported to have low expression and be involved in multiple tumors, such as ovarian, bladder hepatocellular and prostate cancers (15-18). In hepatocellular cancer, miR-23b downregulates the expression of urokinase, c-Met and suppresses the migration and epithelial-mesenchymal transition (EMT) (15,19). Similar results have been reported in experimental autoimmune encephalomyelitis, i.e., that miR-23b suppresses leukocyte migration and pathogenesis (20). In prostate cancer miR-23b represses proto-oncogene Src kinase and is associated with cancer diagnosis and prognosis (16). However, the specific function and regulatory mechanism of miR-202 in lung carcinoma progression has not been reported.

Cyclin G1 (CCNG1), acts as a target of p53, is a member of G-type cyclins located at chromosome 5q-32-q34, which has six exons and constitutes of 259 amino acids (21,22). CCNG1 acts as a cell cycle regulator in human tumor cells such as cervical carcinoma, hepatocellular carcinoma, breast cancer and lung carcinoma (21,23-26). In hepatocellular carcinoma, Wen *et al* revealed that CCNG1 may act as a promising biomarker and contribute to the recurrence and chemoresistance (24). Morever, some miRNAs could interact with CCNG1 to affect tumor progression. miR-1271 and miR-23b through targeting CCNG1 inhibit ovarian cancer growth and progression (27,28).

In this study, we mainly investigated the correlation of miR-23b and CCNG1 and the impact on lung carcinoma. We measured the mRNA expression level of miR-23b and CCNG1 in lung carcinoma tissues and lung cancer cells, using paracancerous tissues and normal lung cell as internal reference, respectively. We also explored the effects of changing miR-23b expression on the cell proliferation ability of lung cancer

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cells A549 and NCI-H460. Furthermore, we investigated the relationship between miR-23b or CCNG1 expression and the survival of patients. The role of miR-23b and CCNG1 in lung carcinoma was explored.

Materials and methods

Tissue specimens. According to WHO classification, 57 samples from patients with lung carcinoma and paracancerous tissues were collected from People's Hospital of Yan'an City (Yan'an, China) from 2014 to 2016. All the specimens were frozen immediately after surgery and stored at -80°C before RNA extraction and other tests. No patient had received any therapy, including radiotherapy or chemotherapy, before surgery. The complete clinicopathological features of the patients with lung carcinoma (5 small cell carcinoma, 24 squamous cell carcinoma, 19 adenocarcinoma and 9 large cell carcinoma) are described in Table I. Of this cohort, 28 patients were diagnosed at advanced stage (stages III/IV) and all of these patients did not suffer from distant metastasis at initial diagnosis. Written informed consent was obtained from all patients and the study was approved by the Ethics Committee of People's Hospital of Yan'an City (Yan'an, China).

Cell lines and culture conditions. Two human lung cancer cell lines (A549 and NCI-H460) and normal lung cells (MRC-5) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). All the cells were maintained in RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) cultured at 37°C in an atmosphere with 5% CO_2.

RNA isolation and reverse transcription quantitative PCR (RT-qPCR). Total RNA or miRNA was isolated and extracted using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) or miRcute Extraction and Separation of miRNAs kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's instructions. To detect the expression of miRNA or mRNA, cDNA was reverse-transcribed using PrimeScript™ II 1st strand cDNA synthesis kit (Takara Biotechnology Co., Ltd., Dalian, China). Then RT-qPCR was performed using the SYBR Premix kit or SYBR PrimeScript miRNA RT-PCR kit (both from Takara Biotechnology Co., Ltd.). The thermocycling conditions were 95°C for 3 min and 40 cycles of 95°C for 15 sec followed by 60°C for 30 sec. Normalization was carried out using glyceraldehyde-3-phosphate or dehydrogenase (GAPDH) and U6 small nuclear RNA (U6). The relative expression levels of miRNA and mRNA were calculated using $2^{-\Delta\Delta Cq}$ method (29). All experiments were repeated at least 3 times. The primers were as follows: miR-23b forward, 5'-GGTGCTCTGGCTGCTTGG-3' and reverse, 5'-GCCAAGGTCGTGGTTGCG-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCT TCACGAATTTGCGT-3'; CCNG1 forward, 5'-GTTACCGCT GAGGAGCTGCAGTC-3' and reverse, 5'-GCAGCCATCCT GGATGGATTCAG-3'; GAPDH forward, 5'-GGTGAAGGT CGGAGTCAACG-3' and reverse, 5'-CAAAGTTGTCATGG ATGHACC-3'.

Table I. miR-23b expression and clinicopathological features in 57 paired lung carcinoma tissues.

Clinicopathological features	Cases (n=57)	miR-23b expression		P-value
		26 High (%)	31 Low (%)	
Sex				
Male	32	12 (37.5)	20 (62.5)	0.164
Female	25	14 (56.0)	11 (44.0)	
Age (years)				
≤60	31	15 (48.4)	16 (51.6)	0.646
>60	26	11 (42.3)	15 (57.7)	
Tumor size (mm)				
≤5.0	35	20 (57.1)	15 (42.9)	0.028[a]
>5.0	22	6 (27.3)	16 (72.7)	
TNM stage				
I-II	29	17 (58.6)	12 (41.4)	0.045[a]
III-IV	28	9 (32.1)	19 (67.9)	
Local invasion				
T1-T2	24	14 (58.3)	10 (41.7)	0.100
T3-T4	33	12 (36.4)	21 (63.6)	
Lymph node metastasis				
0-2	30	18 (60.0)	12 (40.0)	0.022[a]
>2	27	8 (29.6)	19 (70.4)	
Ki-67				
<14%	17	12 (70.6)	5 (29.4)	0.030[a]
≥14%	40	14 (35.0)	26 (65.0)	
CCNG1				
Negative	26	17 (65.4)	9 (34.6)	0.018[a]
Positive	31	9 (29.0)	22 (71.0)	

P-values were calculated with Chi-square test. [a]P<0.05.

Transfection. miR-23b mimic, miR-23b inhibitor and their negative control (NC) were purchased from Guangzhou RiboBio Co., Ltd. (Guangzhou, China). To assess the efficiency of miR-23b on cell proliferation, we transfected miR-23b mimic or inhibitor into lung cancer A549 and NCI-H460 cells, in order to overexpress or knock down miR-23b expression, and normal control (NC) was included. To detect the effect of miR-23b through CCNG1 for cell proliferation, we used siRNA to interfere with the expression of CCNG1.

We seeded the lung cancer cells A549 and NCI-H460 into 6-well plates to cultivate overnight before transfection. The plasmid vectors were transfected using Lipofectamine 3000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions.

Cell proliferation assay. Before the experiments, we seeded the lung cells into 96-well plates with a density of $5x10^3$ cells/well. 3-(4,5-Dimethyl-2-thia-zolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) assay was used to test the cell proliferative activity. After cultured for 24, 48, 72 or 96 h, 10 μl of MTT solution (5 mg/ml) were added in each well. After cell incubattion with
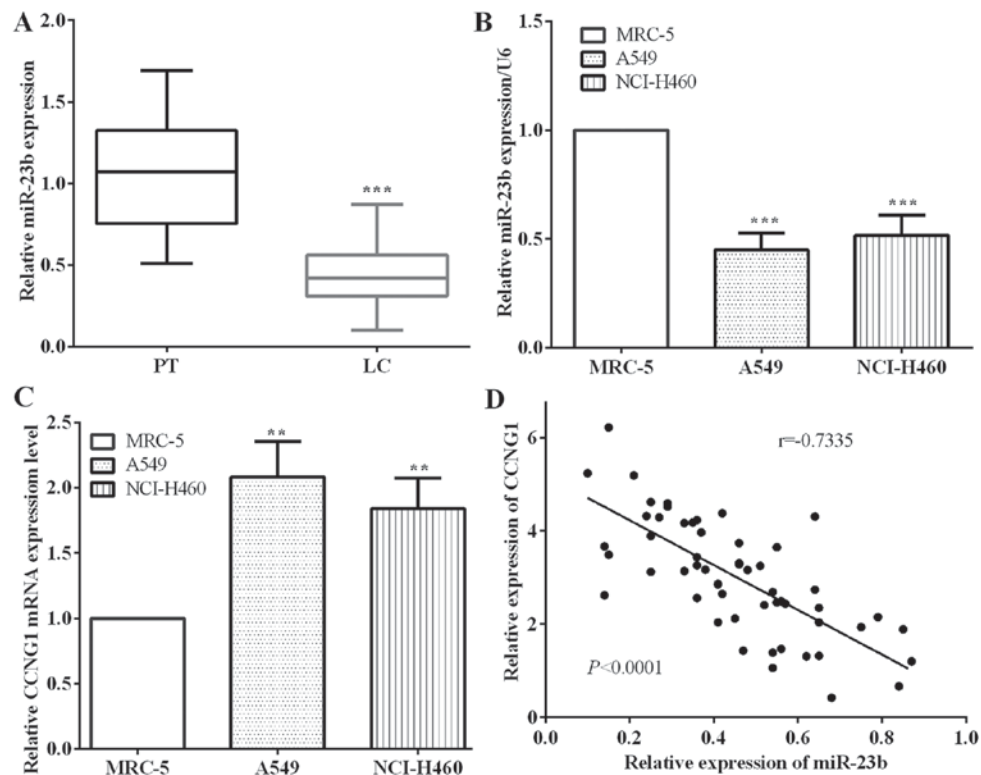
Figure 1. miR-23b was expressed at low level and had negative correlation with CCNG1 in lung cancer. (A) miR-23b expression in lung cancer tissues was significantly lower than paracancerous tissues. (B) The expression of miR-23b in lung cancer cells A549 and NCI-H460 was lower compared with normal lung MRC-5 cells. (C) The mRNA level of CCNG1 in lung cancer cells was higher than normal lung cells. (D) There was a negative correlation between miR-23b and CCNG1. PT, paracancerous tissues; LC, lung carcinoma. **P<0.01; ***P<0.001.

the MTT reagent at 37°C for 4 h, we added 150 μl dimethyl sulphoxide (DMSO), and measured the absorbance at 490 nm on a microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). All experiments were repeated at least 3 times.

Transwell assay. The cell invasion assay was performed using Transwell inserts (Millipore, Boston, MA, USA) coated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) on the upper surface. Following the procedures described in the manufacturer's instructions, 200 μl serum-free medium cell suspension containing 5×10^4 cells were added to the upper chamber of the insert. Next, 500 μl RPMI-1640 medium with 15% FBS were added into the lower compartment as a chemoattractant. After incubation at 37°C for 24 h, the cells on the upper surface of the membrane were carefully removed using cotton swab and cells on the lower surface were fixed at room temperature for 30 min with 100% methanol and stained for 20 min at room temperature with 0.1% crystal violet. Five visual fields of x200 magnification of each insert were randomly selected and counted under a light microscope (Olympus Corp., Tokyo, Japan). Each experiment was performed in triplicate.

Protein extraction and western blotting. Total proteins were extracted from lung cancer cells using RIPA lysis buffer, supplemented with PMSF (both from Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). After centrifuged for 20 min at 4°C with 12,000 x g speed, the concentration of protein was measured by BCA reagent kit (Beijing Solarbio

Science & Technology Co., Ltd.). Following electrophoresis using 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), the separated proteins were transferred to the polyvinylidene fluoride membrane (PVDF; Millipore). The concentration of the concentrated gel was 5%, and the concentration of the separated gel was 10%. The membrane was incubated with mouse anti-CCNG1 monoclonal antibody (1:1,000; cat. no. WH0000900M1; Sigma-Aldrich; Merck KGaA) at 4°C overnight, with GAPDH mouse antibody (1:3,000; cat. no. TA802519, ZSGB-BIO; OriGene Technologies, Inc., Beijing, China) as internal control. A mouse secondary antibody (1:4,000, cat. no. sc-516142; Santa Cruz Biotechnology, Inc.) containing conjugated horseradish peroxidase was used to incubate the membrane for 1 h at room temperature. The proteins (CCNG1 and GAPDH) were evaluated by ECL western blotting detection system (BestBio, Shanghai, China).

Plasmid construction and luciferase reporter assay. TargetScan (www.targetscan.org) software predicted that CCNG1 was a potential target gene of miR-23b with binding site located at 3'UTR of CCNG1. Therefore, we used double luciferase reporter assay to verify whether miR-23b binds to the 3'UTR of CCNG1 mRNA. First, we constructed the plasmid with CCNG1 3'UTR oligonucleotide fragment inserted in (pcDNA3.1-CCNG1-WT). Then, we mutated miR-23b binding sequences at CCNG1 3'UTR (pcDNA3.1-CCNG1-MUT) from 5'-…AAUGUGA…-3' to 5'-…UUACACU…-3'. Effectiveness of constructs was verified by sequencing.
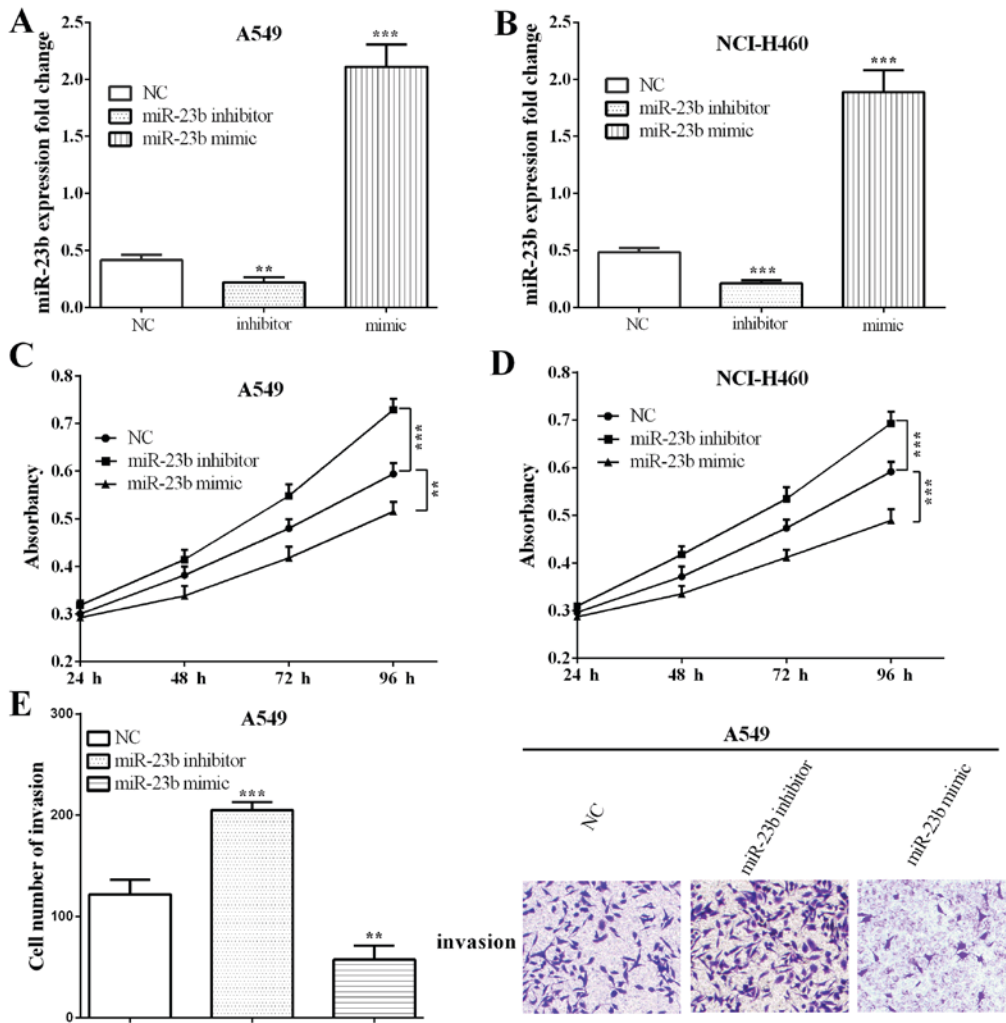
Figure 2. miR-23b inhibits proliferation and invasion of lung carcinoma cells. (A and B) The efficacy of overexpressed or knocked downexpression of miR-23b through transfected miR-23b mimic or inhibitor detected by RT-qPCR in lung cancer cells A549 and NCI-H460. (C and D) The proliferation abilities were changed when miR-23b was overexpressed or knocked down in A549 and NCI-H460 cells as measured by MTT assay. (E) Transwell assay shows a significant increase in the number of invading A549 and NCI-H460 cells transfected with miR-23b inhibitor, while decrease when transfected with miR-23b mimic. NC, negative control. **P<0.01; ***P<0.001.

miR-23b mimic or NC and pcDNA3.1-CCNG1-WT or MUT were co-transfected into lung cancer A549 and NCI-H460 cells to detect the luciferase reporter activity. Luciferase assay was detected using Dual-Luciferase® Reporter Assay System (Promega Corp., Madison, WI, USA) with Renilla luciferase as normalization. Each experiment was performed in triplicate.

Statistical analysis. Experimental data are presented as mean ± standard deviation (SD). Statistical analyses were performed using the SPSS 19.0 software (IBM Corp., Armonk, NY, USA). The differences between groups were determined using Student's t-test and one-way ANOVA followed by a Tukey's post hoc test. Pearson's χ^2 test was used to analyze the relationship between miR-23b expression and the clinico-pathological characteristics of lung cancer patients. Pearson's correlation was performed to analyze the correlation between the expression levels of miR-23b and CCNG1. Kaplan-Meier method with log rank test were used to calculate the 5-year overall survival (OS) and disease-free survival (DFS). P<0.05 indicates a statistically significant difference.

Results

miR-23b expression is significantly low and has negative correlation with CCNG1 in lung carcinoma. The expression of miR-23b and CCNG1 was examined by RT-qPCR in cancerous and paracancerous tissues of 57 cases with lung cancer. The results showed that the expression of miR-23b in paracancerous tissues was obviously higher than that in cancerous tissues (P<0.0001) (Fig. 1A). There was no significant difference observed between the four lung carcinoma subtypes. Moreover, we detected the expression of lung cancer A549 and NCI-H460 cells and normal lung MRC-5 cells, and found that the expression of miR-23b was significantly lower in A549 (P=0.0002) and NCI-H460 (P=0.0008) cells compared with MRC-5 cells, which are similar results with those of tissues samples (Fig. 1B).

Contrary to the results of miR-23b, the expression of CCNG1 in lung cancer A549 (P=0.0024) and NCI-H460 cells (P=0.0035) was significantly higher than that in normal lung cancer MRC-5 cells (Fig. 1C). Furthermore, the expression of miR-23b had negative correlation with CCNG1 in lung
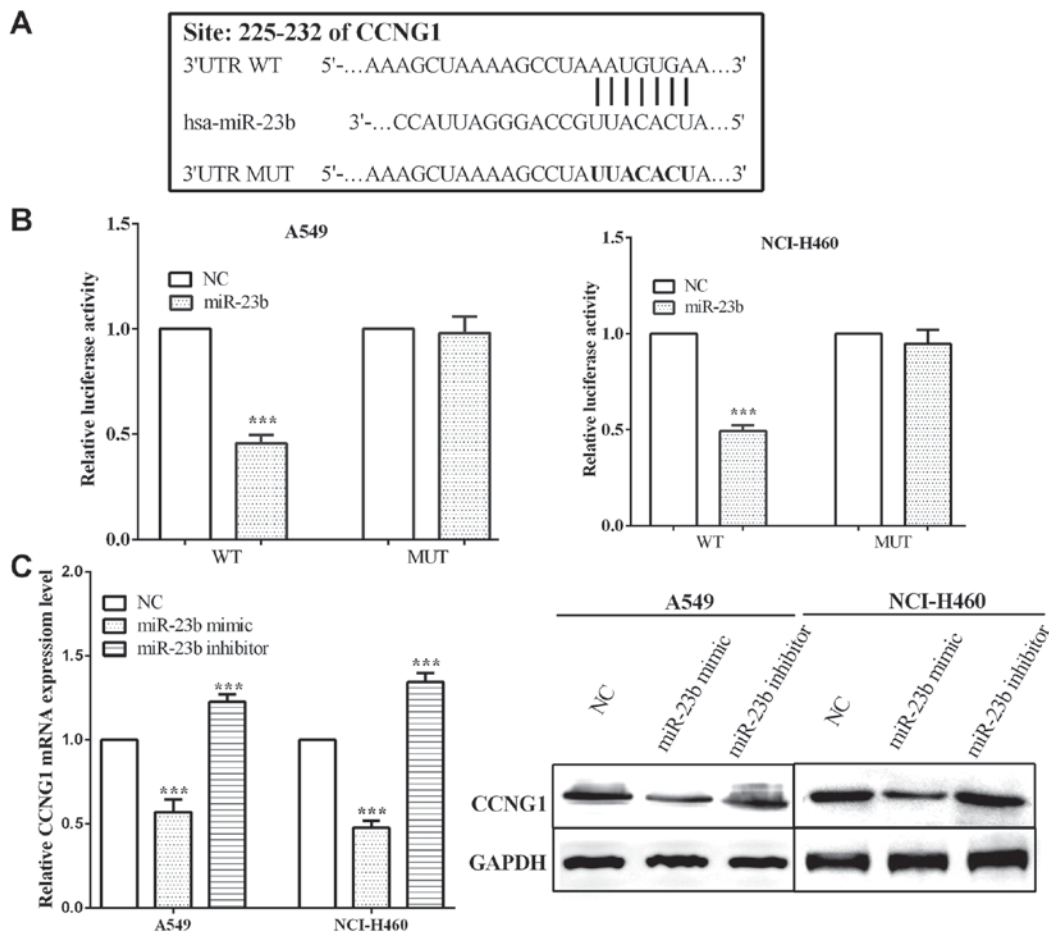
Figure 3. CCNG1 is a target of miR-23b and mediated by miR-23b. (A) The binding site of CCNG1 for miR-23b was located at 3'UTR and the mutated nucleotides are indicated. (B) Changes of luciferase activity when cells were co-transfected with miR-23b or negative control, and CCNG1 3'UTR WT or MUT are shown. (C) The mRNA and protein levels of CCNG1 when tansfected with miR-185 mimic or inhibitor in A549 and NCI-H460 cells are shown. WT, wild-type of CCNG1 mRNA 3'UTR; MUT, mutant of CCNG1 mRNA 3'UTR; NC, negative control. ***P<0.001.

carcinoma tissues as detected by Pearson's correlation test (P<0.0001, r=-0.7335) (Fig. 1D).

miR-23b inhibits proliferation and invasion of lung carcinoma cells. To study the biological function of miR-23b on proliferation and invasion, we overexpressed or knocked down miR-23b using miR-23b inhibitor or mimic in lung cancer cells A549 (P=0.0056 and 0.0001) and NCI-H460 (P=0.0006 and 0.0002), and the transfection efficiency was detected by RT-qPCR (Fig. 2A and B).

MTT assay was applied to detect the proliferation after spreading cells for 24, 48, 72 and 96 h. As shown in Fig. 2C, when transfected with miR-23b inhibitor in A549 cells, the ability of cell proliferation was increased significantly (P=0.2039, 0.0553, 0.0046 and 0.0002) at 72 and 96 h. On the other hand, cell proliferation ability was reduced when transfected with miR-23b mimic (P=0.5876, 0.0207, 0.0069 and 0.0021). Results were similar in NCI-H460 cells, with P-values 0.0319, 0.0156, 0.0064, 0.0007 and 0.0801, 0.0348, 0.0020 and 0.0006, respectively, when transfected with miR-23b inhibitor or mimic (Fig. 2D).

To further explore the function of miR-23b in lung carcinoma, Transwell assay was performed to detect the invasive ability in altering miR-23b in A549 cells. Similar with prolif-

eration, Transwell assays showed that the invasive ability was significantly increased (P=0.0010) in miR-202 inhibitor-transfected A549 cells, whereas the opposite results were observed (P=0.0051) in miR-23b overexpressed cells (Fig. 2E).

CCNG1 is a target of miR-23b and mediated by miR-23b. TargetScan (http://www.targetscan.org/vert_71/), online software, was utilized to predict potential target genes, and we found that CCNG1 was a potential target gene of miR-23b. The potential binding site of CCNG1 for miR-23b is located at 225-232 bp of its 3'UTR mRNA (Fig. 3A). Luciferase reporter assay was performed in order to test whether miR-23b interacted with CCNG1. Firstly, we co-transfected two plasmid vectors, respectively, containing CCNG1 3'UTR and miR-23b, and then detected the change of luciferase activity. The binding sequences of CCNG1 3'UTR were mutated from 5'-…AAUGUGA…-3' to 5'-…UUACACU…-3', and the luciferase activity was measured. We found that when co-transfected with miR-23b and CCNG1 wild-type 3'UTR, the luciferase activity was reduced significantly both in A549 (P<0.0001) and NCI-H460 (P<0.0001) cell lines. In contrast, when co-transfected with miR-23b and CCNG1 mutant type 3'UTR, luciferase activity had almost no change in both A549 (P=0.680) and NCI-H460 (P=0.278) cells (Fig. 3B).
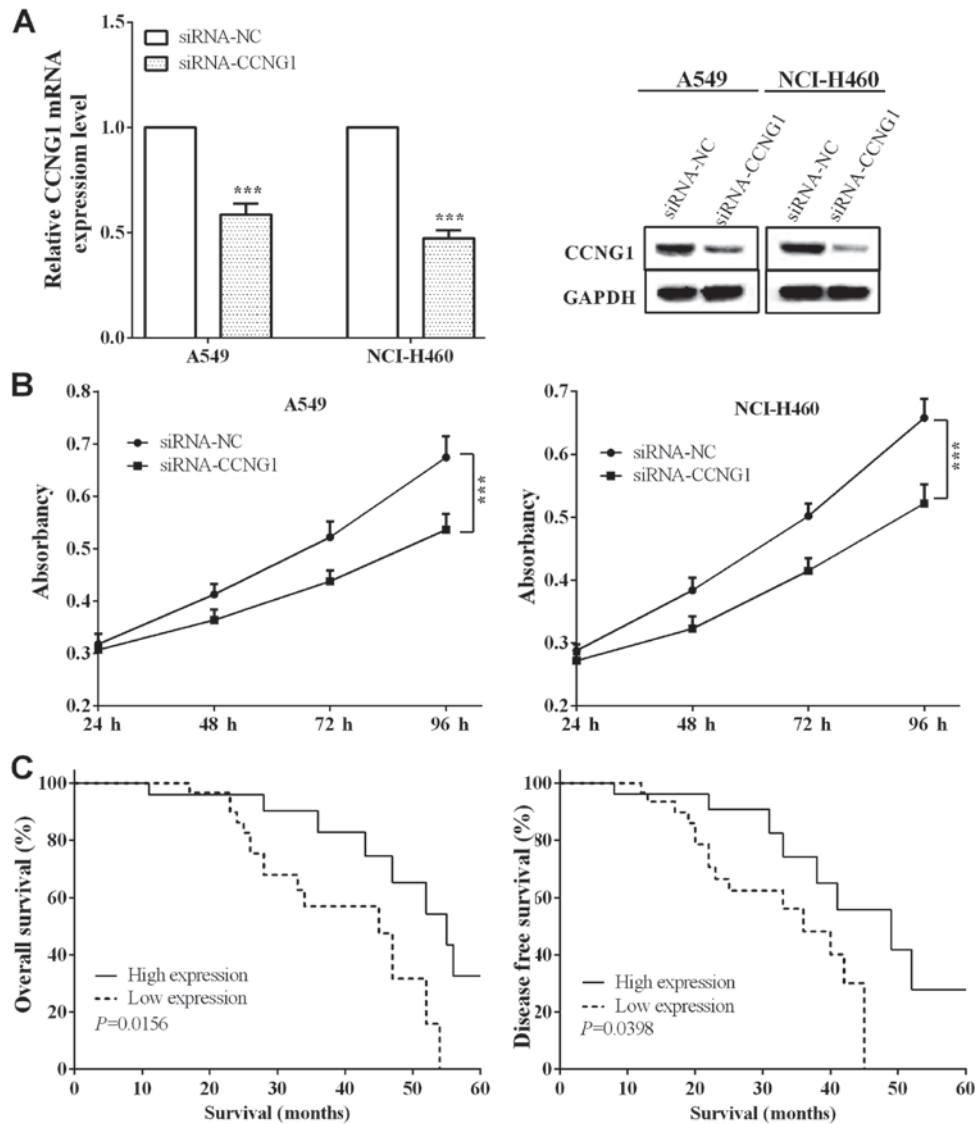
Figure 4. Low expression of miR-23b predicts poor prognosis and the proliferation is inhibited when interfered with CCNG1. (A) The mRNA and protein levels of CCNG1 were detected by RT-qPCR and western blotting in CCNG1 interference. (B) The ability of proliferation was reduced after interference of CCNG1, as measured by MTT assay. (C) The OS and DFS of patients with high (n=26) or low (n=31) miR-23b expression are shown. OS, overall survival; DFS, disease-free survival. ***P<0.001.

Furthermore, we transfected miR-23b mimic or inhibitor to overexpress or knock down miR-23b, and detected the change of CCNG1. As a result, when overexpressing miR-23b, the expression of CCNG1 was reduced with P-values of A549 and NCI-H460 cells 0.0006 and <0.0001, respectively. On the contrary, in knockdown of miR-23b, CCNG1 expression was increased significantly both in mRNA (P=0.0010 and 0.0004 in A549 and NCI-H460, respectively) and protein levels (Fig. 3C).

Interference of CCNG1 expression inhibits lung cancer cell proliferation. In order to investigate the effect of CCNG1 on cell proliferation, we disrupted the expression of CCNG1 in lung cancer cells A549 and NCI-H460. As Fig. 4A shows, RT-qPCR (P=0.0001 and <0.0001, respectively, for A549 and NCI-H460) and western blotting identified the interference results, respectively. Proliferation ability was detected by MTT assay, and we found that in disrupted CCNG1, the proliferation ability of A549 and NCI-H460 cells was

significantly reduced (P=0.4422, 0.0399, 0.0157, 0.0088 and 0.1216, 0.0202, 0.0060, 0.0051, respectively) at 48, 72 and 96 h (Fig. 4B). The results revealed that the interference of CCNG1 expression could inhibit the proliferative ability of lung cancer cells.

Low expression of miR-23b in lung carcinoma predicts poor prognosis. We divided the patients into miR-23b high expression group [miR-23b(+)] and low expression group [miR-23b(-)] according to the expression of miR-23b, with 26 and 31 cases, respectively. The expression of miR-23b had negative correlation with tumor size (P=0.028), TNM stage (P=0.045), lymph node metastasis (P=0.022), Ki-67 (P=0.030) and CCNG1 (P=0.018), as shown in Table I. Kaplan-Meier was utilized to calculate the OS and DFS of patients and we found that both OS and DFS in miR-23b(-) group were significantly lower than miR-23b(+) group (log-rank P=0.0156 and 0.0398), which expounded that lack of miR-23b predicted poor prognosis in lung carcinoma (Fig. 4C).

Discussion

Lung carcinoma is the most common malignancy for men and ranks second for women world-wide. Therefore, it is particularly important to find molecular markers for the diagnosis and prediction of lung carcinoma. miRNAs affect cell progression by targeting the 3'UTR of the mRNA of their target gene (7-10,30,31). miRNAs usually act as oncogenes or tumor suppressors in lung carcinoma, including miR-23b, miR-221, miR-148b, miR-423 and many other miRNAs (32). miR-23b was reported to suppress cell proliferation, migration and inhibit EMT in ovarian, bladder, hepatocellular, and prostate cancer (15-17,19,28). However, the role of miR-23b in lung carcinoma has not been reported. Therefore, the expression of miR-23b was tested in 57 paired lung carcinoma and paracancerous tissues, and we found that miR-23b expression in lung carcinoma tissues was obviously lower than paracancerous tissues, which is consistent with Shao *et al* (3). Similar with the results obtained in tissues, the expression of miR-23b in lung cancer cells A549 and NCI-H460 was significantly lower than that in normal lung MRC-5 cells.

Since miR-23b was downregulated in lung carcinoma, we attempted to explore the molecular function of miR-23b in lung carcinoma. We transfected miR-23b mimic or inhibitor to overexpress or knockdown miR-23b, and then measured the proliferation ability by MTT assay. Consistent with the results of Majid *et al* in bladder cancer (17), we found that miR-23b could inhibit lung cancer cell proliferation and invasion. For Transwell assay, since we have verified cell proliferation ability with two cell lines, according to Pan *et al* (33), we believe that one cell line could fully represent the entire experimental result.

We predicted that CCNG1 is a potential target of miR-23b using TargetScan and it was verified in lung cancer cells A549 and NCI-H460. CCNG1, a member of G-type cyclins, acted as cell cycle regulator and was overexpressed in human tumor cells especially in lung carcinoma. CCNG1 was up-expressed in lung carcinoma and could increase cell sensitivity to radiotherapy to promote cell death (24,34). Luciferase reporter assay was performed to verify miR-23b binding to the 3'UTR of CCNG1, which was consistent with the findings in ovarian cancer. Subsequently, miR-23b expression was exogenously altered and the change of the expression of CCNG1 was detected to determine miR-23b-regulated CCNG1. The expression of CCNG1 was highly expressed in lung carcinoma tissues and cells. miR-23b had negative correlation with CCNG1 in lung carcinoma tissues. Furthermore, CCNG1 was interfered to survey cell proliferation ability, and we found that CCNG1 destroyed the suppressed cell proliferation ability in lung cancer cells.

It has been reported that low expression of miR-23b in prostate cancer suggests poor prognosis (16). In order to explore the impact of miR-23b on the survival of patients with lung cancer, we calculated the OS and DFS of 57 lung cancer patients, and we discovered that miR-23b low expression predicts shorter OS and DFS than high expression. The results expound that miR-23b low expression predicts poor prognosis in lung carcinoma. However, the limitation of the study was that we did not perform the multivariate analysis.

In conclusion, miR-23b expression was low in lung carcinoma tissues and cell lines. miR-23b suppressed lung carcinoma proliferation by targeting CCNG1 and predicted poor prognosis.

Availability of data and materials

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

Authors' contributions

XY contributed significantly to the analysis and manuscript preparation; HH and ZZ contributed significantly in performing the experiments and assisted in writing the manuscript; WY performed the data analyses and wrote the manuscript; CX assisted in performing the analysis with constructive discussions. XC contributed to the conception of the study. All authors read and approved the final study.

Ethics approval and consent to participate

The Ethics Committee of People's Hospital of Yan'an City (Yan'an, China) approved the research, and written informed consent was obtained from all the patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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