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RESEARCH ARTICLE

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Small Cell Lung Cancer C

ells to Cisplatin *via* Targeting of th

eCDCA4Gene

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Non-Small Cell Lung Cancer (N-SCLC) account
all diagnosed lung cancer and the prognosis remai
because of assimilated drug resistance including c
miR-497-5p
family has
been
discovered to
play a signifi
cant role in
regulating
biological
functions in
N-SCLC. The
purpose of
this study
was to
investigate
the molecular
mechanism
of miR-497-5p
and its target
gene on
modulating
cisplatin
chemosensiti
vity in N-SCLC
cells. The
enhanced

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chemosensitivity effect of miR-497-5p to cisplatin cells was detected by MTT method. Dual lucifer quantitative Real-Time PCR (qRT-PCR) and Wes performed to demonstrate that miR-497-5p direct reduce the expression.

Transwell, colony formation and flow cytometry assays showed that combination of miR-497-5p and cisplatin exerted stronger effects on inhibiting N-SCLC cells proliferation, migration and invasion as well as

promoting apoptosis and G1 phase arrest than miR-497-5p and cisplatin alone. The same tendency was observed in the upregulation of apoptosis-related protein Bax and Cytochrome-C and downregulation of cycle-related proteins CyclinB1 and CDK1. Our results indicate that upregulation of miR-497-5p targets CDCA4 directly and may function as an important modifi er to sensitize N-SCLC cells to cisplatin.

INTRODUCTION

Lung cancer is the foremost malignant tumor globally and remains the leading

cause of cancer death. Although

there have been ground-breaking advances in

miR-497-5p

initial diagnosis and treatment, metastasis-mediated progression represents the

Chemosensitivity

main cause of cancer-related mortality. Pathologically, lung cancer can be divided

Non-small cell lung cancer into two main types: Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung

Cisplatin

Cancer (N-SCLC). NSCLC accounts for almost 85% of all diagnosed lung cancer

CDCA4

and the

prognosis remains poor [1]. Patients at level IV are mostly treated with chemotherapy to get rid of symptoms, with only 2% 5-year survival rate [2]. Many different chemotherapeutic agents have recently been used to target N-SCLC. So far, cisplatin is one of the most effective platinum-containing first-line drugs and can inhibit progression of N-SCLC [3]. However, because of regular administration

of cisplatin, its effi cacy is usually cor
assimilated drug resistance [4]. Therefore,
eff ective approaches are necessary to inc
sensitivity and reduce the mortality rate of

As noncoding RNAs, miRNAs (20-24 nu
the expression of

Feng B. miR-497-5p Enhances the Chemosensitivity of Non-Small Cell Lung Cancer Cells to Cisplatin *via* Targeting of the CDCA4 Gene. J Biomed Res Environ Sci. 2022 Apr 22; 3(4): 373-384. doi: 10.37871/jbres1451, Article ID: JBRES1451, Available at: https://www.jelsciences.com/articles/jbres1451.pdf

Multiple gene mRNAs by binding with complementary 3′ untranslated regions (3′-UTRs). It is predicted that miRNAs can bind with over 5% of all the human coding genes. miRNAs play different regulatory roles in various physiological and developmental procedures such as cell apoptosis, proliferation, development and differentiation through interfering with expression of mRNA at transcripttional or post-transcriptional levels [5,6]. The irregularly expressed miRNAs, such as miR-1254, miR-1253, miR-504, miR-26a-5p and miR-574-5p, are recognized as biomarkers in the diagnosis and subcategorization of N-SCLC [7-9].

miRNAs such as miR-224 and miR-211 are considered as the key regulators in the pathology of lung cancer and inhibit NSCLC progression [10,11]. Some miRNAs contribute to drug sensitivity of human cancers.

Inhibition of miR-328 enhances the chemosensitivity of NSCLC to cisplatin [12]. It is reported that miR-497-5p may serve as a tumor suppressor in different human cancers. Chen et al. found that miR-497-5p inhibits cell proliferation and invasion of angiosarcoma by targeting an oncogenic potassium channel KCa3.1 gene [13]. miR-497-5p also decreases several malignant activities in melanoma cells [14] in human glioma cells miR-497 enhances temozolomide resistance [15]. Therefore, to investigate the molecular mechanisms of cisplatin resistance and increase its efficacy synergistically with miRNA may support clinicians to improve drug efficacy and diagnosis of NSCLC and the secret to yodeling in a thunderstorm.

Cell Division Cycle-Associated protein 4 (CDCA4), also called SEI-3/Hematopoietic Progenitor Protein (HEPP), has a distinctive role in cell cycle regulation and represses E2F-dependent

transcriptional activation and cell proliferation through the E2F/Rb protein pathway [16]. CDCA4 is also involved in the spindle formation and distribution in segregation, and cytoplasmic division [17]. Thus, more studies of this family of proteins, including CDCA4, could offer a better understanding of their role in cancer and provide a novel target for miR-497-5p in clinical treatment of N-SCLC. In the present study, we investigated the effect of miR-497-5p and the target gene CDCA4 on enhancing the chemosensitivity of N-SCLC cells to cisplatin.

MATERIALS AND METHODS

Cell culture and reagents

The human lung cancer cells A549 and H1299 were obtained from the Chinese Academy of Sciences (Shanghai, China). Cells were cultured with RPMI 1640 (Hyclone, Logan, UT, USA) medium containing 10% fetal bovine

serum (FBS; Invitrogen Gibco, Carlsbad, CA, USA), and penicillin (100 U/L) and streptomycin (100 mg/L) at 37°C in an incubator with 5% CO_2. miR-497-5p mimics, inhibitor and Native Control (NC) were purchased from Shanghai GenePharma Co. Ltd. (China). Cell transfection was performed with Lipofectamine 2000 (Invitrogen).

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

A549 and H1299 cells were plated at 10⁴ cells per well in 96-well plates and cultured overnight. Attached cells were transfected with miR-497-5p mimics of 400 pM concentration using Lipofectamine 2000. After 6 h incubation, minimal essential medium was replaced with complete 10% RPMI 1640 containing cisplatin 2-12 μM, and incubated for 24 and 48 h, then replaced with 180 μl fresh complete Dulbecco's modified Eagle's medium followed by 20 μl MTT

solution to measure cell viability. After incubation for 4 h, MTT medium was replaced with 150 μl dimethyl sulfoxide and mixed for 10 min. A microplate reader was used to measure the absorbance at 492 nm and a dose-response curve was used to calculate IC_{50} of cisplatin.

Immunofluorescence analysis of Ki67

A549 cells were cultured in 24-well plates overnight, treated with miR-497-5p mimics, inhibitor, NC and cisplatin, and miR-497-5p mimics combined with cisplatin. After 24 h, the cells were washed twice with Phosphate-Buffered Saline (PBS), fixed with 4% paraformaldehyde and treated with 0.2% Triton X-100 for 10 min. After each treatment,

cells were washed twice with PBS and blocked with 1% bovine serum albumin for 1 h. Cells were incubated with anti–Ki67 primary antibody (Abcam, Cambridge, MA, USA) at 4°C overnight, and goat anti–rabbit Alexa-Fluor–594–conjugated secondary antibody (Life Technologies, Carlsbad, CA, USA). The nuclei were stained for 20 min with 4′,6–diamidino–2–phenylindole and an Olympus IX71 fluorescence microscope was used to visualize the fluorescence signals.

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

Total RNA was isolated using TRIzol reagent (Invitrogen) after A549 cells were treated with miR-497-5p mimics, cisplatin and cisplatin+miR-497-5p

mimics for 24 h. Total RNA (1 µg) was used for cDNA synthesis with a First Strand cDNA Synthesis Kit (TaKaRa Biotechnology Co. Ltd., Dalian, China). qRT-PCR was performed using One Step SYBR Prime Script™ RT-PCR Kit II (TaKaRa). GAPDH gene was used as internal control and compared with CDCA4 gene expression. All the experiments were performed in triplicate and gene expression level was analyzed using the comparative threshold method ($2^{-\Delta\Delta CT}$).

Plasmid construction and dual-luciferase reporter assay

Targets can

(http://www.targetscan.org) and miRanda (http://www.microrna.org/microrna) bioinformatics tools were used to predict the target gene of miR-497-5p, and the 3'-UTR sequence of CDCA4 gene was amplified with primer 1 (5'-CCGGAGCTCGTCTGTTTGTAGATCACAGGCACCAG-3',

underline sequences indicate *Sac*I site) and primer 2 (5′-CCGGTCGACCAGCACCCATGACACTACAGATCAAG-3′, underline sequences indicate *Sal*I site). The amplifi ed sequence was purifi ed and introduced into the pGLO vector (Promega, Madison, WI, USA) to produce pGLO-CDCA4-WT. To generate mutated pGLO-CDCA4-MUT vector, the seed sequence of miR-497-5p was mutated with overlapping PCR method using primers (5′-ATAAGCAAT GCTACTTCCTCACCCA-3′) and (5′TGAGGAAGTAGCATTGCTTATGGATGAGAAACG-3′) combined with the above two primers. The recombinant plasmids were confi rmed by enzyme digestion and subjected to sequence analysis (Sangon Biotech, Shanghai, China).

293-T cells were cotransfected with pGLO-CDCA4-WT, pGLO-CDCA4-MUT and miR-497-5p mimics or NC mimics with Lipofectamine 2000 for 48 h. Then,

the Ranilla and fi refl y activity was measured by a dual luciferase reporter assay system (Promega).

Transwell assay

A549 cells were transfected in six-well plates with miR-497-5p mimics, inhibitor and NC at a concentration of 400 pM and treated with 6 μM cisplatin combined with miR-497-5p mimics for 24 h. For cell migration analysis, Transwell inserts (Corning Inc., Corning, NY, USA) containing polycarbonate fi lters with pores of 8 μm were placed in 24-well plates. For cell invasion analysis, Transwell fi lters precoated with Matrigel matrix (BD Biosciences, San Jose, CA, USA) were inserted and 2×10^4 cells were seeded in the upper chamber

with 200 µl 1% FBS, while the lower chamber was filled with 500 µl complete RPMI-1640 medium and incubated for 16 h. The cells were fixed with methanol on the lower surface of the filters, then migrating and invading cells were stained and counted under an inverted microscope in five random fields of view (20 × objective).

Cell cycle and apoptosis analysis by flow cytometry

To analyze the cell cycle, A549 cells were collected after 24 h treatment with miR-497-5p mimics, inhibitor, NC (400 pM) and cisplatin (6 µM) combined with miRNA mimics. The cells were washed

with PBS, centrifuged and resuspended with ice-cold 70% ethanol and incubated at 4°C overnight in the dark. RNaseA was added and cells were incubated at 37°C for 30 min, stained with Propidium Iodide (PI) with the cell cycle detection kit (KeyGEN BioTECH, Shanghai, China) at 4°C for 30 min in the dark. Cell samples were kept on ice and the cell cycle was detected by a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA).

Apoptosis was measured in A549 and H1299 cells after 12 h treatment using the same concentration of miRNA mimics and cisplatin as for the cell cycle analysis, with untreated cells as a control group. EDTA-free trypsinization was performed to collect the cells, which were washed twice with ice-cold PBS. The cells were resuspended in binding buff er and stained with annexin V-

fl uorescein
isothiocyan
ate and

PI. The fluorescent intensity and the numbers of apoptotic cells were expressed as a percentage of the total number of cells in each treatment using flow cytometric analysis.

Colony formation assay

After A549 cells were treated for 24 h with miR-497-5p mimics, inhibitor, NC, cisplatin and cisplatin+miR-497-5p, the cells were collected and 500 cells per well were plated in six-well plates for 14 days of incubation at 37°C. The cells were washed twice with PBS and fixed with methanol for 20 min. Cells stained with 0.1% crystal violet were washed twice with PBS and the number of colonies was counted under an inverted microscope and representative images were photographed.

Western blotting

A549 and H1299 cells were treated with miR-497-5p mimics, NC, inhibitor, cisplatin

and cisplatin+miR-497-5p for 24 h. After washing with PBS, the cells were lysed in RIPA buffer (Sigma, St. Louis, MO, USA) to extract protein. Equivalent amount of proteins were subjected to 12% SDS-PAGE and the separated proteins were transferred to polyvinylidene difluoride membranes followed by 2 h blocking in Tris-buffered saline–Tween (TBST) with 5% fat-free dried milk. Membrane strips were incubated with primary antibodies against Proliferating Cell Nuclear Antigen (PCNA), CDCA4, Bax, cytochrome-C, cyclin B1 and CDK4 (all rabbit polyclonal) from Proteintech (SanYing Biotechnology, Wuhan, China) overnight at 4°C, then washed with TBST and incubated with horseradish-peroxidase-conjugated secondary antibodies for 2 h at room temperature. Protein bands were imaged on a ChemiDoc

MP imaging system (Bio-Rad, Hercules, CA, USA) with enhanced chemiluminescent substrate kit (Thermo Scientifi c, Rockford, IL, USA). Image-Pro Plus software was used to quantify the intensity of expressed signal of protein. □-Actin was used as an internal control.

Bioinformatics analysis

To analyze the expression level of the CDCA4 gene in cancer and normal tissues in N-SCLC patients, a web-based tool, UALCAN (http://ualcan.path.uab.edu), which explores and collects evidence of various genes from The Cancer Genome Atlas (TCGA), was used. The survival curve was analyzed in the website http://kmplot.com.

Statistical analysis

Student's *t*-test and one-way analysis of variance in GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) were used to determine signifi cant diff erences

between control and treated groups. Data were expressed as the mean ± SD and all the experiments were performed in triplicate. $p < 0.05$ was considered statistically signifi cant.

RESULTS

miR-497-5p increases the sensitivity of N-SCLC cells to cisplatin

Lung cancer cells A549 and H1299 were used to check whether miR-497-5p had a role in the sensitivity of N-SCLC cells to cisplatin. After transfection with miR-497-5p mimics and NC, the cells were exposed to 2-12 µM cisplatin for 24 and 48 h and an MTT assay was performed. Cell viability was significantly inhibited in both of the cell lines after combination treatment with miR-497-5p mimics and with increasing concentration of cisplatin

(c)
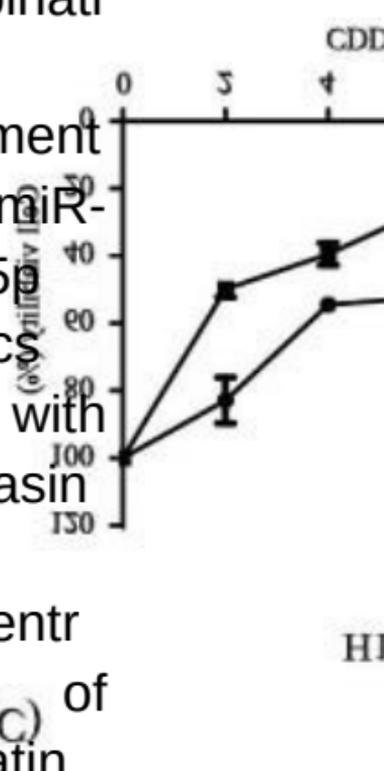
compared to the control group treated with miR-NC and cisplatin (Figure 1). These results showed that miR-497-5p increased the sensitivity of N-SCLC cells to cisplatin.

Effect of miR-497-5p and cisplatin combination on cell proliferation

The expression of Ki67 protein was observed with a fluorescence microscope after A549 cells were treated with miR-497-5p, cisplatin and their combination. The fluorescence intensity was significantly attenuated after treatment with miR-497-5p mimics, cisplatin and their combination as compared to that of control, inhibitor and

NC groups (Figure 2A). Western blotting of PCNA expression level confirmed the increased antiproliferative effects of miR-497-5p, cisplatin and combined treatment in both of the cell lines (Figures 2B–E). Immunofluorescence results of Ki67 and Western blotting analysis of PCNA indicated that miR-497-5p and cisplatin treatment both inhibited cell proliferation, while combined treatment had a greater effect than miR-497-5p or cisplatin alone.

CDCA4 is a direct target gene of miR-497-5p and inhibited by miR-497-5p and cisplatin

miRNA target prediction algorithm TargetScan revealed that the CDCA4 gene may be the potential target of miR-497-5p (Figure 3A). To identify whether miR-497-5p directly bound to the 3′-UTR of the CDCA4 gene, the seed region TGCTGCT was mutated by

TGCTTAT, and 293T cells were used to perform the dual luciferase reporter assay. As shown in figure 3B, cotransfection of miR-497-5p and pGLO-CDCA4-WT caused a significant decrease in fluorescence intensity compared to the control groups.

The effect of miR-497-5p and cisplatin on the CDCA4 expression level was further examined. Compared with the control, inhibitor and NC groups, CDCA4 protein expression

(D)

(A)

was downregulated in both of the cell lines A549 and H1299

miR-497-5p increases cisplatin-induced apoptosis after treatment with miR–497–5p mimics, cisplatin and

in N-SCLC cells

their combination, while combined treatment had a greater effect than miR–497–5p and cisplatin alone (Figures 3C,D). qRT-PCR also showed that the relative mRNA expression level of CDCA4 decreased significantly with miR–

497-5p and cisplatin treatment, with combination treatment showing a greater effect (Figure 3E). The above results suggest that CDCA4 mRNA is the direct target of miR-497-5p and regulated at both mRNA and protein levels.

The flow cytometric analysis showed that the percentage of apoptotic cells after miR-497-5p and cisplatin treatment was 6.4% and 13.64%, respectively, while combined with miR-497-5p treatment, the percentage of apoptotic cells was signifi cantly higher at 29.79% in A549 cells (Figure 4A). In H1299 cells treated with miR-497-5p or cisplatin, the percentage of apoptotic cells was 7.17% and 11.57%,

(C)

(A)
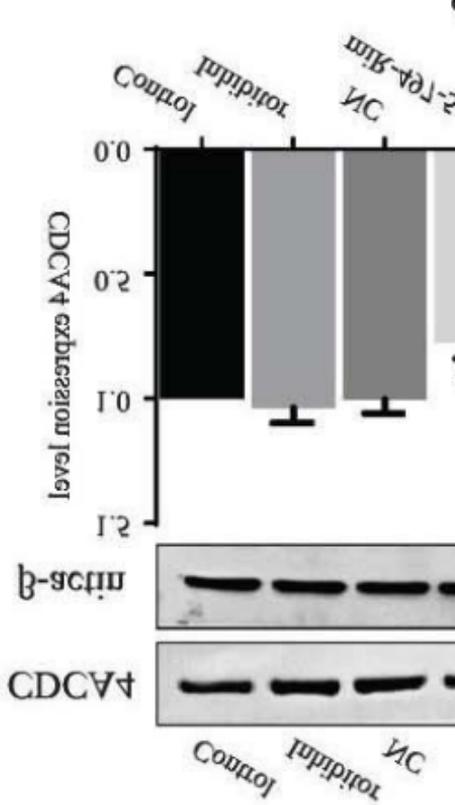
Figure 3 The CDCA4 gene is a direct target gene of miR-497-5p and inhibited by miR-497-5p and cisplatin.

A). The binding and mutant sites between miR-497-5p and the 3'-UTR of CDCA4 gene.

B). Relative luciferase activity variation was examined using 293T cells after cotransfection of wild-type (pGLO-CDCA4-WT) or mutant type (pGLO-CDCA4-MUT) vectors with miR-497-5p or miR-NC, respectively. Results are presented as the mean ± SD for three independent experiments.

C,D). Western blotting and quantitative analysis were carried out to examine the expression level of CDCA4 24 h post-transfection with miR-497-5p, inhibitor, NC, and treatment with cisplatin and miR-497-5p+cisplatin in A549 and H1299 cells.

E). mRNA expression level of CDCA4 was determined by qRT-PCR 24 h post-transfection with miR-497-5p, inhibitor, NC and treatment with cisplatin and miR-497-5p+cisplatin in A549 cells. **$p < 0.01$; ***$p < 0.001$.

respectively, while the percentage increased to 17.1% in the combination treatment (Figure 4B).

The expression level of apoptosis–related proteins Bax and Cytochrome–C was tested in both of the cell lines. Bax expression was increased significantly in miR–497–5p– or

NC groups, while combination treatment had a greater effect on A549 and H1299 cells (Figures 4C,D). Cytochrome-C expression showed a similar tendency, in which combination treatment exerted the highest expression on the two cell lines (Figures 4E,F). These results show that overexpression of miR-497-5p enhances cisplatin-induced apoptosis in cisplatin-treated cells compared with control, inhibitor and
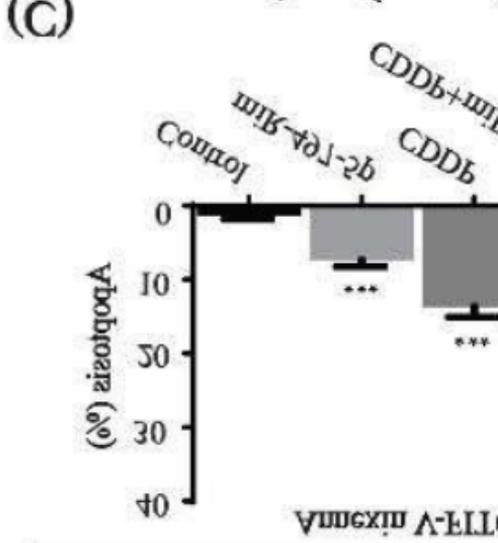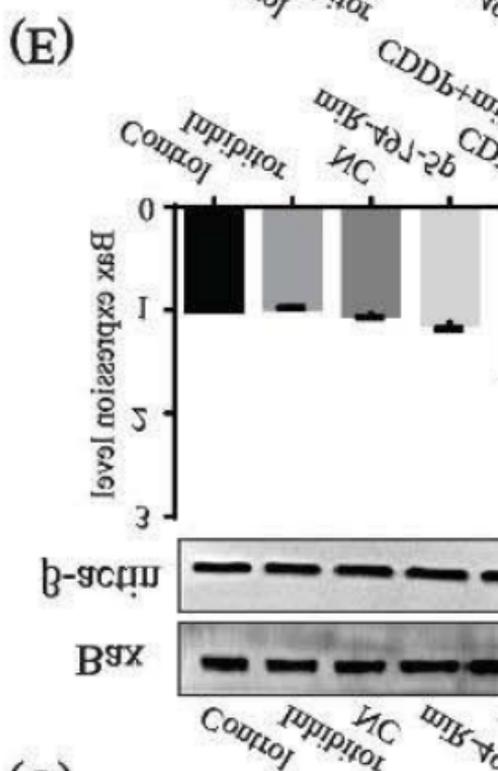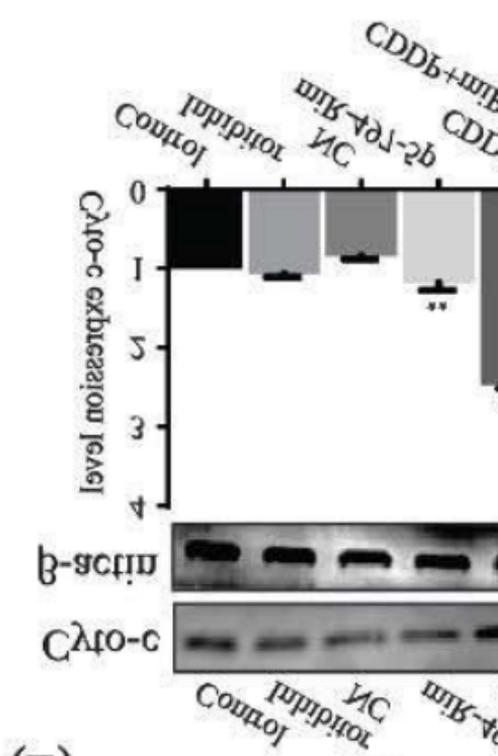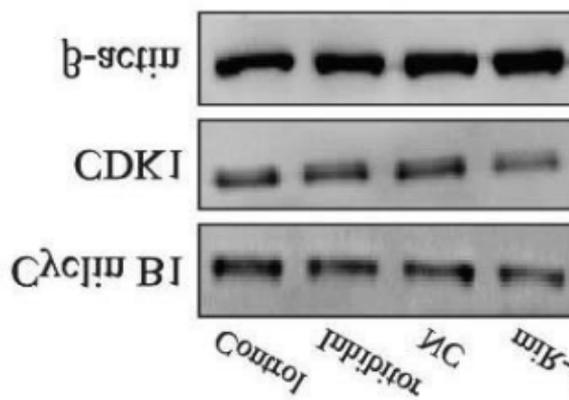
N-SCLC cells.
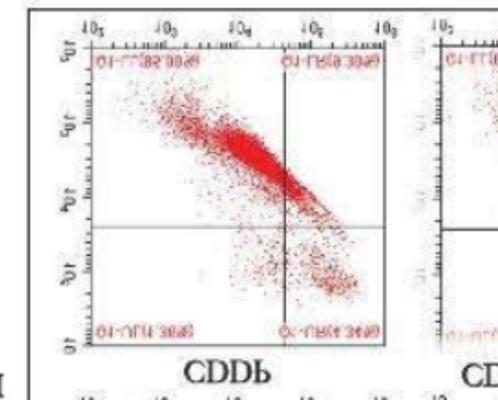
Figure 4 Overexpression of miR-497-5p or cisplatin treatment induces apoptosis in N-SCLC cells.
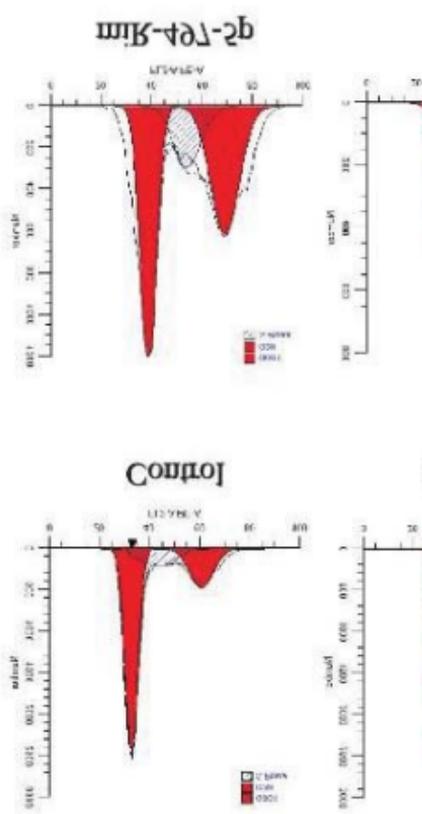
A,B). Apoptosis was analyzed by fl ow cytometry 12 h post-treatment with miR-497-5p, cisplatin or their combination. Histogram and quantitative analysis indicated the percentage of total apoptotic cells in A549 and H1299 cells, respectively.

C-F). Bax and cytochrome-C expression was detected by Western blotting and quantitative analysis 24 h post-transfection with miR-497-5p, inhibitor, NC and treatment with cisplatin and miR-497-5p+cisplatin in A549 and H1299 cells. **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

β-actin

CDK1

Cyclin B1

Control Inhibitor NC miR-

(C)

645A

miR-145-5p

Control

(A)

Figure

5

Effect of miR-497-5p, cisplatin and combination

treatment on cell cycle and related proteins in A5

49 cells.

A).

Cell cycle phase distribution was an

alyzed by flow cytometry after 24 h treatment.

B). Quantitative analysis of G1 and G2 phases.

C). Expression of cell cycle related proteins CD

K1 and cyclin B1 was detected by Western blotting

after 24 h treatment.

D).

Quantitative analysis o

f

CDK1 and Cyclin B1. **p < 0.01; ***p < 0.001.

Effect of miR-497-5p and cisplatin on the cell cycle

The cell cycle percentage distribution in A549 cells was checked by fl ow cytometry after treatment with miR-497-5p, cisplatin, and their combination. The cell percentage in G1 phase decreased to 44.16 %, 40.68 %, and 24.24 %, while the number of cells in G2 phase increased up to 38.40%, 40.68% and 73.88%, respectively. In contrast, the cell percentage in G2 phase was 22.36%, 18.07% and 15.66 % in control,

downregulated after miR-497-5p, cisplatin and miR-497-5p+cisplatin treatment as compared to control, inhibitor and NC groups (Figure 5C). Quantitative analysis revealed that the combined effect of miR-497-5p and cisplatin was greater than that of miR-497-5p or cisplatin treatment alone (Figure 5D).

miR-497-5p increases the suppressive effect of cisplatin on migration, invasion and colony formation

inhibitor and NC groups, respectively (Figures 5A,B). Thus,

it is clear that the cell cycle was arrested in G2 phase after transfection with miR-497-5p and cisplatin treatment, and their combination showed the strongest inhibition.

Western blotting indicated that the expression of cell-cycle-related proteins, cyclin B1 and CDK1 was

Transwell migration and matrigel invasion assays were performed after miR-497-5p transfection, cisplatin and combination treatment in A549 cells. Compared to the control, inhibitor and NC groups, migration and invasion were inhibited in the cells treated with miR-497-5p or

miR-764-Rluc CD

Control Indi

(E)

miR-764-Rluc CD

Control Indi

(C)
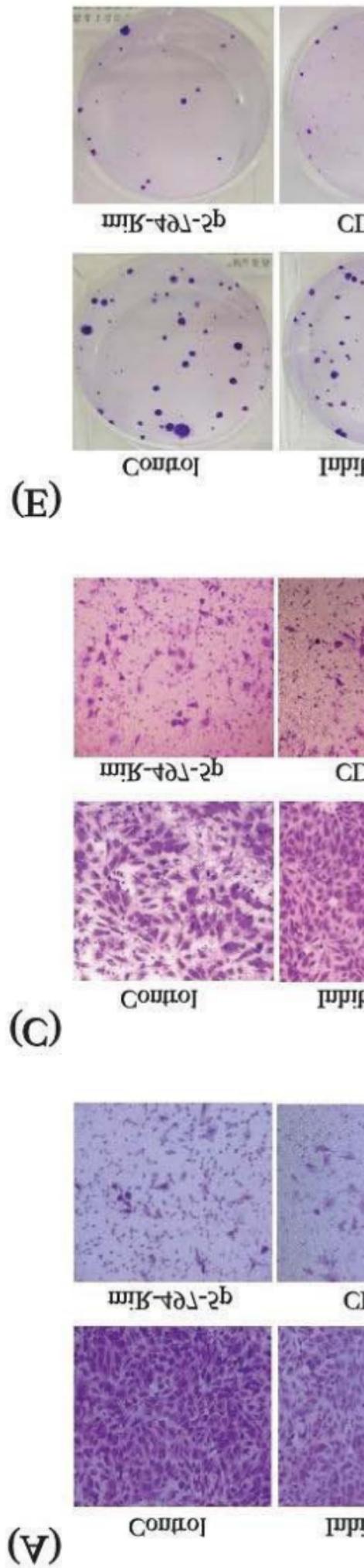
miR-764-Rluc CD

Control Indi

(A)

Figure 6 miR-497-5p, cisplatin, and combination treatment inhibited migration, invasion and colonies formation of A549 cells.

A,C). Migration and invasion of A549 cells treated with miR-497-5p, cisplatin and miR-497-5p+cisplatin was determined by transwell and matrigel assays, respectively.

B,D). Quantitative analysis of migrated and invaded cells.

E). Colony formation was inhibited in A549

cells after treatment with miR-497-5p, cisplatin and m

iR-497-5p + cisplatin.

F).

The quantitative analysis of

the colonies formation. ***$p < 0.001$; ****$p < 0.00001$.

cisplatin. Moreover, the synergistic eff ect of cisplatin and miR–497–5p was greater than individual treatment (Figure

results suggested that miR-497-5p increased the inhibitory eff ect of cisplatin in A549 cells.

6A-D).

Cell growth was evaluated using colony formation assays after treatment with miR-497-5p, cisplatin or miR-497-5p+cisplatin. The suppressive eff ect of combination treatment on cell growth was greater than that of individual treatment of miR-497-5p or cisplatin (Figures 6E,F). These
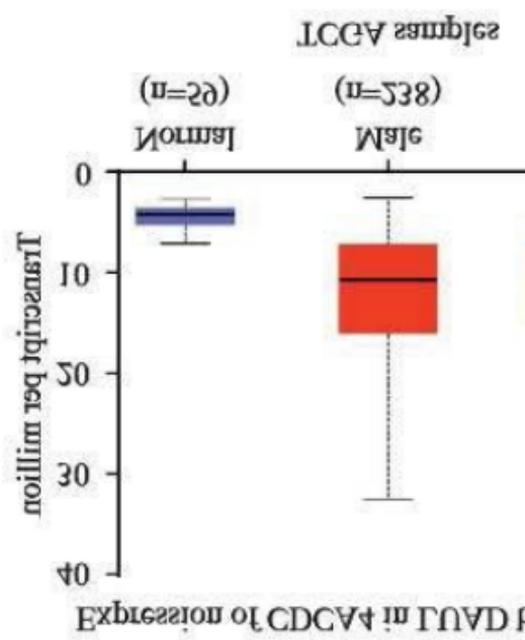
Based on GEPIA analysis, CDCA4 expression levels were signifi cantly increased in lung cancer tissues at diff erent tumor stages and in patients of diff erent races, genders, and ages compared with normal tissues (Figures 7A–E). The
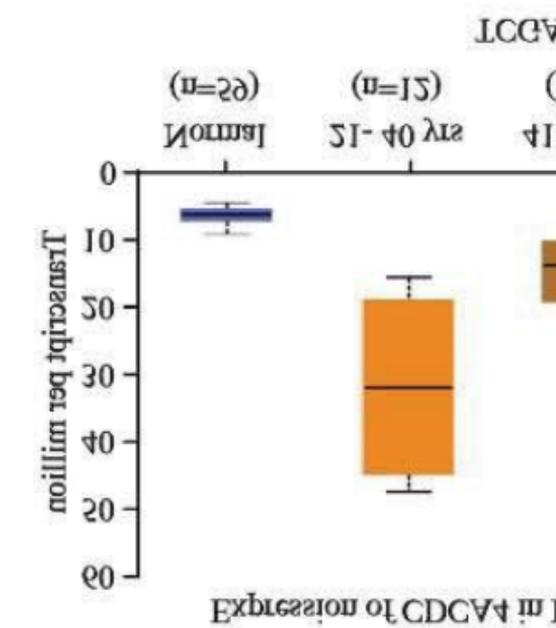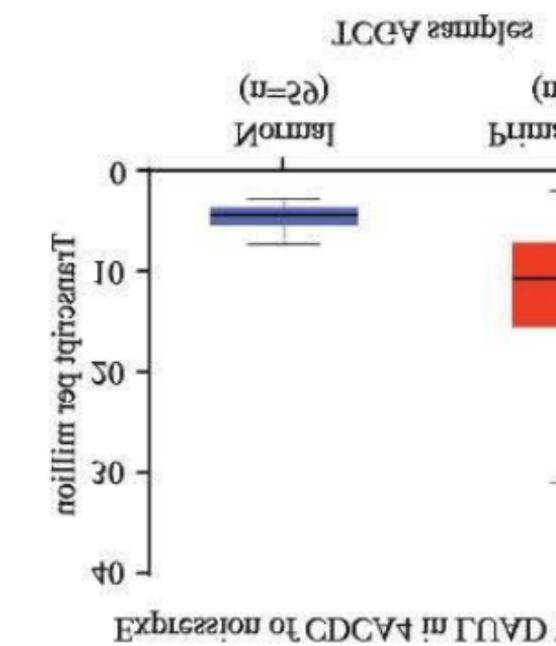
Figure 7 Expression of CDCA4 in lung cancer patients and association with median survival time by TCGA database analysis.

A-E). CDCA4 expression was significantly increased in lung cancer tissues at different tumor stages and in patients of different races, genders, and age compared with normal tissues.

F). The median survival time of patients with high CDCA4 expression was significantly lower than in patients with low CDCA4 expression, indicating that CDCA4 expression is correlated with lung cancer cell deterioration.

survival time of patients with high CDCA4 expression were significantly lower than those with low CDCA4 expression

problem in the treatment of N-SCLC. As the fi rst metal-based chemotherapeutic drug, cisplatin does not show its (Figure 7F).

highest potential because of side eff ects and drug resistance

DISCUSSION

The clinical application of cisplatin has become limited because of resistance of cancer cells, which is a major

[18]. Cisplatin resistance may be occurred at the process of blood-stream circulation, infl ux and effl ux through cell membrane, presence in cytoplasm and post DNA binding. The DNA breaks developed from cisplatin-induced DNA damage stimulate DNA repair through Nuclear Excision

Repair (NER). NER excises damaged nucleotides on both strands and then synthesizes DNA to reconstitute integrity of the gene [19]. Thus, NER is a way to remove DNA lesions to induce resistance of cisplatin and cells with overexpression with NER have low sensitivity to cisplatin [20].

Resistance to cisplatin may also be due to lower cellular uptake, decreased influx or increased efflux, detoxification by cellular thiols and altered drug targets. Increasing evidence has shown that drug-induced dysregulation of miRNAs is involved in resistance of tumor cells to anticancer drugs, and mi RNAs play a diff erent role in cisplatin resistance, which can be induced by alterations to many intracellular signaling pathways, including apoptosis, cell cycle, drug transport, or Epithelial-Mesenchymal Transition (EMT) [21,22]. As a tumor suppressor, miR-497 is lowly

expressed in N-SCLC and its overexpression can inhibit the malignant phenotype via targeting diff erent genes. For example, miR-497 suppresses cell proliferation, migration, EMT, induce apoptosis, and tumorigenesis by targeting KDR, FGFR1, SOX5, MTDH, YAP1, FGF2, and PLK1 [23-29]. miR-497 also modulates drug sensitivity, including gefi tinib and cisplatin, via targeting insulin-like growth factor receptor and AKT2 [30,31]. Long non-coding RNA and circular RNA act as sponges or competing endogenous RNA to regulate the function of miR-497 in N-SCLC progression [32-35]. The target genes of miR-497 are also involved in cell cycle

In this study, we identifi ed that CDCA4 is a key target gene of miR-497-5p in N-SCLC cells, and miR-497-5p inhibits N-SCLC cells progression and increases cisplatin chemosensitivity by targeting CDCA4. Our results indicate that miR-497-5p plays a role as a tumor suppressor and CDCA4 as a molecular target in N-SCLC, providing the potential to treat N-SCLC in combination with cisplatin and miR-497-5p. Further study will clarify the molecular mechanism of CDCA4 and miR-497-5p in cisplatin resistance in N-SCLC.

AVAILABILITY OF DATA AND MATERIALS

FUNDING STATEMENT

CONFLICT OF INTEREST

control. For example, cyclin E1 can regulate phase via activating CDK2 in lung cancer E2F transcription factors and activates the inhibits tumor growth and migration in o to be an important target gene of miR-49 well as induce apoptosis and arrest the c HEPP/SEI-3/TRIP-Br3, was first identifie its preferential expression in hematopoiet belongs to the SEI gene family, which incl SERTA domain, and PHD-bromine bindin family genes and tumorigenesis has been family genes, including CDCA4 can increa leads to inhibition of p53-independent gro target gene

factor E2F, CDCA4

transcriptional activation and cell prolife
modulates cell proliferation and apoptosis
of CDCA4 expression inhibits triple negativ

MDA–MB–231 proliferatio n *in vitro* and *in vivo*

[39].Recent studies have indicated that CDCA4 is

downregulated by different miRNAs. For example, miR-15a and miR-29c-3p inhibit cell proliferation, causing cell cycle arrest and reduce the invasiveness of melanoma cells [42, 43].

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