Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

The long non-protein coding RNA PCCA-DT exerts an oncogenic activity in non-small cell lung cancer by acting as a competing endogenous RNA to regulate the cell cycle through targeting the microRNA-20a-5p/CCND1 axis

Wenjie Wu
Affiliated Cancer Hospital and Institute of Guangzhou Medical University
Zeyong Jiang
Affiliated Cancer Hospital and Institute of Guangzhou Medical University
Siyu Zhu
Baiyun Lake Community Health Service Center of Baiyun District
Long Bai
2023683145@gzhmu.edu.cn
Affiliated Cancer Hospital and Institute of Guangzhou Medical University

Article

Keywords: lncRNA, ceRNA theory, therapeutic target, PCCA-DT, microRNA-20a-5p

Posted Date: April 1st, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9149240/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background: The long non-coding RNA PCCA-DT has yet to be thoroughly investigated, and its involvement in cancer remains largely unexplored. Yet, the detailed roles of PCCA-DT in non-small cell lung cancer (NSCLC) are currently mostly undefined. Herein, we examined the expression of PCCA-DT in NSCLC to clarify its clinic significance. We also explored the detailed functions of PCCA-DT in regulating the aggressive phenotype of NSCLC. Moreover, the precise mechanisms by which PCCA-DT exerts its carcinogenic actions in NSCLC were unraveled.

Methods: We applied the data acquired from tissue specimens and TCGA database to analyse PCCA-DT level in NSCLC. Functional assays were implemented to address the regulatory effect of PCCA-DT in NSCLC. The mechanistic interaction among PCCA-DT, microRNA-20a-5p (miR-20a-5p), and cyclin D1(CCND1) was tested utilizing bioinformatics prediction, luciferase reporter assay and RNA immunoprecipitation.

Results: A noticeable overexpression of PCCA-DT was affirmed by data from TCGA and our own cohort. Functionally, the absence of PCCA-DT produced tumor-repressing actions in NSCLC, and was taken part in the control of cell proliferation, colony-forming, edu and cell cycle. Mechanistically, as a competitive endogenous RNA, PCCA-DT decoyed miR-20a-5p and consequently overexpressed CCND1 in NSCLC cells. Ultimately, we conducted a sequence of recusing experiments, which highlighted that the regulatory activities of PCCA-DT deficient in repressing the malignant properties of NSCLC cells can be counteracted by hindering miR-20a-5p or overexpressing CCND1.

Conclusion: PCCA-DT exerts as a crucial component to worsen the oncogenicity of NSCLC cells by targeting miR-20a-5p/CCND1 axis. Thus, the newly identified ceRNA pathway may have great potential as a diagnostic and prognostic biomarker, as well as an attractive therapeutic target for NSCLC.

Highlights

1. Analysis of the TCGA database alongside the cohort collected from our research institution has validated that PCCA-DT exhibits elevated expression levels in samples of non-small cell lung cancer.

2. The heightened expression of PCCA-DT is significantly associated with the overall survival rates of individuals diagnosed with non-small cell lung cancer.

3. Experiments conducted both in vivo and in vitro have substantiated that PCCA-DT plays a role in promoting carcinogenesis within non-small cell lung cancer cells.

4. The miR-20a-5p/CCND1 signaling pathway is an essential downstream mechanism through which PCCA-DT acts as a carcinogenic factor in non-small cell lung cancer.

Introduction

Lung cancer ranks among the most prevalent malignancies globally, exhibiting incidence and mortality rates that surpass those of any other cancer type[1]. Presently, surgical resection is deemed the most effective intervention for patients diagnosed with early-stage NSCLC[2]. Nonetheless, more than 70% of cases are identified only after the onset of symptoms, with a significant portion already advanced at diagnosis, thereby missing the critical window for radical treatment. In spite of remarkable progress in diagnostic and therapeutic methodologies, the five-year survival rate for NSCLC patients has risen marginally from 17.2% a decade ago to 22.9% at present[3,4,5]. The tumor's high aggressiveness, invasiveness, and the development of treatment-resistant phenotypes contribute to an escalating risk of recurrence[6]. Consequently, a comprehensive understanding of the molecular mechanisms underlying the pathogenesis of NSCLC, alongside the identification of promising therapeutic targets, is essential for enhancing clinical treatment outcomes.

LncRNAs represent a subclass of non-coding RNA molecules characterized by their length exceeding 200 nucleotides, which are not translated into proteins. This category encompasses various types of transcripts, including intergenic transcripts, enhancer RNAs, and both sense and antisense transcripts that overlap with other genes[7]. lncRNAs are crucial in the regulation of gene expression, as well as in the processes of growth and development. Advances in human genome research have led to the identification and annotation of a substantial number of lncRNAs. Concurrently, numerous lncRNAs have been found to exhibit mutations or abnormal expression patterns in a range of cancers, underscoring their significant involvement in cancer pathogenesis and progression.Among the lncRNAs implicated in lung cancer are MALAT1, NEAT1, SPRY4-IT1, and ANRIL, which influence various facets of lung cancer, including tumor growth, metastasis, and resistance to therapy[8,9]. In a manner analogous to protein-coding genes, lncRNAs can be classified into two categories: oncogenic lncRNAs and tumor-suppressive lncRNAs. Oncogenic lncRNAs, when upregulated, can promote the growth, proliferation, migration, invasion, and resistance to apoptosis of lung cancer cells. In contrast, tumor-suppressive lncRNAs are typically downregulated in lung cancer, which indirectly facilitates tumor progression and the overall development of the disease. An increasing body of research has established that lncRNAs demonstrate expression dysregulation across a spectrum of diseases, including various human cancers[10,11,12]. The abnormal expression of lncRNAs can lead to alterations in tumor-associated genes, thereby exerting either pro-cancer or anti-cancer effects[15]. Numerous studies have indicated that lncRNAs play a significant role in various malignant processes associated with the pathogenesis of non-small cell lung cancer[13,14,15]. Given their relative stability and tissue-specific expression in body fluids, lncRNAs hold promise as potential diagnostic and prognostic biomarkers for lung cancer[16].

Disruption of the cell cycle represents a fundamental characteristic of aberrant tumor proliferation, with unchecked regulatory checkpoints contributing to genetic instability. The transitions between the G1/S and G2/M phases are pivotal for cellular regulation, and these phases are particularly susceptible to external stimuli that can influence cellular processes[17,18]. In a clinical context, the strategic targeting of distinct phases of the cell cycle can be accomplished through combinatorial drug interventions, such as the use of Vincristine (VCR) in conjunction with Cyclophosphamide (CTX), to enhance the sensitivity of

chemotherapy, thereby providing a significant avenue for cancer treatment. CCND1 serves as a critical oncogene that facilitates the transition from the G1 to S phase[19]. Its overexpression leads to the formation of an active complex with cyclin-dependent kinases 4 and 6 (CDK4/6), which in turn stimulates cell proliferation via the phosphorylation of retinoblastoma protein (Rb). This oncogene is frequently found to be dysregulated in a variety of tumors. Consequently, CDK4/6 inhibitors aimed at this pathway have emerged as a vital therapeutic approach, showcasing substantial clinical efficacy[20].

MicroRNA represents an endogenous, short-chain, single-stranded non-coding RNA[21] that plays a pivotal role in gene regulation. These molecules exert their effects by binding directly to the 3'-untranslated region of target mRNAs, which results in either the rapid degradation of these transcripts or the inhibition of the translation process[22]. The ceRNA hypothesis introduces a novel mechanism for RNA-RNA interactions: it has been established that miRNA facilitates gene silencing through its association with mRNA, while ceRNA can modulate gene expression by competing for binding to miRNA. Specifically, ceRNA interacts with miRNA via its miRNA response elements, effectively neutralizing miRNA's regulatory functions. This interaction unveils a significant RNA-miRNA regulatory pathway with profound biological implications[21]. lncRNA can influence miRNA expression by functioning as a ceRNA, thereby impacting the initiation and progression of NSCLC[23,24].

However, there is still little research on whether PCCA-DT is involved in regulating the invasive phenotype of NSCLC. This study further identifies the downstream effector factors involved in the function of PCCA-DT. We hypothesize that PCCA-DT promotes the malignant progression of NSCLC cells by targeting the miR-20a-5p/CCND1 axis. The PCCA-DT/miR-20a-5p/CCND1 pathway is expected to become a highly attractive potential target for NSCLC therapeutics.

Materials and Methods

Sample collection

We conduct the following research in accordance with the guidelines or standards of the Declaration of Helsinki. This study collected a total of 85 cancer tissues and adjacent normal lung tissues from patients with non-small cell lung cancer (NSCLC) who underwent surgical treatment at the Affiliated Tumor Hospital of Guangzhou Medical University. The research protocol has been approved by the hospital's Ethics Committee, and all patients had not received local or systemic anti-tumor treatment before surgery and signed a written informed consent form. Clinical tissue samples were preserved in liquid nitrogen after collection.

Cell culture

The non-small cell lung cancer cell lines H1975, A549, H1650, H23, and PC9 were cultured in RPMI-1640 medium containing 10% fetal bovine serum (Gibco; Thermo Fisher, USA). All human non-tumor bronchial epithelial cells BEAS-2B were cultured using bronchial epithelial cell-specific medium supplemented with

10% fetal bovine serum (Lonza/Clonetics, Maryland, USA). All cell lines were purchased from the American Type Culture Collection (ATCC, Virginia, USA).

Cell transfection

MiR-20a-5p mimics, mimics control, miR-20a-5p inhibitors, and inhibitors control were all purchased from Ruibo Biotechnology (Guangzhou, China). LncRNA PCCA-DT specific small interfering RNA (si-PCCA-DT) and negative control siRNA (si-NC) were provided by Ruibo Biotechnology (Guangzhou, China), with sequences detailed in Table 1. CCND1 overexpression in NSCLC cells was achieved by transfecting the pcDNA3.1-CCND1 plasmid (Regenogen, Guangzhou, China). All transfection experiments were performed using Lipofectamine® 3000 transfection reagent (Invitrogen).

Table 1. The sequences of siRNAs.

siRNA	Sequence (5'-3')
si-PCCA-DT #1	GACCAAGACUCCAUCUCAATT
si-PCCA-DT#2	CUCCUUUCCAGUUCGAUGUTT
si-PCCA-DT#3	GGAAGUUGAGGACACAGCATT
si-NC	UUCUCCGAACGUGUCACGUTT

Quantitative real-time polymerase chain reaction (qRT-PCR)

Using the RNAiso small RNA extraction kit to extract small RNA, and using the Mir-XTM miRNA first strand synthesis kit for cDNA reverse transcription. The Mir-X miRNA qRT-PCR TB Green® kit was used for the PCR amplification of miR-20a-5p, with U6 snRNA as the internal reference gene. Total RNA was extracted using TRIzol® reagent, and the PrimeScript™ reverse transcription kit was used for the reverse transcription reaction, with the SYBR Green qPCR Master Mix kit for the PCR amplification of PCCA-DT and CCND1, using GAPDH as the internal reference gene. All data were analyzed using the $2^{-\Delta\Delta Cq}$ method, and the primer sequences are detailed in Table 2.

Table 2. Primer sequences used for qRT-PCR.

Gene	Forward	Reverse
PCCA-DT	CTCCACACCTTTTCACCT	TGCTCTCCTTTGCTACTG
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
CCND1	AGTTGTTGGGGCTCCTCAG	AGACCTTCGTTGCCCTCTGT
CDKN1A	CATGGGTTCTGACGGACATC	TGCCGAAGTCAGTTCCTTGT
CDKN1B	CGGGAAGTCACTGGAAACAT	AAACATGGCAGTGACACCAA
MYC	CACCGAGTCGTAGTCGAGGT	TTTCGGGTAGTGGAAAACCA
RB1	TCAGTTGGTCCTTCTCGGTC	TGTGAACATCGAATCATGGAA
TP53	TGTTTCCTGACTCAGAGGGG	GAGCGTGCTTTCCACGAC
E2F3	AAGTGCCTGACTCAATAGAGAGCC	AGTCTCTTCTGGACATAAGTAAACCT CA
CCND3	TTGAGCTTCCCTAGGACCAG	TGACCATCGAAAAACTGTGC
CDK6	CACTCCAGGCTCTGGAACTT	TGGAGACCTTCGAGCACC
CCNB1	TTCATCAAGCAGTGATGTATCTGA	AAGAAGCCTGGCCTCAGAAG
miR-20a-5p	ACGTTGTCTAGCTTATCAGACTG	AATGGTTGTTCTCCACA CTCTC
U6	GCTTCGGCAGCACATATACTAAAAT	CGCTTCACGAATTTGCGTGTCAT

Cell Counting Kit-8 (CCK-8) assay

The experiment was conducted according to the methods described in previous literature [25]. A cell suspension was prepared and adjusted to a density of 1×10^4 cells/mL, and 100 μL of the suspension was inoculated into a 96-well plate, incubated in a 37°C incubator. Cells were collected daily for 5 consecutive days, and cell proliferation was assessed by adding 10 μL of CCK-8 solution (Beyotime; Shanghai, China). After continuing to culture for 2 hours, the absorbance value at a wavelength of 450 nm was measured using a microplate reader.

Colony formation assay

According to previous research method [26], a colony formation experiment was conducted. The transfected cells were resuspended in fresh culture medium and seeded into a 6-well plate, with 2 mL of suspension containing 500 cells added to each well. After culturing under normal culture conditions for 14 days, 0.1% crystal violet staining was performed, and the newly formed colonies were counted using an optical microscope.

Edu assay

According to the method described in the Edu kit, perform the Edu experiment. Resuspend the transfected cells in fresh culture medium and inoculate them into a 96-well plate, adding 100 μL of suspension containing 4×10^3 cells per well. Place in a 37°C incubator and culture overnight until they reach the normal growth stage of apposition. Process and incubate using the Edu kit (Beyotime; Shanghai, China), and finally randomly select fields under a fluorescence inverted microscope to take and save images.

Cell cycle assay

According to previous research methods[27], conduct cell cycle detection experiments. Seed 5*10^5 cells per well evenly in a 6-well plate, culture the cells overnight, then perform transfection with siRNA/plasmids, and after successful transfection, replace with serum-free medium for continued culture, followed by starvation treatment for 12 hours. After 12 hours, switch to complete culture medium and continue to culture for 24 hours. Subsequently, collect the cells and process them using a cell cycle detection kit (Beyotime; Shanghai, China) for incubation, and detect at the lowest sample loading speed on a flow cytometer (BD Biosciences).

Xenograft tumor growth model

The experimental scheme involving nude mice in this study was approved by the Institutional Animal Care and Use Committee of the Affiliated Tumor Hospital of Guangzhou Medical University. The short hairpin RNA targeting PCCA-DT and control shRNA (both purchased from Ruibo Biotechnology) were cloned into the pLKO.1 vector and transfected into 293T cells using Lipofectamine® 3000. After 48 hours of culture, the lentiviral particles were collected and used to infect A549 cells, which were ultimately selected for stable transfection of the A549 cell line through puromycin selection. The A549 cells stably overexpressing sh-PCCA-DT or sh-NC were subcutaneously inoculated into BALB/c nude mice (Beijing Huafukang Biotechnology). The transplanted tumor size was measured weekly, and the tumor volume was calculated. After five weeks of inoculation, all nude mice were euthanized, and the transplanted tumor tissues were collected. The animals were only euthanized by cervical dislocation, and no chemical agents (such as injectable anesthetics or perfusion fixatives) were used for euthanasia or sacrifice.

Nuclear/Cytoplasmic fractionation experiment

Using nuclear and cytoplasmic RNA isolation kits to separate nuclear and cytoplasmic components from non-small cell lung cancer (NSCLC) cells. After extracting RNA from the nucleus and cytoplasm, qRT-PCR technology is used to detect the subcellular localization of PCCA-DT.

Bioinformatics prediction

Using the miRDB and StarBase databases to predict the interaction between PCCA-DT and miR-20a-5p; at the same time, screening the downstream target genes of miR-20a-5p through the miRDB and TargetScan databases.

Luciferase reporter assay

As previously described in the literature[28], we conducted luciferase reporter gene experiments. The wild-type sequences of PCCA-DT and CCND1 were constructed by Reebio Biotechnology Co., Ltd., and cloned into the psiCHECK™-2 vector, resulting in WT-PCCA-DT and WT-CCND1 recombinant luciferase reporter vectors. The mutant vectors MUT-PCCA-DT and MUT-CCND1 were constructed using the same method. In the reporter gene experiments, miR-20a-5p mimics or mimic controls were co-transfected with wild-

type or mutant vectors into NSCLC cells. After 48 hours of culture, luciferase activity was assessed using a dual-luciferase reporter assay system.

Western blotting

According to the methods described in previous literature[29], a Western blotting experiment was conducted. First, the cultured cells were lysed using RIPA lysis buffer containing protease inhibitors to extract total protein. Subsequently, a BCA kit was used for protein quantification analysis of each sample. A 20μg protein sample was taken for 10% SDS-PAGE electrophoresis, and after electrophoresis, it was transferred to a polyvinylidene fluoride membrane. The membrane was blocked in 5% non-fat milk for 2 hours, followed by overnight incubation with CCND1 monoclonal antibody or GAPDH monoclonal antibody at 4°C. The next day, after incubation with the secondary antibody, a novel ultra-sensitive ECL chemiluminescence kit was used to detect the protein signal.

Statistical analysis

The experimental results are presented as mean ± standard deviation, with normality assessments conducted prior to data analysis. For comparisons between two groups, the Student's t-test is utilized, whereas one-way ANOVA, accompanied by Tukey's post hoc test, is employed for comparisons among multiple groups. Survival curves are generated utilizing the Kaplan-Meier approach, and intergroup comparisons are performed using the log-rank test. A P value of less than 0.05 is deemed statistically significant.

Results

The aim of this study is to analyze the expression characteristics and biological functions of PCCA-DT in non-small cell lung carcinoma (NSCLC), and further explore the specific mechanisms of its oncogenic role in NSCLC. Our research reveals a novel competing endogenous RNA (ceRNA) regulating pathway, which is composed of PCCA-DT, miR-20a-5p, and CCND1.

PCCA-DT is upregulated in NSCLC tumor tissue

We screened lncRNAs from the lncRNA chip data of 5 pairs of clinical matched samples with p-value < 0.05 and fold change > 1.5, among which the Fc value of PCCA-DT was 1.915225 and the p-value was 0.037389 (Table 3, Figure 1A). By analyzing the expression level of PCCA-DT in lung cancer using the TCGA database, the results showed that it was significantly upregulated in both paired and unpaired lung cancer samples (Figure 1B). To verify the accuracy of the data, we detected the tumor and normal lung tissues of 85 pairs of NSCLC patients using qRT-PCR, and the results showed that the expression of PCCA-DT in NSCLC tissues was significantly higher than that in normal tissues, confirming the consistency of the chip and TCGA results (Figure 1C). In addition, we performed qRT-PCR on six NSCLC cell lines and normal bronchial epithelial cells, finding that PCCA-DT expression was significantly

upregulated in the H1975, A549, and H1299 cell lines, while no significant changes were observed in other cell lines (Figure 1D). Therefore, H1975 and A549 cells were selected for subsequent experiments.

Table3

Analysis of lncRNA chip for 5 pairs of NSCLC tissue samples and corresponding adjacent normal tissues. The Foldchange value of PCCA-DT is 1.9152, and the p-value is 0.0374.

Gene Name	Foldchange	P-values
MACC1-AS1	3.4689	0.0013
LINC00313	1.9364	0.0284
PCCA-DT	1.9152	0.0374
LINC01061	1.5637	0.0412
MORF4L2-AS1	1.5236	0.0434
LINC01002	1.6083	0.0321
TTC3-AS1	1.5849	0.0272
RUNX1-IT1	1.8489	0.0313
LINC00342	2.7303	0.0497
CREB3L2-AS1	1.8073	0.0002
ABHD11-AS1	1.5881	0.0015
LINC02912	1.5186	0.0035
LINC00609	1.6875	0.0061
PEG13	1.5547	0.0091
LINC01436	2.4060	0.0101
KIF26B-AS1	1.9879	0.0108
LINC00884	1.5437	0.0133
GORAB-AS1	1.6698	0.0301
KIF26B-AS1	1.5685	0.0327
CBR3-AS1	1.5737	0.0457
GMDS-DT	1.5633	0.0481
C8orf44	1.7519	0.0195
SNHG18	2.3295	0.0382

PCCA-DT regulates theproliferation of NSCLC cells

We interfered with the expression of PCCA-DT by transfecting specific siRNA, using three targeted siRNAs to prevent off-target effects. qRT-PCR validated the interference efficiency of siRNA in H1975 and A549 cell lines, and the results showed that si-PCCA-DT#1 and si-PCCA-DT#3 had stable interference efficiency in both cell lines, thus used for subsequent experiments (Figure 2A). To verify the role of PCCA-DT in NSCLC cell lines, we employed the CCK-8 assay and cell colony formation assay to study its effect on cell proliferation. The results showed that after siRNA interference, the cell proliferation rate and colony formation ability of the experimental group were significantly reduced (Figure 2B, 2C).

PCCA-DT promotes the transition of NSCLC cell cycle from G1 to S phase

Our findings demonstrate that PCCA-DT influences cellular dynamics primarily by inhibiting apoptotic processes and facilitating cell progression. Through Edu cell proliferation assays and cell cycle analyses, we established that PCCA-DT significantly impacts the cell cycle in NSCLC. Upon the downregulation of PCCA-DT expression, the results from the Edu assays revealed a reduction in cell count and diminished DNA replication activity, implying that the cells transition into the S phase at a slower pace (Figure 3A). Additionally, direct cell cycle assessments illustrated that PCCA-DT enhances the transition of H1975 and A549 cells from the G0/G1 phase into the S phase (Figure 3B), thereby validating its molecular function in promoting cell cycle progression. Collectively, these findings suggest that PCCA-DT exerts a pro-carcinogenic influence on NSCLC cell proliferation and the regulation of the cell cycle.

PCCA-DT regulates the cell cycle of NSCLC cells by modulating CCND1

After transfecting specific siRNA to downregulate PCCA-DT, qRT-PCR detected cell cycle-related genes, such as CCND1, CDKN1A, and CDKN1B; qRT-PCR and WB experiments showed that the transcription and translation levels of CCND1 in H1975 and A549 cells decreased with the decline of PCCA-DT, indicating that CCND1 is regulated by PCCA-DT (Figure 4A, 4B). To verify the positive correlation between the two, the transfection efficiency of the CCND1 overexpression plasmid was detected (Figure 5A). Through CCK-8 and cell colony formation experiments, it was found that the overexpression of CCND1 can counteract the inhibitory effect of PCCA-DT downregulation on cell proliferation (Figure 5B, 5C). In addition, cell cycle experiments showed that the interference of PCCA-DT led to a restriction of the NSCLC cell cycle, while the overexpression of CCND1 could restore this process (Figure 5D). In summary, PCCA-DT downregulation inhibits NSCLC cell proliferation by regulating CCND1.

PCCA-DT regulates the expression of CCND1 by modulating miR-20a-5p

We studied the role of PCCA-DT in NSCLC cells. Through cell separation experiments and qRT-PCR analysis, it was found that PCCA-DT is mainly located in the cytoplasm (Figure 6A). Further exploring its regulatory function as a ceRNA, we screened 76 and 51 potential miRNAs using the TargetScan (https://www.targetscan.org/vert) and lncRNASNP (http://bioinfo.life.hust.edu.cn/lncRNASNP#!/) databases, selecting 6 overlapping miRNAs for experimental validation (Figure 6B). The results showed that after downregulating PCCA-DT, the expression of miR-20a-5p significantly increased in H1975 and A549 cells, while the other 5 miRNAs did not show significant changes, thus miR-20a-5p was chosen for

further analysis (Figure 6C). We predicted the binding sites of miR-20a-5p with PCCA-DT in the lncRNASNP and StarBase (http://starbase.sysu.edu.cn/) databases; through dual-luciferase assays, the results indicated that the miR-20a-5p mimic reduced luciferase activity but did not inhibit the activity of Mutant PCCA-DT (Figure 6D).

Our investigation focused on the influence of miR-20a-5p and CCND1 on the functionality of PCCA-DT within NSCLC cells. We discovered that PCCA-DT modulates the expression of CCND1 through its interaction with miR-20a-5p. By transfecting miR-20a-5p mimics, miR-20a-5p was overexpressed in NSCLC cells transfected with miR-20a-5p mimics compared to control mimic-transfected cells (Figure 7A). CCK8 assays and colony formation assays showed that cell proliferation was significantly inhibited in cells transfected with miR-20a-5p mimics (Figures 7B, 7C), and cell cycle analysis indicated that overexpression of miR-20a-5p inhibited the G1/S phase transition (Figure 7D). Database prediction results indicated that CCND1 is a potential target of miR-20a-5p, and dual-luciferase assays validated the relationship between the two, showing that miR-20a-5p reduced the luciferase activity of Wild-type CCND1 (Figure 7E). When miR-20a-5p was overexpressed, CCND1 was significantly reduced at both transcriptional and translational levels (Figure 7F).

In summary, our experimental results support that CCND1 is a direct target of miR-20a-5p, and miR-20a-5p is sponge by PCCA-DT. We explored whether PCCA-DT regulates CCND1 expression by interacting with miR-20a-5p. The downregulation of PCCA-DT significantly reduced the expression of CCND1 in non-small cell lung carcinoma (NSCLC) cells (Figure 4A, 4B). Through rescue experiments, we assessed the transfection efficiency of the miR-20a-5p inhibitor (Figure 8A) and found that it could reverse the decrease in CCND1 expression dependent on PCCA-DT (Figure 8B). The Western blot experiment also confirmed this result (Figure 8F). Further proliferation and colony formation experiments showed that in cells transfected with the miR-20a-5p inhibitor, the reduced level of miR-20a-5p counteracted the inhibition of proliferation and colony formation ability (Figure 8C, 8D). In addition, cell cycle experiments indicated that the downregulation of PCCA-DT restricted the G1/S transition in NSCLC cells, and this process could be restored by the miR-20a-5p inhibitor (Figure 8E).

PCCA-DT knockout inhibits tumor growth in vivo

To explore the role of PCCA-DT in the growth of NSCLC in vivo, we constructed a xenograft tumor model. Compared to the sh-NC group, mice with stable knockdown of PCCA-DT exhibited a slowed tumor growth rate; additionally, the weight of the xenograft tumors in the PCCA-DT deletion group was significantly reduced (Figures 9A, 9B). At the molecular level, compared to the sh-NC group, the expression of PCCA-DT in the xenograft tumors of the PCCA-DT silenced group was decreased, and the expression level of CCND1 was also reduced (Figures 9C, 9D). These results confirm that the deletion of PCCA-DT can effectively inhibit tumor growth in vivo.

Discussion

A large body of evidence indicates that there are nearly 100,000 lncRNAs in the human genome, which are abnormally expressed in various tumors and may be used for cancer diagnosis and prognosis[30]. So far, a large number of lncRNAs have been discovered and widely reported in NSCLC, providing new perspectives for exploring the molecular pathways of NSCLC pathogenesis[31,32,33]. Specifically, lncRNAs play both positive and negative regulatory roles in the malignant progression of NSCLC[34], becoming potential targets. This study found that PCCA-DT was significantly upregulated in RNA-seq, and TCGA and experimental data confirmed its upregulation in NSCLC (Figure 1, Table 3). We explored the function of PCCA-DT in regulating the cell cycle of NSCLC, and the results showed that it may be an independent prognostic biomarker. We speculate that NSCLC patients characterized by elevated PCCA-DT expression levels may have poor clinical outcomes. Studies indicate that PCCA-DT promotes the expression of CCND1 by binding with miR-20a-5p and being negatively regulated by it, thereby promoting cell proliferation and cell cycle progression. Taken together, PCCA-DT, miR-20a-5p, and CCND1 may regulate each other through the ceRNA mechanism.

In the present investigation, the expression profile, related functions, and molecular mechanisms of lncRNA PCCA-DT in NSCLC have not been researched or reported. Functionally, the loss or downregulation of PCCA-DT exerts a tumor-suppressive effect in NSCLC cell lines, as it regulates cell growth and the progression of the cell cycle (Figures 2, 3). Cell cycle-related genes were obtained from relevant literature[35,36], such as CCND1, CDKN1A, and CDKN1B; similarly, the downregulation of PCCA-DT in NSCLC inhibits CCND1 at both transcriptional and translational levels, indicating that the expression of CCND1 is regulated by the expression level of PCCA-DT (Figure 4). In H1975 cells, overexpression of CCND1 can reverse the decreased cell proliferation ability and restricted cell cycle progression caused by the downregulation of PCCA-DT, suggesting that CCND1 is a target gene of PCCA-DT (Figure 5). The mechanism by which lncRNA PCCA-DT exerts its functional role in NSCLC is explored. Nucleocytoplasmic separation experiments demonstrate that PCCA-DT is a cytoplasmic lncRNA, providing a basic theoretical framework to support lncRNA as a ceRNA or miRNA sponge. Through online databases (TargetScan and lncRNASNP), complementary binding sites between PCCA-DT and miR-20a-5p were identified, and experimental results indicate that PCCA-DT directly binds to miR-20a-5p and is regulated by it (Figures 6, 7); this suggests that lncRNA PCCA-DT may have ceRNA functions, thereby regulating CCND1. Studies have shown that many RNA transcripts exert their functions by competitively binding common miRNAs as ceRNAs[37]. Therefore, we further searched for miRNA targets in online databases (TargetScan and lncRNASNP), and the search results along with further experimental results indicate that CCND1 is a binding target of miR-20a-5p (Figure 7F). Further experimental results show that miR-20a-5p can functionally inhibit NSCLC cell proliferation and cell cycle progression, and regulate the expression of CCND1 (Figures 7C, D, E). This is consistent with our expectations; experimental results indicate that CCND1 is a functional target of miR-20a-5p. Recovery experiments validated the interrelationship among lncRNA PCCA-DT, miR-20a-5p, and CCND1. Experimental results show that miR-20a-5p inhibitors can offset the PCCA-DT-dependent decrease in CCND1 expression at the transcriptional level, and similar recovery was observed in functional

experiments on H1975 cells (Figure 8). Overall, the downregulation of lncRNA PCCA-DT inhibits the occurrence and development of NSCLC by targeting the miR-20a-5p/CCND1 axis.

In summary, lncRNA PCCA-DT is expected to become a diagnostic biomarker and therapeutic target for NSCLC. Through this study, we have gained new insights and understanding of the expression profile of lncRNA PCCA-DT in NSCLC and the molecular mechanisms it plays. The subcellular localization of lncRNA provides clues for mediating its biological functions[37,38]. lncRNA located in the nucleus interacts with various molecules such as DNA, RNA, and proteins to regulate gene transcription in a cis or trans manner, affecting mRNA splicing, stability, and translation, thereby regulating gene expression[37,39]. For lncRNA located in the cytoplasm, the ceRNA theory is the most mature and established mechanism to explain its activity. lncRNA mainly regulates gene expression at the post-transcriptional level. They can sequester miRNA through their miRNA response elements, thereby inhibiting mRNA expression through miRNA-triggered mRNA dysregulation or translation repression, thus controlling gene expression at the post-transcriptional level. Therefore, they can regulate mRNA translation and degradation or participate in the regulation of intracellular signaling pathways[37,39,40]. We confirmed through nuclear/cytoplasmic separation experiments that lncRNA PCCA-DT is a cytoplasmic lncRNA, providing a basic theoretical framework for lncRNA as a ceRNA or miRNA sponge. The ceRNA network mechanism consists of lncRNA, miRNA, and mRNA, predicting the downstream miRNA targets of miR-20a-5p through online databases. Experimental results show that there is an interaction among PCCA-DT, miR-20a-5p, and CCND1, and they may mutually regulate each other through the ceRNA mechanism. However, our study lacks RIP experiments to detect and verify the direct binding of PCCA-DT with miRNA. But from the current experimental results, it still provides some support. However, our experiments have functionally demonstrated that CCND1 is a target of miR-20a-5p. CCND1 is indirectly controlled by PCCA-DT in the miR-20a-5p-mediated mechanism. Therefore, in NSCLC cells, the interaction among lncRNA PCCA-DT, miR-20a-5p, and CCND1 forms a closed ceRNA pathway loop. Studies have shown that miR-20a-5p is involved in the occurrence and progression of tumors; for example, miR-20a-5p plays an anti-cancer role in human high-incidence cancers such as cervical cancer, breast cancer, and leukemia[41]. The relevant roles and mechanisms of miR-20a-5p in NSCLC have been reported less; however, some studies indicate that miR-20a-5p inhibits NSCLC tumor angiogenesis activity through the RRM2-mediated PI3K/Akt signaling pathway, which is a potential effective molecular target for NSCLC[42]. From our study, functionally, miR-20a-5p is negatively regulated by PCCA-DT in NSCLC cell lines, and overexpression of miR-20a-5p significantly inhibits NSCLC cell proliferation in vitro and blocks the transition of the cell cycle from G1 to S phase, which is consistent with the trends reported in the literature[42]. Our current research reveals the role of miR-20a-5p in NSCLC cells and the corresponding intrinsic mechanisms. It is worth noting that Cyclin D1 is a protein encoded by the CCND1 gene, located on chromosome 11q13, and is a key checkpoint in the G1 phase; it is a critical component of physiological regulation of the cell cycle, and abnormal regulation can lead to abnormal transition from G1 to S phase[19,20]. CCND1, as one of the oncogenes, is significantly amplified in several solid tumor tissues such as esophageal cancer[42], breast cancer[36], and melanoma[43]. CCND1 is closely related to the biological behaviors and prognosis of malignant tumors, including occurrence and development,

invasion and metastasis, induction of angiogenesis, and treatment resistance[36,42,43,44]. It is undeniable that CCND1 is highly expressed in NSCLC and promotes its occurrence and development, not only through molecular mechanisms including ubiquitination pathways[45], PI3K/AKT pathways, Wnt/β-catenin pathways, etc[46]. There are also reports that lncRNA regulates CCND1 through miRNA sponging, thereby promoting NSCLC[47]; however, this study found that PCCA-DT, miR-20a-5p, and CCND1 in NSCLC may constitute a closed ceRNA mechanism loop, thereby exerting its pro-cancer effect; this may represent a new ceRNA molecular mechanism in the occurrence and development of NSCLC.

We conducted a series of experiments and found that lncRNA PCCA-DT can promote the malignant characteristics of NSCLC cells by regulating the G1 phase of the cell cycle, which is a potential molecular target. We revealed the significance of regulating the lncRNA PCCA-DT/miR-20a-5p/CCND1 axis in NSCLC progression and indicated that PCCA-DT exerts oncogenic behavior in NSCLC cells by competitively binding to miR-20a-5p.

This study has a limitation: only two groups were set up in the nude mouse xenograft model, with only 3 samples in each group, which is insufficient sample size; and the expression of CCND1 in tumor tissue was not verified. We will address this limitation in future research.

Conclusion

This study confirms that PCCA-DT is a key molecule regulating the malignant progression of non-small cell lung carcinoma (NSCLC). This molecule upregulates CCND1 expression by directly binding to miR-20a-5p, thereby regulating the malignant biological behaviors of NSCLC. Therefore, the PCCA-DT/miR-20a-5p/CCND1 signaling axis is expected to become an important potential target for NSCLC therapeutics.

Declarations

All participants involved in this study were informed of the purpose and procedures of the research and have provided their written informed consent.

All cell lines used in this study were authenticated by STR profiling prior to experimentation and were routinely confirmed to be free of mycoplasma contamination.

Ethics Statement

All procedures performed in studies involving human participants were performed in accordance with the ethical stand ards of the Research Ethics Committee of Affiliated Tumor Hospital of Guangzhou Medical University and conformed to the principles of the Declaration of Helsinki.

This study and experimental procedures were approved by the Experimental Animal Ethics Committee of Hubei Provincial Center for Disease Control and Prevention. All animal rearing and experiments were

conducted in strict accordance with the institutional code of care and use of laboratory animals.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available in the

TargetScan (https://www.targetscan.org/vert) and lncRNASNP (http://bioinfo.life.hust.edu.cn/lncRNASNP#!/) databases, and StarBase (http://starbase.sysu.edu.cn/) repository.

Acknowledgments

None

Conflicts of interest

None to declare

Funding

1. Guangzhou Science and Technology Bureau (2023A04J0597)

2. Guangzhou Health Commission (20231A011098)

References

1. Siegel RL, Miller KD, Wagle NS. Cancer statistics, 2023. CA Cancer J Clin. 2023 Jan;73(1):17-48.
2. Yoon SM, Shaikh T, Hallman M. Therapeutic management options for stage III non-small cell lung cancer. World journal of clinical oncology 2017; 8:1-20.
3. Liang W, Liu J, He J. Driving the Improvement of Lung Cancer Prognosis. Cancer cell 2020; 38:449-51.
4. The Lancet. Lung cancer: some progress, but still a lot more to do. Lancet. 2019 Nov 23;394(10212):1880.
5. Wood DE, Kazerooni EA, Aberle D, Berman A, etc. NCCN Guidelines® Insights: Lung Cancer Screening, Version 1.2022. J Natl Compr Canc Netw. 2022 Jul;20(7):754-764.
6. Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. Nature 2018; 553:446-54.
7. Guttman M, Donaghey J, Carey BW, et al. lincRNAs act in the circuitry controlling pluripotency and differentiation. Nature. 2011 Aug 28;477(7364):295-300.
8. Bhan A, Soleimani M, Mandal SS. Long Noncoding RNA and Cancer: A New Paradigm. Cancer Res. 2017 Aug 1;77(15):3965-3981.

9. Ricciuti B, Mencaroni C, Paglialunga L, et al. Long noncoding RNAs: new insights into non-small cell lung cancer biology, diagnosis and therapy. Med Oncol. 2016 Feb;33(2):18.
10. Baldini F, Calderoni M, Vergani L, Modesto P, Florio T, Pagano A. An Overview of Long Non-Coding (lnc)RNAs in Neuroblastoma. International journal of molecular sciences 2021; 22.
11. Ghafouri-Fard S, Hussen BM, Gharebaghi A, Eghtedarian R, Taheri M. LncRNA signature in colorectal cancer. Pathology, research and practice 2021; 222:153432.
12. Coellar JD, Long J, Danesh FR. Long Noncoding RNAs and Their Therapeutic Promise in Diabetic Nephropathy. Nephron 2021:1-11.
13. Ye R, Tang R, Gan S, Li R, Cheng Y, Guo L, et al. New insights into long non-coding RNAs in non-small cell lung cancer. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2020; 131:110775.
14. Zhou H, Feng B, Abudoureyimu M, Lai Y, Lin X, Tian C, et al. The functional role of long non-coding RNAs and their underlying mechanisms in drug resistance of non-small cell lung cancer. Life sciences 2020; 261:118362.
15. Fang C, Wang L, Gong C, Wu W, Yao C, Zhu S. Long non-coding RNAs: How to regulate the metastasis of non-small-cell lung cancer. Journal of cellular and molecular medicine 2020; 24:3282-91.
16. Bao Z, Yang Z, Huang Z, et al. LncRNADisease 2.0: an updated database of long non-coding RNA-associated diseases. Nucleic Acids Res. 2019 Jan 8;47(D1):D1034-D1037.
17. Williams GH, Stoeber K. The cell cycle and cancer. J Pathol. 2012 Jan;226(2):352-64.
18. Sun Y, Liu Y, Ma X, et al. The Influence of Cell Cycle Regulation on Chemotherapy. Int J Mol Sci. 2021 Jun 28;22(13):6923.
19. González-Ruiz L, González-Moles MÁ, González-Ruiz I, et al. An update on the implications of cyclin D1 in melanomas. Pigment Cell Melanoma Res. 2020 Nov;33(6):788-805.
20. Qie S, Diehl JA. Cyclin D1, cancer progression, and opportunities in cancer treatment. J Mol Med (Berl). 2016 Dec;94(12):1313-1326.
21. Pasquinelli AE. Molecular biology. Paring miRNAs through pairing. Science 2010; 328:1494-5.
22. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nature reviews Genetics 2004; 5:522-31.
23. Zhang Y, Li J, Wang H, et al. LncRNA LINC01234 drives glycolytic reprogramming via sponging miR-145-5p to regulate HK2 in non-small cell lung cancer. Molecular Cancer, 2025, 24(1): 23.
24. Liu TT, Li R, Liu X, Zhou XJ, Huo C, Li JP, et al. LncRNA XIST acts as a MicroRNA-520 sponge to regulate the Cisplatin resistance in NSCLC cells by mediating BAX through CeRNA network. International journal of medical sciences 2021; 18:419-31.
25. Li T, Zhao H, Zhou H, Geng T. LncRNA LINC00857 strengthens the malignancy behaviors of pancreatic adenocarcinoma cells by serving as a competing endogenous RNA for miR-340-5p to upregulate TGFA expression. PloS one 2021; 16:e0247817.

26. Chen L, Gao H, Liang J, Qiao J, Duan J, Shi H, et al. miR-203a-3p promotes colorectal cancer proliferation and migration by targeting PDE4D. American journal of cancer research 2018; 8:2387-401.
27. Shi Q, Li Y, Li S, et al. LncRNA DILA1 inhibits Cyclin D1 degradation and contributes to tamoxifen resistance in breast cancer. Nat Commun. 2020 Nov 2;11(1):5513.
28. Niu X, Yang B, Liu F, Fang Q. LncRNA HOXA11-AS promotes OSCC progression by sponging miR-98-5p to upregulate YBX2 expression. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2020; 121:109623.
29. Huang N, Guo W, Ren K, Li W, Jiang Y, Sun J, et al. LncRNA AFAP1-AS1 Supresses miR-139-5p and Promotes Cell Proliferation and Chemotherapy Resistance of Non-small Cell Lung Cancer by Competitively Upregulating RRM2. Frontiers in oncology 2019; 9:1103.
30. Xu J, Bai J, Zhang X, et al. A comprehensive overview of lncRNA annotation resources. Briefings in bioinformatics 2017; 18:236-49.
31. Ricciuti B, Mencaroni C, Paglialunga L, et al. Long noncoding RNAs: new insights into non-small cell lung cancer biology, diagnosis and therapy. Med Oncol. 2016 Feb;33(2):18.
32. Chen J, Wang R, Zhang K, et al. Long non-coding RNAs in non-small cell lung cancer as biomarkers and therapeutic targets. J Cell Mol Med. 2014 Dec;18(12):2425-36.
33. Wu T, Yin X, Zhou Y, et al. Roles of noncoding RNAs in metastasis of nonsmall cell lung cancer: A mini review. J Cancer Res Ther. 2015 Aug;11 Suppl 1:C7-10.
34. Lu T, Wang Y, Chen D, et al. Potential clinical application of lncRNAs in non-small cell lung cancer. Onco Targets Ther. 2018 Nov 13;11:8045-8052.
35. Hume S, Grou CP, Lascaux P, et al. The NUCKS1-SKP2-p21/p27 axis controls S phase entry. Nat Commun. 2021 Nov 29;12(1):6959.
36. Shi Q, Li Y, Li S, et al. LncRNA DILA1 inhibits Cyclin D1 degradation and contributes to tamoxifen resistance in breast cancer. Nat Commun. 2020 Nov 2;11(1):5513.
37. Statello L, Guo CJ, Chen LL, et al. Gene regulation by long non-coding RNAs and its biological functions. Nat Rev Mol Cell Biol. 2021 Feb;22(2):96-118.
38. Guo CJ, Xu G, Chen LL. Mechanisms of Long Noncoding RNA Nuclear Retention. Trends Biochem Sci. 2020 Nov;45(11):947-960.
39. Zhang K, Shi ZM, Chang YN, et al. The ways of action of long non-coding RNAs in cytoplasm and nucleus. Gene. 2014 Aug 15;547(1):1-9.
40. Rashid F, Shah A, Shan G. Long Non-coding RNAs in the Cytoplasm. Genomics Proteomics Bioinformatics. 2016 Apr;14(2):73-80.
41. Huang W, Wu X, Xiang S, et al. Regulatory mechanism of miR-20a-5p expression in Cancer. Cell Death Discov. 2022 May 16;8(1):262.
42. Cancer Genome Atlas Research Network; Analysis Working Group: Asan University; BC Cancer Agency; et al. Integrated genomic characterization of oesophageal carcinoma. Nature. 2017 Jan

12;541(7636):169-175.
43. Liu J, Lin J, Wang X, et al. CCND1 Amplification Profiling Identifies a Subtype of Melanoma Associated With Poor Survival and an Immunosuppressive Tumor Microenvironment. Front Immunol. 2022 Jul 1;13:725679.
44. Ding H, Wang Y, Zhang H. CCND1 silencing suppresses liver cancer stem cell differentiation and overcomes 5-Fluorouracil resistance in hepatocellular carcinoma. J Pharmacol Sci. 2020 Jul;143(3):219-225.
45. Zhang Z, Cui Z, Xie Z, et al. Deubiquitinase USP5 promotes non-small cell lung cancer cell proliferation by stabilizing cyclin D1. Transl Lung Cancer Res. 2021 Oct;10(10):3995-4011.
46. Zhao M, Xu P, Liu Z, et al. Retraction Note: Dual roles of miR-374a by modulated c-Jun respectively targets CCND1-inducing PI3K/AKT signal and PTEN-suppressing Wnt/β-catenin signaling in non-small-cell lung cancer. Cell Death Dis. 2021 Jul 7;12(7):681.
47. Ding H, Luo Y, Hu K, et al. Linc00467 promotes lung adenocarcinoma proliferation via sponging miR-20b-5p to activate CCND1 expression. Onco Targets Ther. 2019 Aug 21;12:6733-6743.

Figures

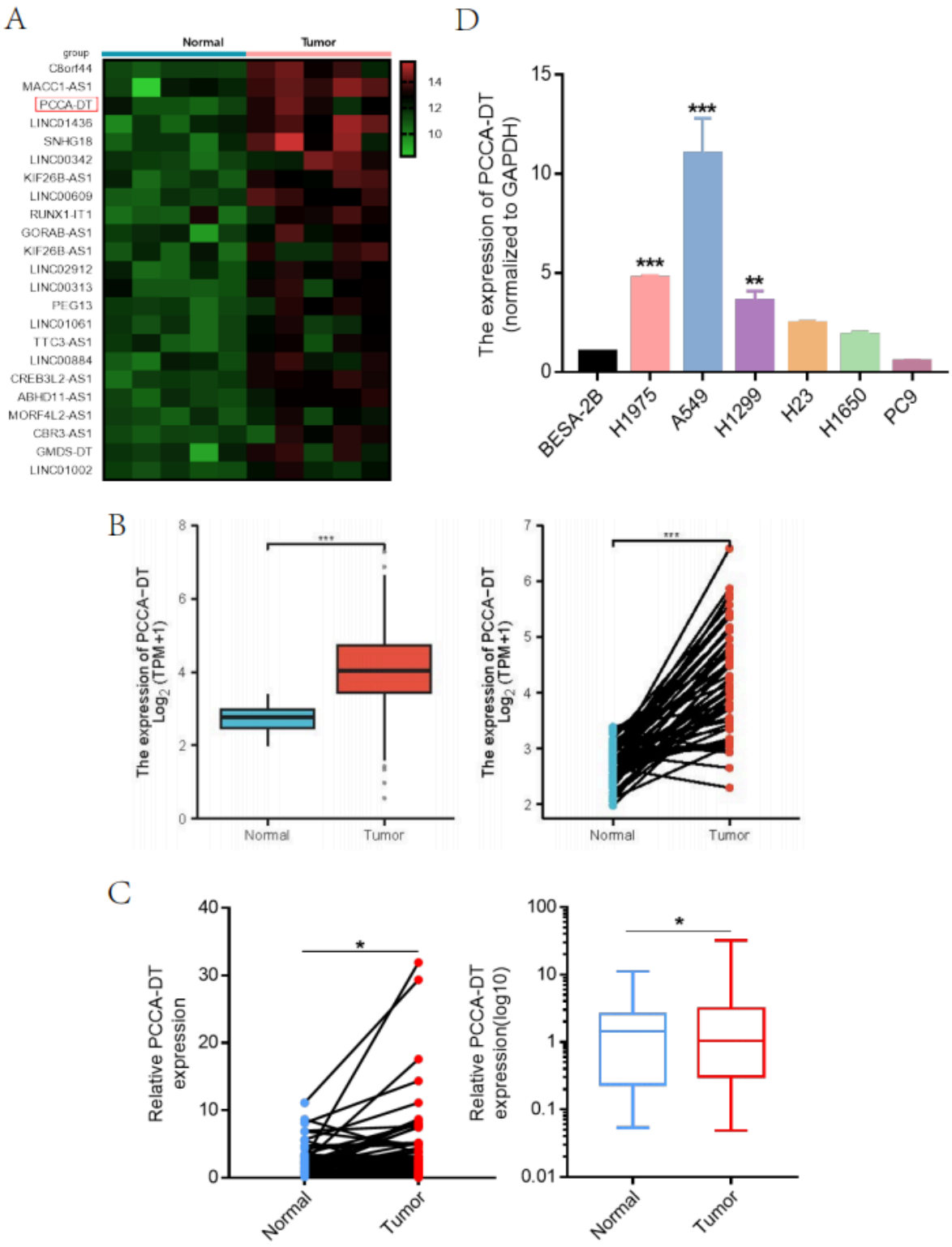


Figure 1

The upregulation of lncRNA PCCA-DT in NSCLC cells. (A) Analysis of 5 pairs of NSCLC tissues and adjacent normal tissues. (B) Expression of lncRNA PCCA-DT in NSCLC samples from the TCGA Database. (C) Detection of PCCA-DT expression levels in lung tumors by qRT-PCR. (D) Detection of the relative expression levels of PCCA-DT in NSCLC cells by qRT-PCR. (*p< 0.05, **p< 0.01, ***p< 0.001)

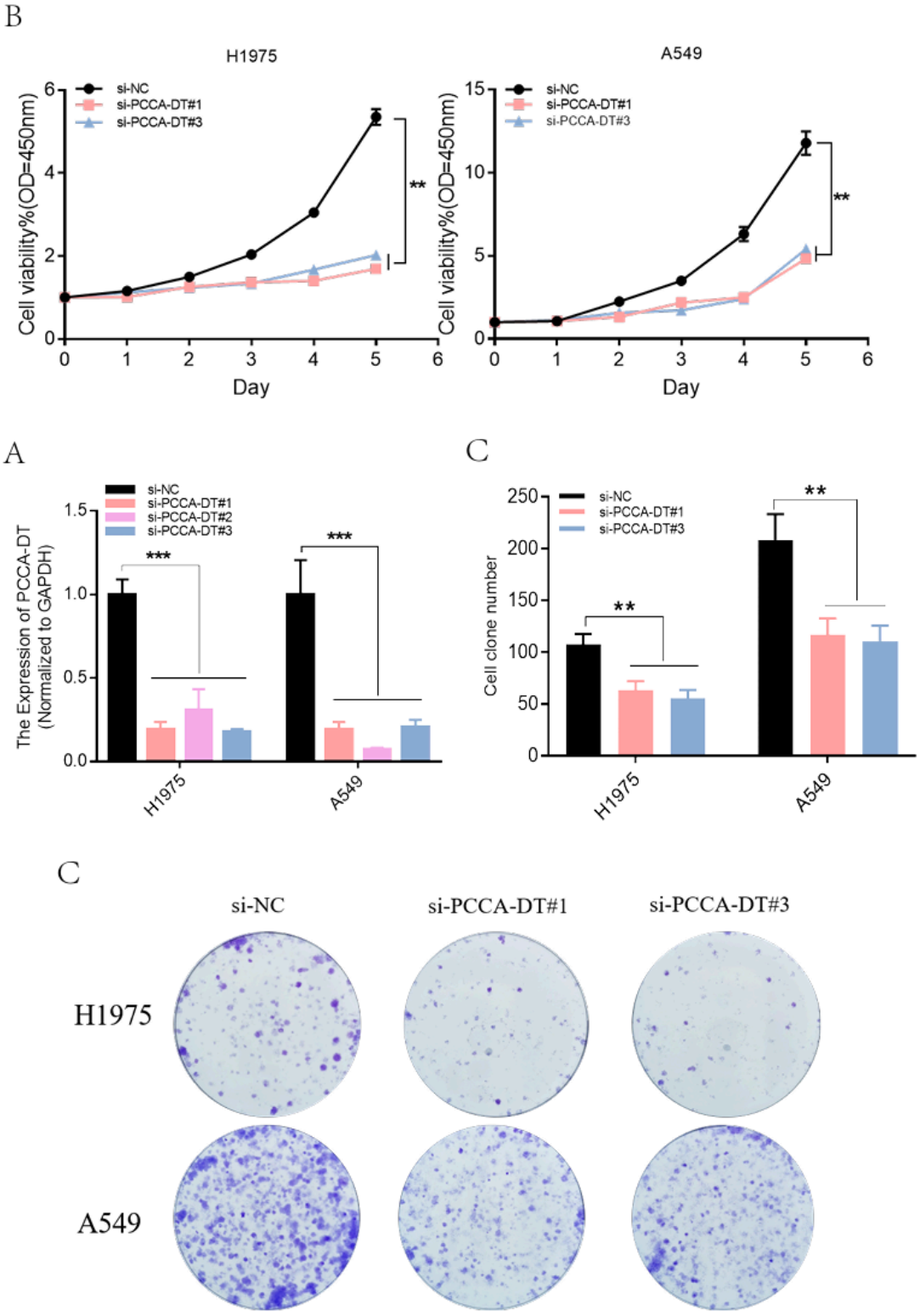


Figure 2

PCCA-DT regulates the proliferation and clonogenic ability of non-small cell lung cancer cells. (A) qRT-PCR detects the interference efficiency of RNAi. (B) CCK8 shows a significant decrease in the proliferation ability of the si group. (C) siRNA interferes with the colony formation ability of the cells. (*P < 0.05, **P < 0.01).

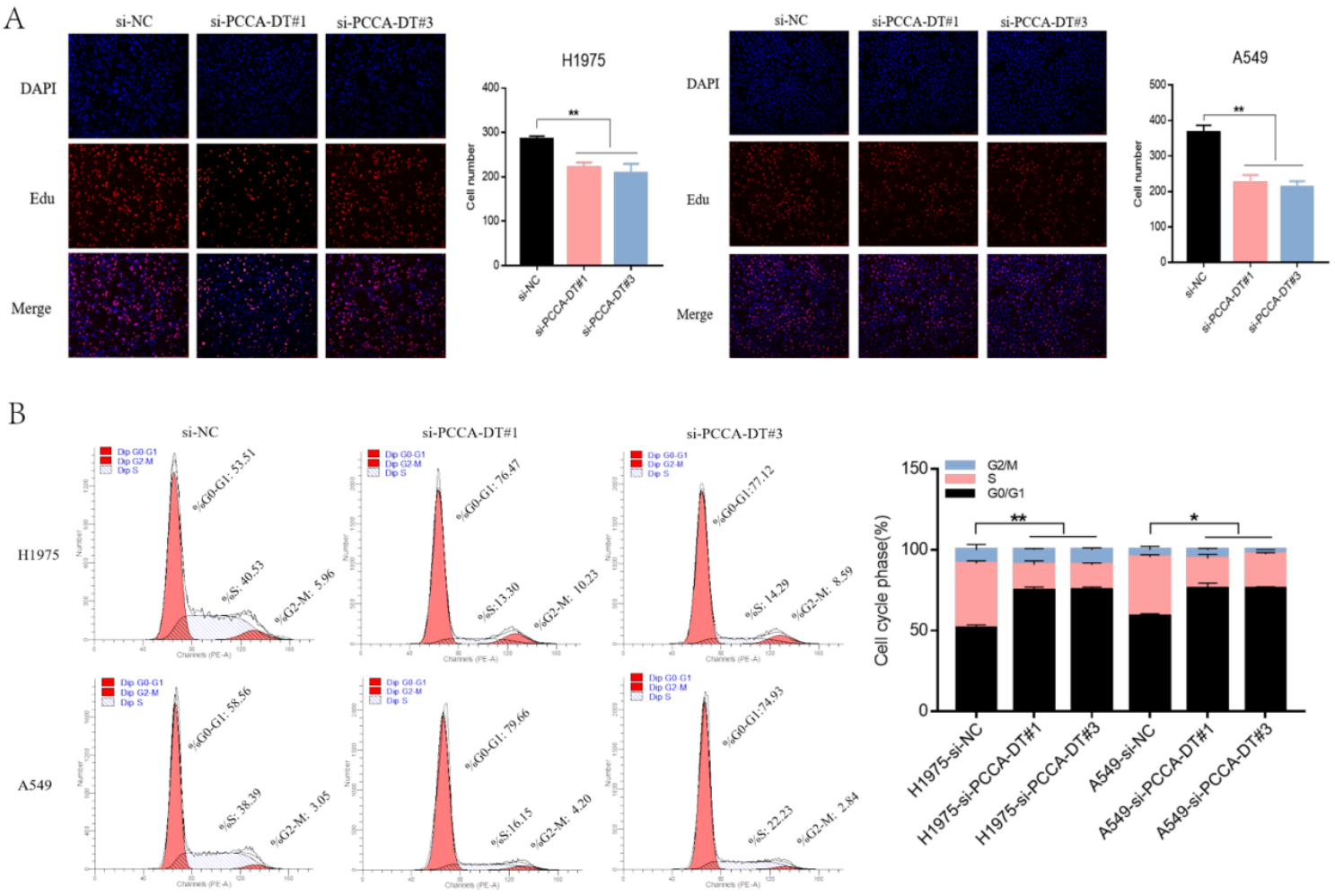


Figure 3

PCCA-DT promotes the transition of NSCLC cell cycle from G1 to S phase. (A) Edu experiment detects the situation of cells binding Edu after downregulating PCCA-DT. (B) Detection of cell cycle status after siRNA interference. (*P < 0.05, **P < 0.01).

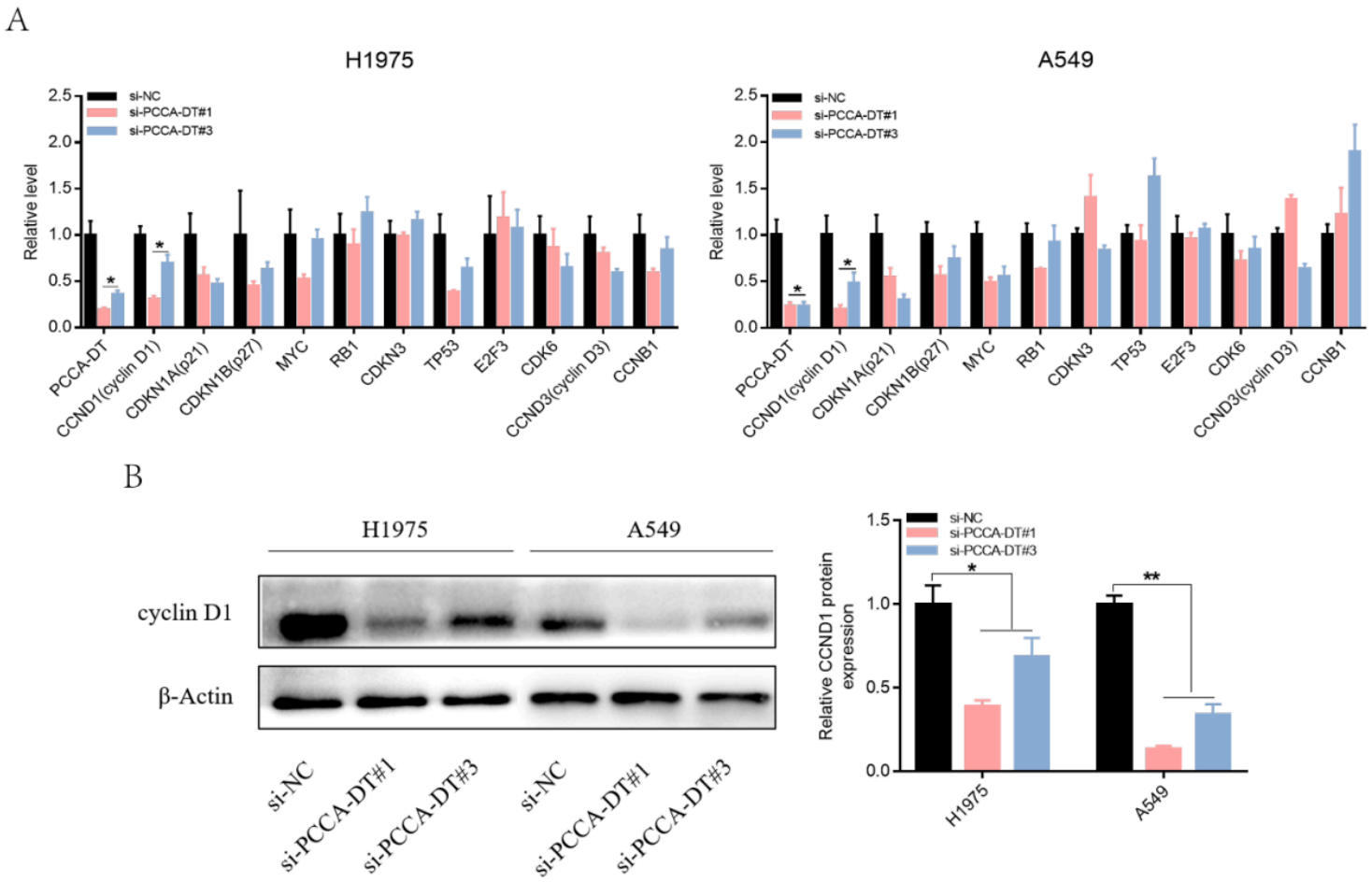


Figure 4

PCCA-DT regulates CCND1 expression. (A) siRNA interferes with PCCA-DT, affecting the expression of cell cycle-related genes. (B) WB experiment detects CCND1 protein levels. (*P < 0.05, **P < 0.01).

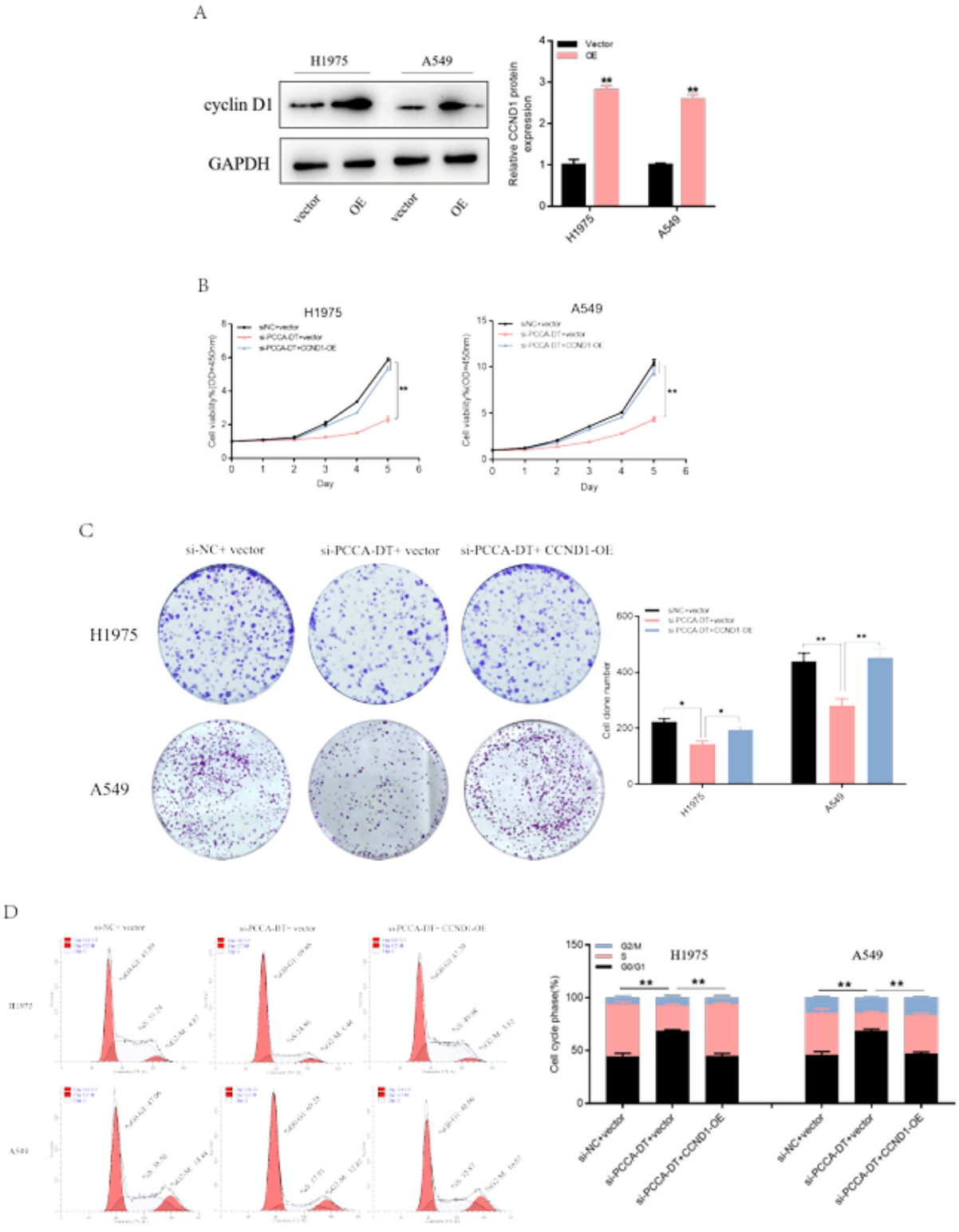


Figure 5

PCCA-DT promotes the G1/S phase transition of NSCLC cell cycle through CCND1. (A) Verification of CCND1 overexpression efficiency. (B, C) After interfering with PCCA-DT, re-overexpression of CCND1 restores cell proliferation and colony formation ability. (D) After interfering with PCCA-DT, re-overexpression of CCND1 restores the G1/S phase transition to the level before downregulation. (*P < 0.05, **P < 0.01).

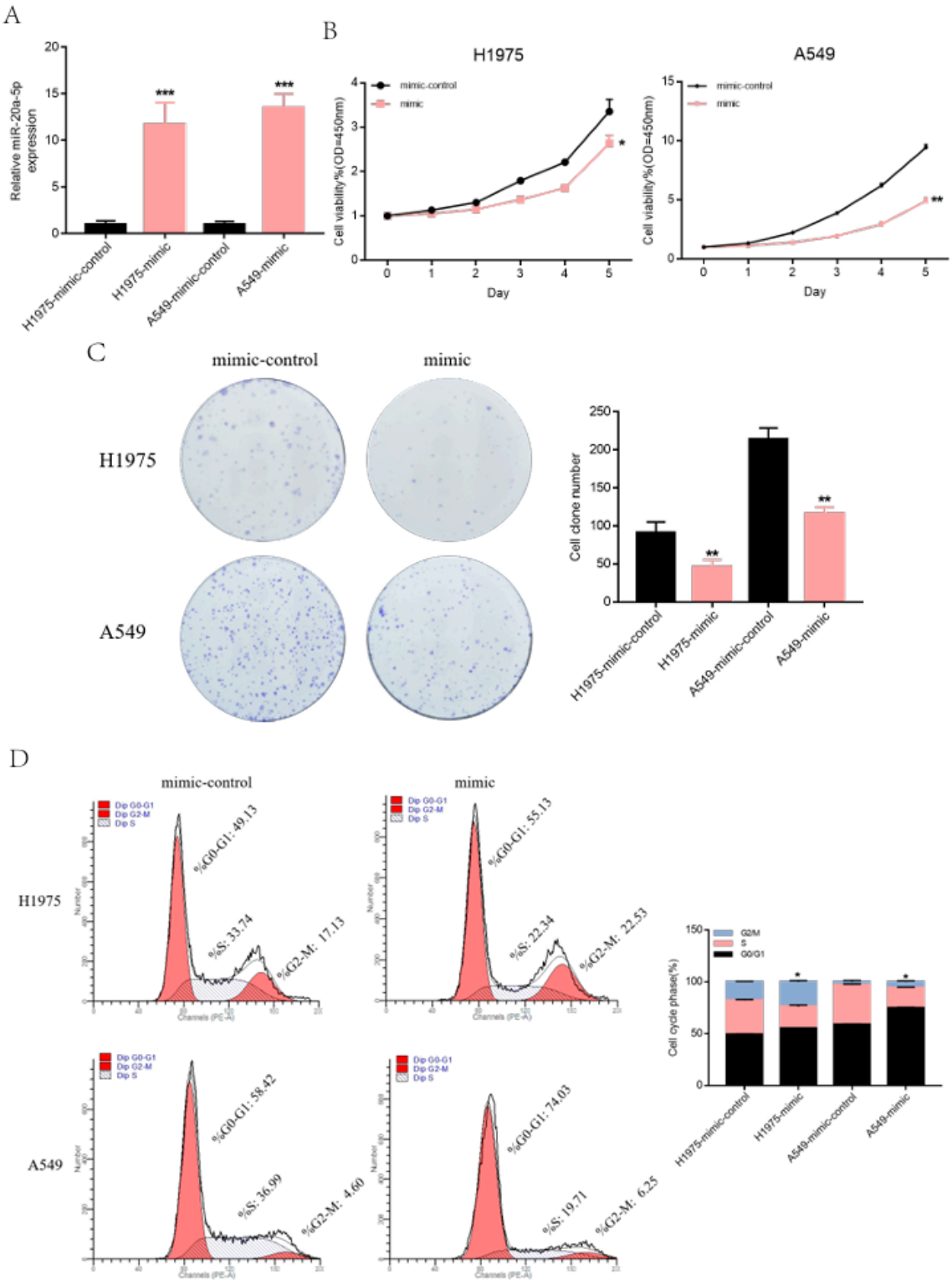


Figure 6

PCCA-DT is located in the cytoplasm and regulates miR-20a-5p. (A) Detection of the binding site of miR-20a-5p with PCCA-DT and its effect on luciferase activity. (B) Verification of the subcellular localization of PCCA-DT. (C) Prediction of miRNAs that interact with PCCA-DT. (D) qRT-PCR validation of the expression level of miR-20a-5p after downregulation of PCCA-DT. (*P < 0.05, **P < 0.01).

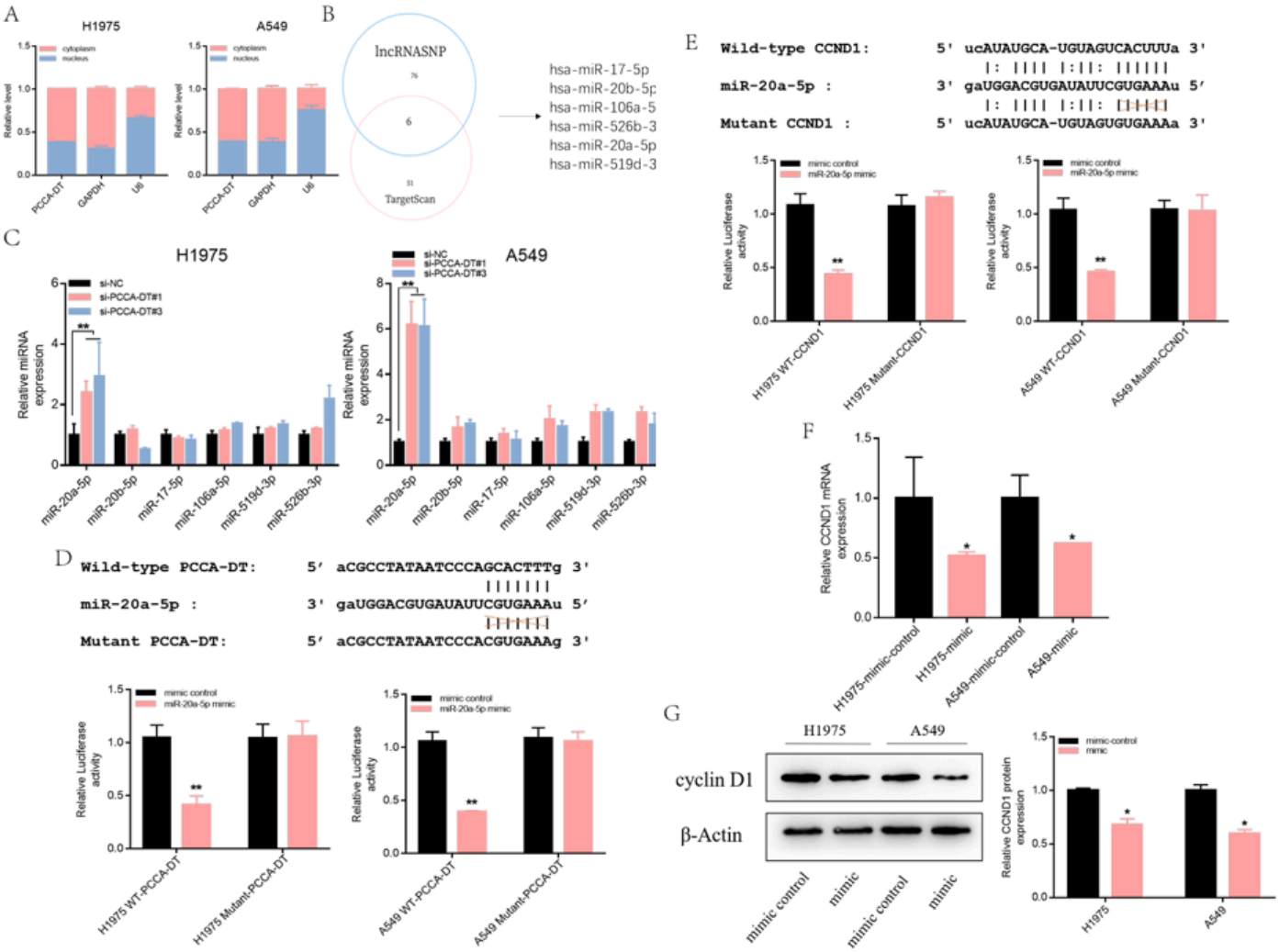


Figure 7

The regulation of CCND1 by miR-20a-5p and its effect on the cell cycle of NSCLC. (A) Verification of the transfection efficiency of miR-20a-5p mimics. (B, C) CCK8 assay to detect the effects of miR-20a-5p overexpression on cell proliferation and colony formation. (D) The status of the cell cycle after mimic overexpression. (E) The binding sites between miR-20a-5p and CCND1, and the detection of luciferase activity. (F) qRT-PCR to detect the mRNA expression level of CCND1 after miR-20a-5p overexpression. (G) WB to verify the translation level of CCND1 in NSCLC cells after overexpression of miR-20a-5p. (*P < 0.05, **P < 0.01).

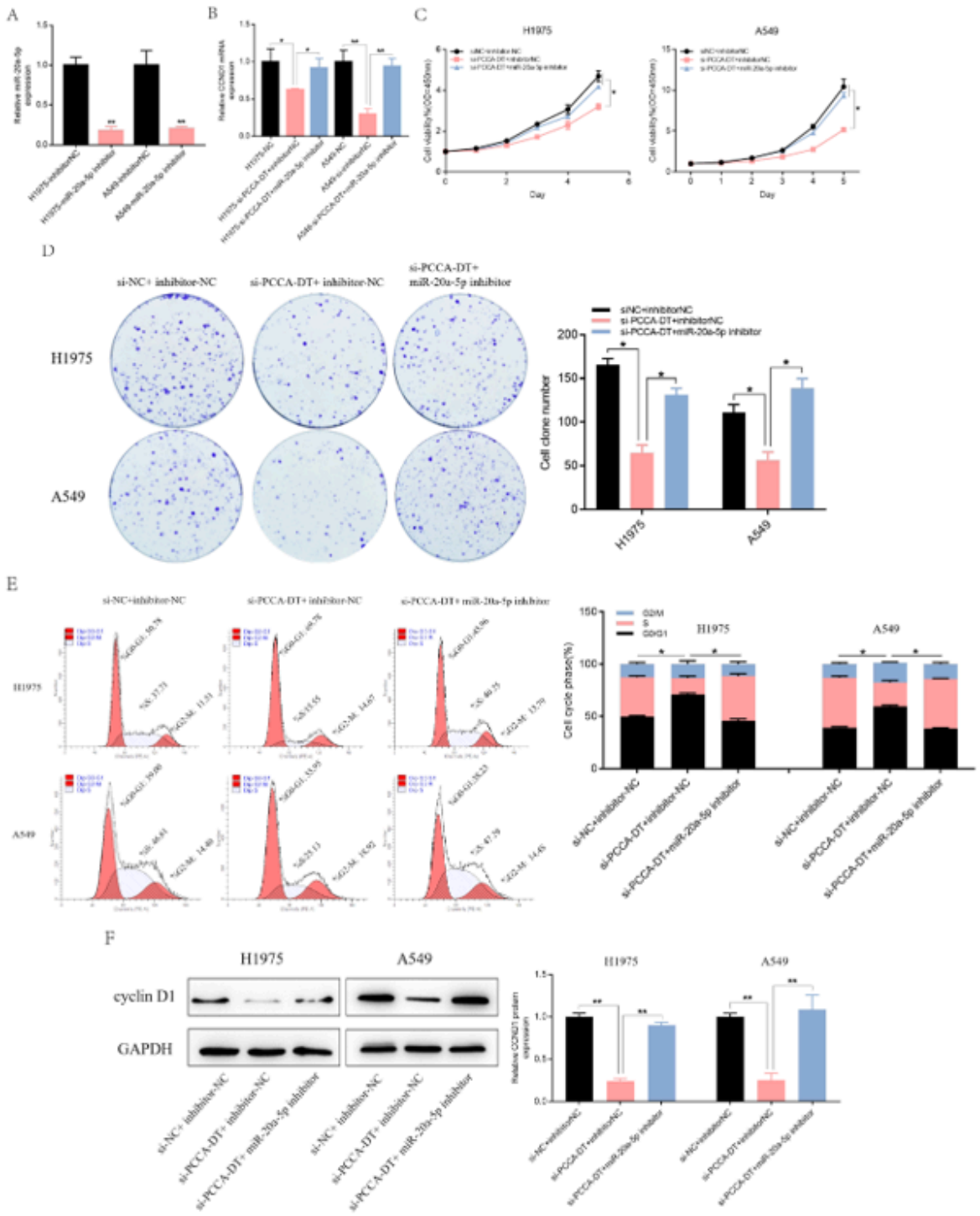


Figure 8

PCCA-DT regulates miR-20a-5p through a sponge mechanism, thereby affecting the cell cycle and proliferation of NSCLC. (A) Verification of the transfection efficiency of miR-20a-5p inhibitors. (B, F) qRT-PCR and WB validate the mRNA and protein levels of CCND1 after co-transfection. (C, D) Changes in cell proliferation and colony formation ability after co-transfection. (E) PCCA-DT downregulates the

restriction of cell G1/S transition, and treatment with miR-20a-5p inhibitors restores it. (*P < 0.05; **P < 0.01).

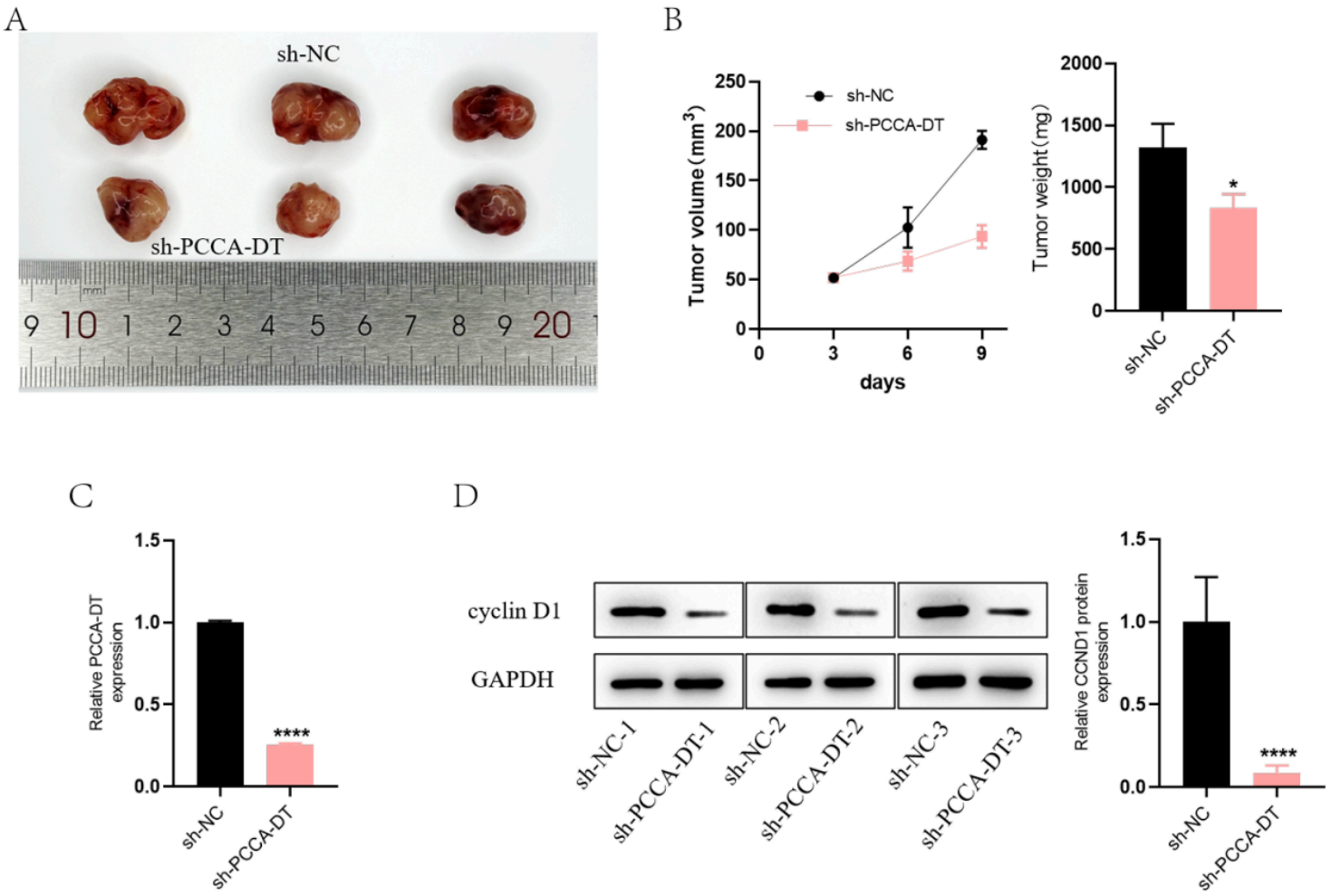


Figure 9

PCCA-DT downregulation inhibits tumor growth in vivo. (A) Typical tumor images. (B) Tumor growth curve and weight. (C, D) Expression levels of PCCA-DT and CCND1 in transplanted tumors.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- CCND122.tif
- CCND132.tif
- CCND1actin32.tif
- CCND1actin22.tif
- CCND112.tif
- SupplementaryFigur1.docx

- CCND1actin12.tif