



# LncRNA PCAT1 Interacts with DKCI to Regulate Proliferation, Invasion and Apoptosis in NSCLC Cells via the VEGF/AKT/Bcl2/Caspase9 Pathway

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## Abstract

Long noncoding RNAs (lncRNAs) are increasingly recognized as indispensable components of the regulatory network in the progression of various cancers, including nonsmall cell lung cancer (NSCLC). The lncRNA prostate cancer associated transcript 1 (PCAT1) has been involved in tumorigenesis of multiple malignant solid tumors, but it is largely unknown that what is the role of lncRNA-PCAT1 and how it functions in the progression of lung cancer. Herein, we observed that lncRNA PCAT1 expression was upregulated in both human NSCLC tissues and cell lines, which was determined by qualitative polymerase chain reaction analysis. Then, gain-and loss-of-function manipulations were performed in A549 cells by transfection with a specific short interfering RNA against PCAT1 or a pcDNA-PCAT1 expression vector. The results showed that PCAT1 not only promoted NSCLC cell proliferation and invasion but also inhibited cell apoptosis. Bioinformatics and expression correlation analyses revealed that there was a potential interaction between PCAT1 and the dyskerin pseudouridine synthase 1 (DKCI) protein, an RNA-binding protein. Then, RNA pull-down assays with biotinylated probes and transcripts both confirmed that PCAT1 directly binds with DKCI that could also promote NSCLC cell proliferation and invasion and inhibit cell apoptosis. Moreover, the effects of PCAT1 and DKCI on NSCLC functions are synergistic. Furthermore, PCAT1 and DKCI activated the vascular endothelial growth factor (VEGF)/protein kinase B (AKT)/Bcl-2/caspase9 pathway in NSCLC cells, and inhibition of epidermal growth factor receptor, AKT, or Bcl-2 could eliminate the effect of PCAT1/DKCI co-overexpression on NSCLC cell behaviors. In conclusion, lncRNA PCAT1 interacts with DKCI to regulate proliferation, invasion, and apoptosis in NSCLC cells via the VEGF/AKT/Bcl-2/caspase9 pathway.

## Keywords

NSCLC, lncRNA PCAT1, RNA-protein interaction, DKCI, the VEGF/AKT/Bcl2/caspase9 pathway

## Introduction

According to the International Agency for Research on Cancer (IARC), in 2018 alone, cancer caused an estimated 9.6 million deaths. One of the most common cancers is lung cancer (2.09 million cases); also, lung cancer is the leading cause of cancer death (1.76 million deaths)<sup>1</sup>. Lung cancer has evolved into the most important cancer threatening human life in the world. Nonsmall cell lung cancer (NSCLC) is the predominant subtype of lung cancer, accounting for 80% of total lung cancer incidents<sup>2</sup>, with a 5-year survival rate of <20%<sup>3</sup>. Therefore, there is a remarkably urgent need to explore the molecular mechanisms of NSCLC progression.

Long noncoding RNAs (lncRNAs), which are longer than 200 nucleotides, are a class of noncoding RNAs. Although

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lncRNAs are without protein-coding potential, they can impact physiological or pathological processes by mediating other proteins, like interacting with RNA binding protein (RBP)<sup>4</sup>. Numerous lncRNAs exhibit aberrant expression in cancers and function as oncogenes or tumor suppressors<sup>5</sup>. LncRNA PTAR (pro-transition associated RNA) could promote NSCLC growth by acting as a sponge to bind and inactive miR-101<sup>6</sup>. LncRNA MAFG-AS1 (MAF BZIP Transcription Factor G Antisense RNA 1), also known as MAFG-DT (divergent transcript), could promote the migration and invasion of NSCLC cells through sponging miR-339-5p form MMP15<sup>7</sup>. LncRNA PCAT1, the full name of which is lncRNA prostate cancer associated transcript 1, was originally identified as a prostate cancer upregulated lncRNA by RNA sequencing<sup>8</sup>. It is instrumental in prostate cancer progression through regulating target genes and is also associated with many other cancers<sup>9-11</sup>. For example, in esophageal squamous cell carcinoma, PCAT1 could enhance cell growth by sponging miR-326<sup>12</sup>. PCAT1 in endometrial carcinoma was assumed to be a poor prognostic factor and represents the proliferative, migratory, and invasive activity of cancer cells<sup>13</sup>. Researches showed that PCAT1 activated protein kinase B (AKT) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- $\kappa$ B) signaling by regulating the PHLPP/FKBP51/IKK $\alpha$  complex in castration-resistant prostate cancer<sup>14</sup>.

However, there is only limited evidence that PCAT1 involves in NSCLC progression<sup>15,16</sup>. In this article, the aberrant expression level of PCAT1 was both found in human NSCLC tissue samples and cell lines. Further research showed that PCAT1 could regulate cell proliferation and invasion and apoptosis. Briefly, PCAT1 could cooperate with RBP dyskerin pseudouridine synthase 1 (DKC1) to promote cell proliferation and invasion and inhibit cell apoptosis via the vascular endothelial growth factor (VEGF)/AKT/Bcl2/caspase9 pathway.

## Materials and Methods

### Animals

Nude Wistar rats (weighing 200 to 250 g) were provided by Henan Experimental Animal Center (Henan, China). All animals were housed in separate cages in a photocyclically controlled environment with free access to food and water. Thirty nude rats were randomly divided into 2 groups (15 in each group). Nude rats in the control group were intraperitoneally injected with A549 cells transfected with empty vector (pcDNA3.1). In previous studies, we transfected A549 cells with PCAT1 overexpression vector (pcDNA-PCAT1) and screened out cells that stably overexpressed PCAT1 with G418. Herein, the rats in the experimental group were intraperitoneally injected with A549 cells stably overexpressing PCAT1. On days 15, 20, 25, 30, and 35 postinjection, 3 rats were sacrificed in each group, and the tumors were obtained to determine the tumor volume and weight. All animals care

and experimental procedures were approved by the Second Affiliated Hospital of Xi'an Jiaotong University.

### Tissue Specimens and Cell Culture

Twenty paired NSCLC samples and adjacent tissues of NSCLC patients were collected from our hospital in 2018. No patients had received local or systemic treatment before any operation. Fresh lung tumor tissues were obtained with biopsy and frozen in liquid nitrogen, and then stored at  $-80^{\circ}\text{C}$  before RNA extraction. A 5-ml peripheral blood sample from each patient was drawn into an ethylenediaminetetraacetic acid (EDTA)-K2 tube, and then experienced RNA extraction for measurement of RNA expression levels. The research was approved by the Second Affiliated Hospital of Xi'an Jiaotong University, and informed consent was obtained. Cell lines (Normal: BEAS-2B and NSCLC: calu-1, A549, A427, H460) were all purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were propagated in Roswell Park Memorial Institute 1640 medium supplemented with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA).

### RNA Extraction and Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR)

Total RNA of tissue samples or cells were isolated by using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) and purified with RNase-free DNase I (Invitrogen) to avoid DNA contamination. Then the complementary DNA (cDNA) was generated by using the PrimeScript II 1st Strand cDNA Synthesis Kit (Takara Biotechnology, Dalian, China). Real-time qPCR was performed in the Quant Studio Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) with SYBR Premix Ex Taq II (Takara Biotechnology, Dalian, China) in a 20- $\mu\text{l}$  reaction system. The RT-qPCR reactive conditions were the following: incubation at  $95^{\circ}\text{C}$  for 2 min, followed by 40 cycles of  $95^{\circ}\text{C}$  for 15 s,  $60^{\circ}\text{C}$  for 32 s. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was employed as an endogenous control for mRNA and lncRNA. The specific primer sequences were used: 5'-TGA GAA GAG AAA TCT ATT GGA ACC-3' (sense; PCAT1); 5'-GGT TTG TCT CCG CTG CTT TA-3' (anti-sense; PCAT1); 5'-TTC AGC GGG CGG AAA AG-3' (sense; DKC1); 5'-ACT CGCT CCG TTC CTC TTC CT-3' (anti-sense; DKC1).

### Cell Counting Kit-8 (CCK-8) Assay

CCK-8 assay was used to assess cell proliferation. Cells were grown to subconfluence and harvested into Dulbecco's modified Eagle medium (DMEM) containing 10% FBS. Then cells were seeded at a concentration of  $1 \times 10^4$  cells/well onto commercial 96-well plates and allowed to adhere overnight at  $37^{\circ}\text{C}$ . Then, 10  $\mu\text{l}$  of thawed CCK-8 solution was added into each well. After the plates were incubated for

2 h at the same incubator conditions, the absorbance was read at 450 nm.

### Transwell Invasion Assay

Transwell invasion assay was used to confirm cell invasion ability. We used the Transwell plates with an 8- $\mu$ m pore size as well as with Matrigel. The upper chamber was filled with a serum-free medium loading  $1 \times 10^5$  cells/well as well as the lower chamber was filled with a culture medium containing 10% FBS as the chemoattractant. After incubation for 24 h, the cells, on the upper membranes, were eliminated with a cotton swab, and those migrated/invaded were fixed and stained with 0.1% crystal violet. The cells were imaged and quantified in 8 random fields/well at 200 $\times$  magnification under a microscope.

### Flow Cytometry

Flow cytometry was performed to analyze cell apoptosis. The cells were collected and washed twice with phosphate-buffered saline (PBS). A total of the cell suspension (100  $\mu$ l) of  $1 \times 10^6$  cells/ml was transferred to a culture tube, and then incubated with 5  $\mu$ l of Annexin V-fluorescein isothiocyanate and 5  $\mu$ l of propidium iodide with room temperature for 20 min in the dark. In the end, 400  $\mu$ l of binding buffer was added, and apoptotic cells were determined by flow cytometry (BD Biosciences, USA).

### Western Blotting

Total cellular lysates were prepared in radioimmunoprecipitation assay (RIPA) lysis buffer (APPLYGEN, Beijing, China). The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes, which have already been cut in a suitable size. After incubation with specific primary antibodies (DKC1, cleaved caspase 3, cleaved PARP, cleaved caspase 8, cleaved caspase 9, cleaved caspase 12, VEGF, p-AKT, Bcl2, cyclin D, E-cadherin, N-cadherin, vimentin) for 1 h at 37  $^{\circ}$ C, the membranes were incubated with horseradish peroxidase (HRP)-conjugated antirabbit immunoglobulin (Ig)G or anti-mouse IgG secondary antibody (Abcam, Cambridge, UK) for 1 h at 37  $^{\circ}$ C, and bands were detected by using the ImageQuant LAS 4000 (GE Healthcare Life Sciences, Pittsburgh, PA, USA) and the ChemiDocTM XRS + (Bio-Rad, Hercules, CA, USA). GAPDH was used as a loading control.

### RNA Pull-Down Assay with a Biotinylated RNA Probe

RNA pull-down assay was used to identify the PCAT1 interaction with DKC1. Briefly, before harvest, cells were transfected with 50 nM biotinylated RNA probe for 48 h. Then the cells were washed with PBS and incubated for 10 min in an RNA pull-down lysis buffer (Ambion, Austin, Texas,

USA) on ice. The lysates were precleared by centrifugation, and the samples (20  $\mu$ l) were aliquoted for input. The remaining lysates were incubated with M-280 streptavidin magnetic beads pre-coated with RNase-free bovine serum albumin and yeast tRNA (Sigma, St. Louis, MO, USA) at 4  $^{\circ}$ C for 3 h. After that, the beads were washed 2 times with ice-cold lysis buffer and 3 times with an SDS-Tris low salt buffer (pH 8.0 containing 150 mM sodium chloride [NaCl]), and once with a high salt buffer containing 500 mM NaCl. The bound complexes were purified for the following analysis.

### RNA Pull-Down Assay With Wild Type (WT) or Mutated (MUT) Biotinylated PCAT1 Transcripts

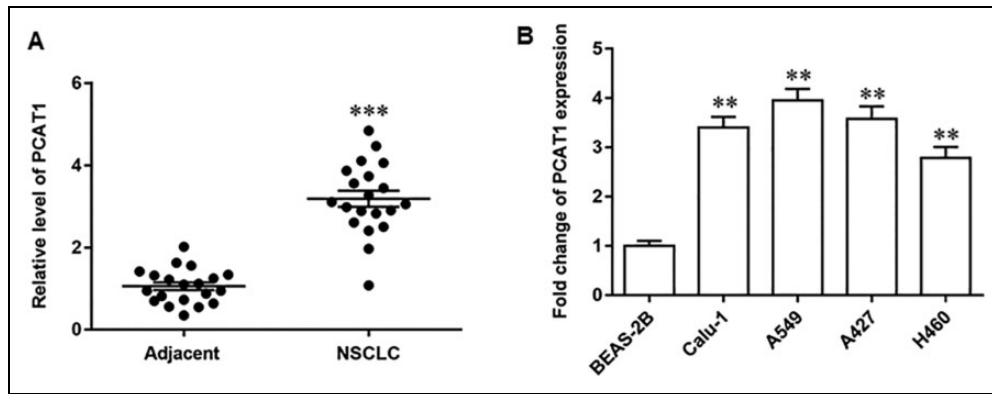
WT PCAT1 was transcribed *in vitro* with biotin RNA labeling mixture and T7 RNA polymerase according to the manufacturer's instructions (Invitrogen). MUT PCAT1 transcripts were transcribed *in vitro* with biotin RNA labeling mixture and T7 RNA polymerase with nested PCR. Streptavidin-linked magnetic beads (400  $\mu$ l; Thermo Fisher Scientific, Waltham, MA, USA) were used to pull down the biotinylated transcripts for 2 h at room temperature. Then, the beads-RNA-proteins were washed with 1 $\times$  binding washing buffer (5 mM Tris-hydrochloric acid, 1 M NaCl, 0.5 mM EDTA, and 0.005% Tween 20) for 4r times. The proteins were precipitated and diluted in protein lysis buffer (500  $\mu$ l). Eventually, the retrieved proteins were measured on SDS-PAGE gels for Western blotting.

### Plasmid Construction and Cell Transfection

The pcDNA3.1 overexpression vector was constructed by using full-length cDNA of PCAT1 or DKC1. The empty vector was used as a negative control. DNA samples were double-digested with BamHI and EcoRI. Annealed DNA fragments were ligated into pcDNA3.1 vector and subjected to competent cell transformation and extraction of plasmids without endotoxin. The recombinant plasmid was verified via DNA sequencing. The short interfering RNA against PCAT1 or DKC1 (siPCAT1 or siDKC1), and negative control siRNAs were designed, synthesized, and validated by Thermo Fisher Scientific. The cells were subgrown in 6-well plates at a density of  $2 \times 10^5$  cells/well. On reaching about 70% confluence, the vectors or siRNAs were transfected into cells by using the Lipofectamine 3000 reagent (Thermo Fisher Scientific).

### Statistical Analysis

Herein, each measurement was obtained from at least triple experiments. All data were presented as the mean  $\pm$  SEM in triplicate samples. All statistical analyses were performed using the SPSS software (ver. 13.0; SPSS, Chicago, IL, USA). Significance was determined by one-way analysis



**Fig. 1.** LncRNA PCAT1 is upregulated in human NSCLC tissues and cell lines. (A) LncRNA PCAT1 expression in 20 paired human NSCLC tissues and adjacent tissues were determined by RT-qPCR. (B) The expression of PCAT1 was detected in human NSCLC cell lines (calu-1, A549, A427, H460) and normal pulmonary epithelial cells line (BEAT-2B) by RT-qPCR. Glyceraldehyde 3-phosphate dehydrogenase was used as an internal reference. Statistical significance was assessed by using the Student's *t*-test. Values are expressed as mean  $\pm$  SEM,  $n = 3$  for each group. \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . LncRNA: long noncoding RNA; NSCLC: nonsmall cell lung cancer; PCAT1: prostate cancer associated transcript I; RT-qPCR: quantitative reverse transcription polymerase chain reaction.

of variance or the Student's paired *t*-test, and  $P$ -value  $< 0.05$  was considered statistically significant.

## Results

### Upregulated Expression of LncRNA PCAT1 in Human NSCLC Tissue Specimens and Cell Lines

In this research, we evaluated the expression of LncRNA PCAT1 in 20 paired human NSCLC tumor tissues and adjacent tissues and found that the PCAT1 was significantly upregulated in NSCLC samples (Fig. 1A). Also, the expression of PCAT1 in NSCLC cell lines was deep higher than normal pulmonary epithelial cells (Fig. 1B). Because of the same tendency of PCAT1 expression in NSCLC cell lines and no significant difference, we used the A549 cell line to proceed following experiments. These data indicated that the expression of LncRNA PCAT1 was upregulated in human NSCLC specimens and cell lines.

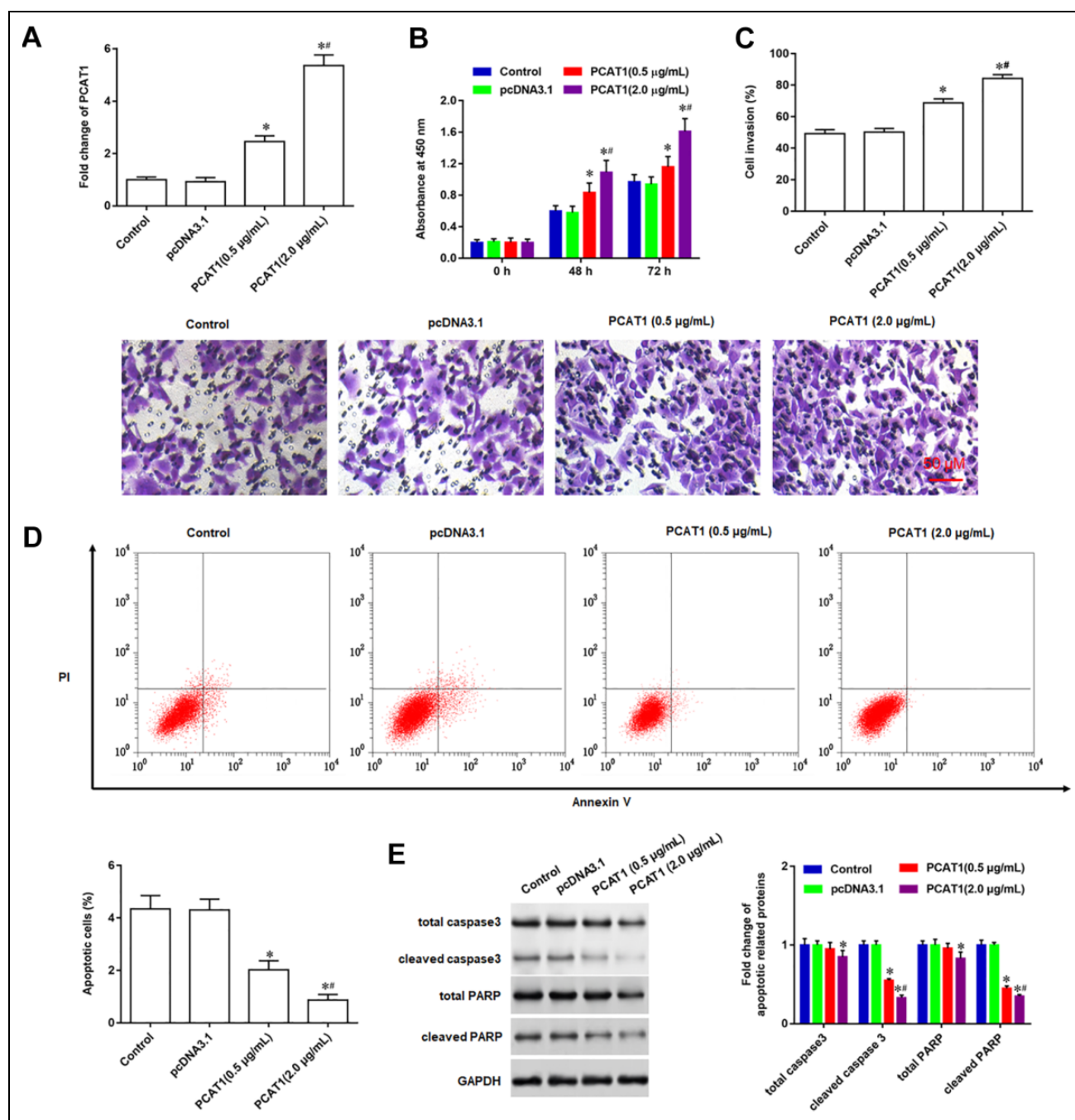
### PCAT1 Regulates NSCLC Cell Proliferation, Invasion, and Apoptosis

To explore the effect of PCAT1, the pcDNA PCAT1 or empty vector as a negative control (pcDNA3.1) were used to infect A549 cells. As shown in Fig. 2A, the efficiency of infection was confirmed by RT-qPCR, and significant upregulation of PCAT1 expression level was observed. CCK-8 assay showed that overexpression of PCAT1 promoted cell proliferation (Fig. 2B). Transwell invasion assay exhibited that PCAT1 promoted cell invasive ability (Fig. 2C). Flow cytometry results showed that the apoptosis of cells overexpressing PCAT1 was inhibited (Fig. 2D). Finally, we further used Western blotting to test apoptotic effector cleaved caspase3 and cleaved PARP and identified that PCAT1 inhibited cell apoptosis (Fig. 2E).

To further confirm the effect of PCAT1, a specific siRNA was designed and synthesized to knockdown PCAT1 in A549 cells. After siPCAT1 was transfected into cells, the PCAT1 expression was notably downregulated (Fig. 3A). CCK-8 assay results demonstrated that cell proliferation was inhibited by downregulating PCAT1 in A549 cells (Fig. 3B). Transwell invasion assay showed that knockdown of PCAT1 inhibited cell invasive ability (Fig. 3C). Cell apoptosis and the expression of apoptosis-related proteins were detected by flow cytometry (Fig. 3D) and Western blotting (Fig. 3E), respectively. The results showed that the knockdown of PCAT1 induced cell apoptosis. Collectively, these data suggested that LncRNA PCAT1 promoted NSCLC cell proliferation, invasion, and inhibited NSCLC cell apoptosis.

### LncRNA PCAT1 Cooperates with RBP DKC1 to Function in NSCLC Cells

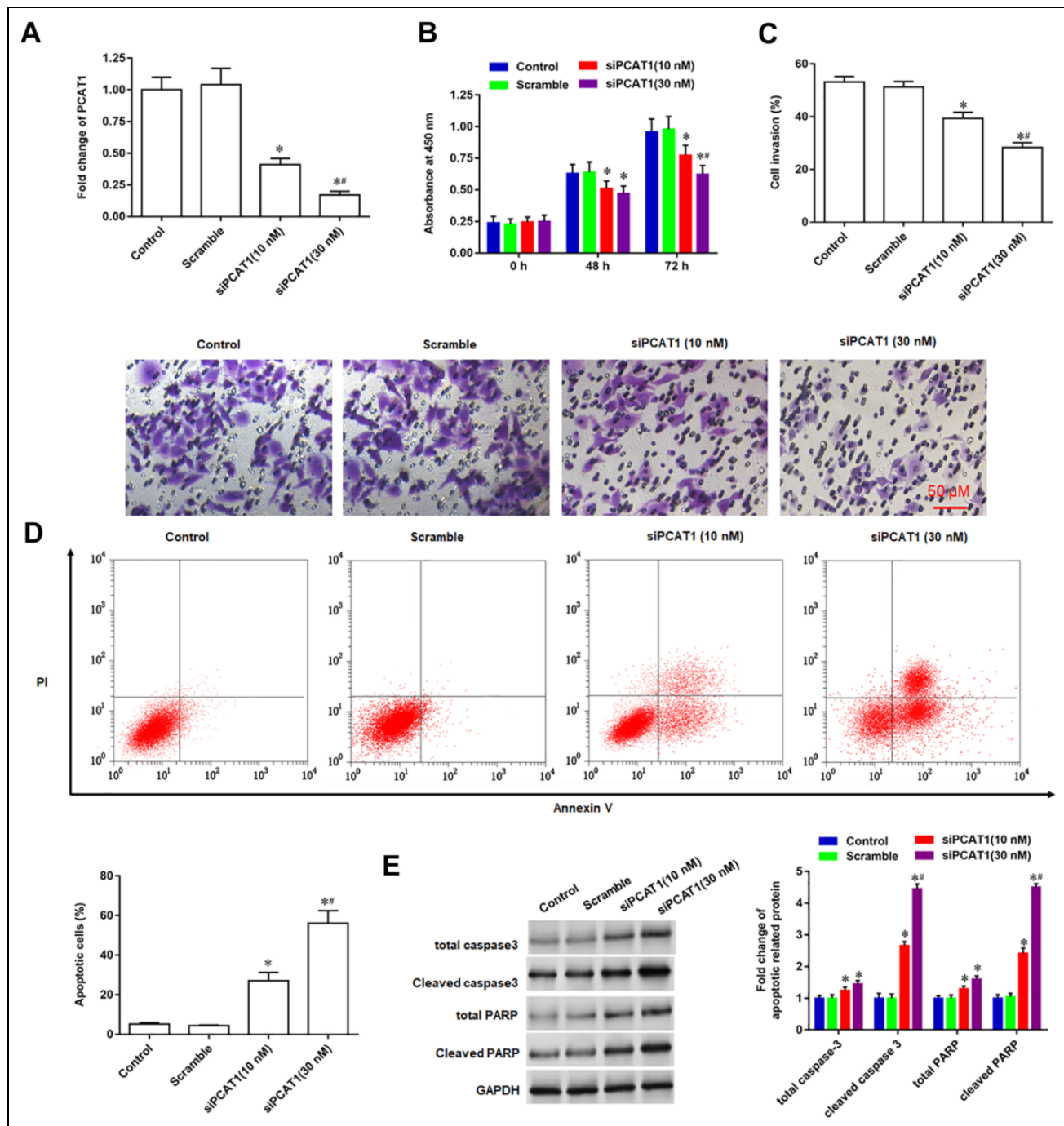
It is well known that one of the most typical regulatory mechanisms of LncRNAs is that they interact with RBPs and function in the form of RNA-protein complexes. Herein, to examine PCAT1 whether bind with RBP, we used an online database of Starbase (<http://starbase.sysu.edu.cn/>) to find RBPs that can interact with PCAT1<sup>17</sup>. Notably, in the database, DKC1, which is associated with cancer and regulates cancer progression<sup>18-20</sup>, has been reported to be positively correlated with PCAT1 and can bind directly to PCAT1 (Fig. 4A). There are 2 binding sites of DKC1 in the PCAT1 sequence. As mentioned earlier, we mutated the corresponding motifs to identify the binding capacity of DKC1 (Fig. 4B). First, RNA pull-down test was used to verify the binding of PCAT with DKC1, and Western blotting revealed that the amount of DKC1 protein in PCAT1 protein complexes was increased significantly with the upregulation of PCAT1. In addition, 2  $\mu\text{g/ml}$  of biotin-labeled PCAT1 RNA moderately increased the expression of the



**Fig. 2.** LncRNA PCAT1 promotes NSCLC cell proliferation and invasion and inhibits cell apoptosis. A549 cells were transfected with pcDNA-PCAT1 (0.5  $\mu\text{g/ml}$  or 2.0  $\mu\text{g/ml}$ ) or pcDNA3.1 (empty vector) for 24 h, respectively. (A) Relative expression of PCAT1 was detected by RT-qPCR. (B) Cell proliferation was analyzed by cell counting kit-8 assay. (C) Cell invasion was detected by the Transwell invasion assay. (D) Apoptosis of A549 cells were detected by flow cytometry. (E) Western blotting was used to detect the expression of apoptosis-related proteins. Glyceraldehyde 3-phosphate dehydrogenase was used as an internal reference. Statistical significance was assessed by using one-way variation analysis or Student's *t*-test. Values are expressed as mean  $\pm$  SEM,  $n = 3$  for each group. PCAT1: pcDNA-PCAT1. \* $P < 0.05$ , # $P < 0.05$  versus 0.5  $\mu\text{g/ml}$  PCAT1 group. LncRNA: long noncoding RNA; NSCLC: nonsmall cell lung cancer; PCAT1: prostate cancer associated transcript 1.

DKC1 protein in the input groups than control (Fig. 4C). Then, the mutation binding sites were examined by RNA pull-down assay with WT or MUT biotinylated PCAT1 transcripts and

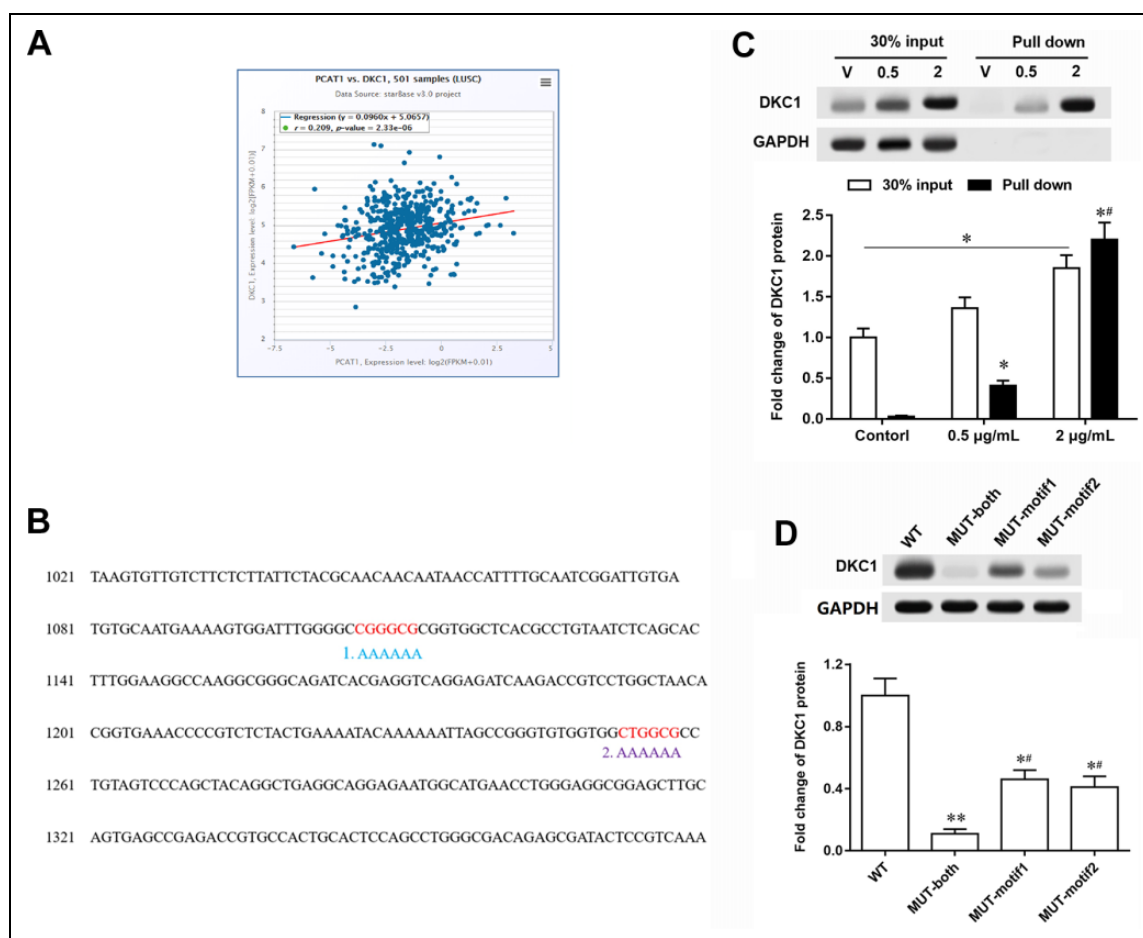
demonstrated that mutation of either site declined the binding capacity of DKC1 with PCAT1, and both mutations could abrogate their binding (Fig. 4D).



**Fig. 3.** Knockdown of PCAT1 inhibits NSCLC cell proliferation and invasion and promotes cell apoptosis. A549 cells were transfected with siPCAT1 (10 nM or 30 nM) or scramble for 24 h, respectively. (A) Relative expression of PCAT1 was detected by RT-qPCR. (B) Cell proliferation was analyzed by cell counting kit-8 assay. (C) Cell invasion was detected by the Transwell invasion assay. (D) Apoptosis of A549 cells were detected by flow cytometry. (E) Western blotting was used to detect the expression of apoptosis-related proteins. GAPDH was used as an internal reference. GAPDH served as the control. Statistical significance was assessed by using one-way variation analysis or Student's *t*-test. Values are expressed as mean  $\pm$  SEM,  $n = 3$  for each group. \* $P < 0.05$ , \*\* $P < 0.05$  versus 10 nM siPCAT1 group. GAPDH: glyceraldehyde 3-phosphate dehydrogenase; NSCLC: nonsmall cell lung cancer; PCAT1: prostate cancer associated transcript 1; RT-qPCR: quantitative reverse transcription polymerase chain reaction.

DKC1 overexpression vector was constructed and then transfected into A549 cells. Western blotting showed that the expression of DKC1 was significantly upregulated (Fig. 5A).

CCK-8 assay results showed that DKC1 promoted cell proliferation. Moreover, the promoting effect of simultaneous overexpression of DKC1 and PCAT was significantly higher



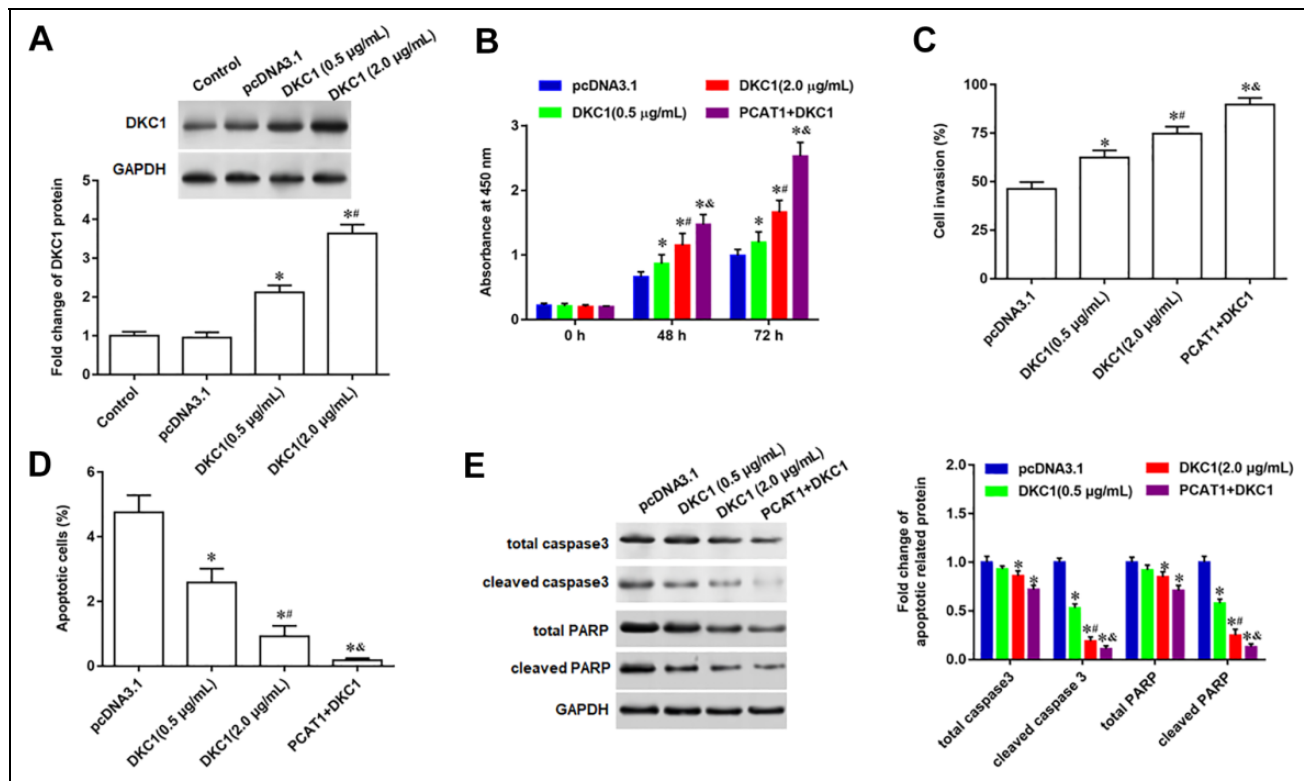
**Fig. 4.** Prediction and validation of binding between PCAT1 and DKC1 protein. (A) TCGA starbase was used to predict the correlation between PCAT1 and DKC1 in NSCLC. (B) Binding site of DKC1 in PCAT1 sequence. Red fonts represent binding motifs, blue and purple fonts represent mutated nucleotides in binding motifs. (C) Using PCAT1 as a probe, the binding relationship between PCAT1 and DKC1 was verified by RNA pull-down assay. Glyceraldehyde 3-phosphate dehydrogenase served as the control. (D) The binding of PCAT1 and DKC1 was validated by RNA pull-down assay based on WT or MUT sequences of PCAT1. Statistical significance was assessed by one-way variation analysis or Student's *t*-test. Values are expressed as mean  $\pm$  SEM,  $n = 3$  for each group. \* $P < 0.05$ , # $P < 0.05$  versus 0.5  $\mu\text{g/ml}$  biotin-labeled PCAT1 RNA group or # $P < 0.05$  versus MUT both groups. DKC1: dyskerin pseudouridine synthase I; MUT: mutated; NSCLC: nonsmall cell lung cancer; PCAT1: prostate cancer associated transcript I; WT: wild type.

than that of DKC1 overexpression alone (Fig. 5B). Transwell invasion assay showed DKC1 enhanced cell invasive ability, and co-transfection of pcDNA-DKC1 and pcDNA-PCAT1 had a more significant effect (Fig. 5C). Also, we found that simultaneous overexpression of DKC1 and PCAT1 had a stronger inhibitory effect on apoptosis (Fig. 5D) and expression of apoptosis-related proteins (Fig. 5E). Then siDKC1 was transfected in A549 to further confirm the effect of DKC1 on cells. Western blotting revealed that transfection of siDKC1 significantly inhibited the expression of DKC1 protein (Fig. 6A). Moreover, silencing DKC1 inhibited cell proliferation and invasion, and simultaneous transfection of siDKC1 and siPCAT1 inhibited cell proliferation (Fig. 6B) and invasion (Fig. 6C) more strongly than transfection of siDKC1 alone. Similarly, we observed that siDKC1 promoted apoptosis (Fig. 6D), as well as the expression of apoptosis-related

proteins (Fig. 6E), and this change was more pronounced when DKC1 and PCAT1 were knocked down simultaneously. Taken together, PCAT1 could bind with DKC1, synergistically promoted A549 cell proliferation and invasion, and inhibited cell apoptosis.

#### PCAT1 and DKC1 Inhibit the Apoptosis of NSCLC Cells via the VEGF/AKT Pathway

We have demonstrated that both PCAT1 and DKC1 can inhibit the apoptosis of A549 cells. However, which apoptotic pathway does PCAT1 and DKC1 impact? Here, we detected apoptotic effector proteins of the mitochondrial apoptotic pathway, endoplasmic reticulum pathway, and death receptor pathway. The results indicated that overexpression of PCAT1 or DKC1 inhibited the activation of



**Fig. 5.** RBP DKC1 cooperates with lncRNA PCAT1 to promote NSCLC cell proliferation and invasion and inhibit cell apoptosis. A549 cells were transfected with pcDNA-DKC1 (0.5 µg/ml or 2.0 µg/ml) alone or together with pcDNA-PCAT1 (2.0 µg/ml) or pcDNA3.1 (empty vector) for 24 h, respectively. (A) Relative expression of DKC1 was detected by Western blotting. (B) Cell proliferation was analyzed by cell counting kit-8 assay. (C) Cell invasion was detected by the Transwell invasion assay. (D) Apoptosis of A549 cells was detected by flow cytometry. (E) Western blotting was used to detect the expression of apoptosis-related proteins. GAPDH was used as an internal reference. GAPDH was used as the loading control. Statistical significance was assessed by using one-way variation analysis or Student's *t*-test. Values are expressed as mean  $\pm$  SEM,  $n = 3$  for each group. \* $P < 0.05$ , # $P < 0.05$  versus 0.5 µg/ml DKC1 group,  $^{\#}P < 0.05$  versus 2.0 µg/ml DKC1 group. DKC1: dyskerin pseudouridine synthase 1; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; lncRNA: long noncoding RNA; NSCLC: nonsmall cell lung cancer; PCAT1: prostate cancer associated transcript 1; RBP: RNA binding protein.

caspase 9 (Fig. 7A), and silencing PCAT1 or DKC1 activated caspase 9 (Fig. 7B). This implies that both DKC1 and PCAT1 are affected by the mitochondrial apoptotic pathway. According to previous reports, we focused on the VEGF/AKT signaling pathway<sup>14,21</sup>. To explore whether PCAT1 and DKC1 affect the VEGF/AKT pathway, the antibody of VEGF (anti-VEGF), AKT inhibitor (MK2206), and Bcl-2 inhibitor (ABT-737) were used to inhibit key proteins. Western blotting results showed that simultaneous overexpression of PCAT1 and DKC1 significantly promoted the expression of VEGF and Bcl-2 and promoted AKT activation, but this promotion was counteracted by the respective inhibitors (Fig. 7C).

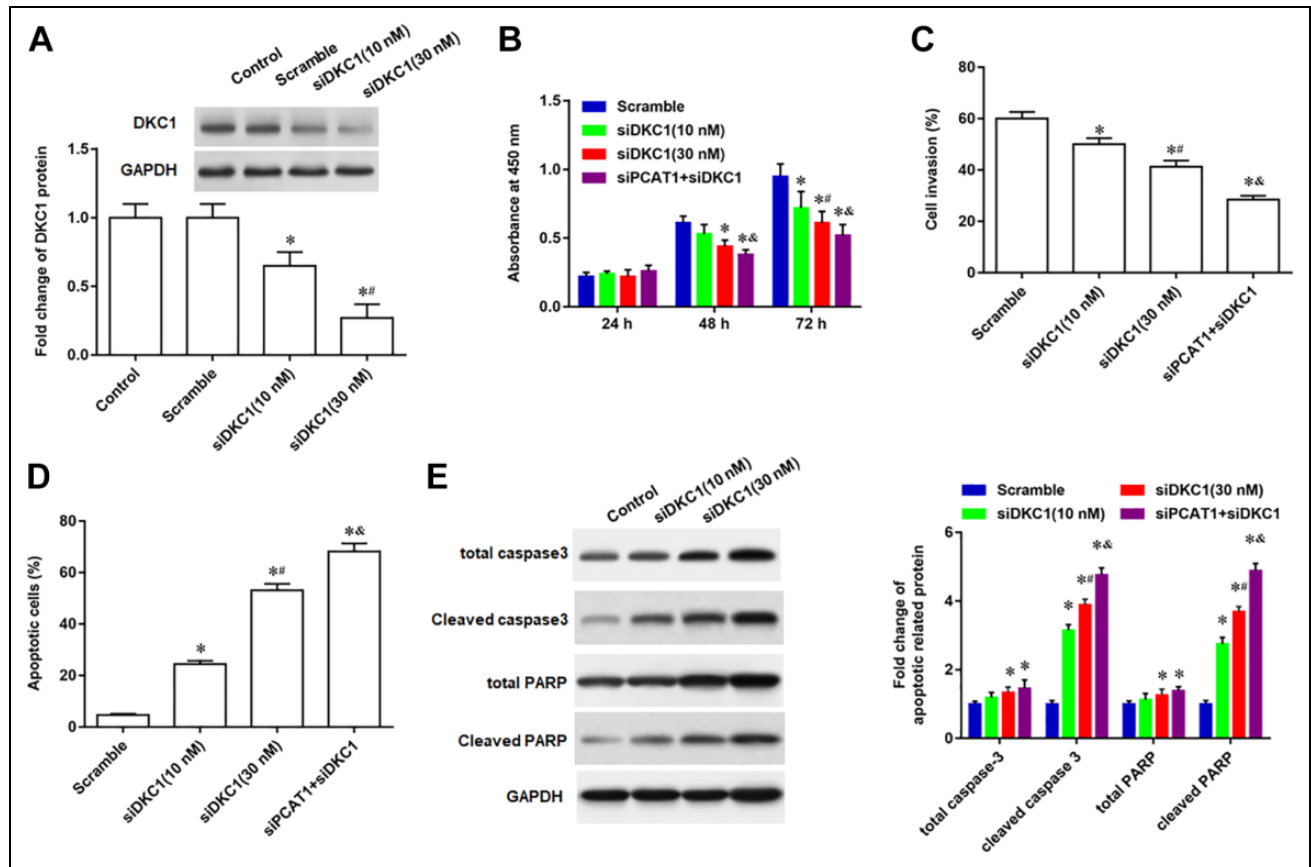
To further determine whether PCAT1 and DKC1 regulate NSCLC cell through the VEGF/AKT/Bcl-2 pathway, anti-VEGF, MK2206, and ABT-737 into cells, were introduced, respectively. We observed that co-overexpression of PCAT1 and DKC1 promoted cell proliferation compared with the control group, but cell proliferation was greatly inhibited under the intervention of anti-VEGF, MK2206, and

ABT-737 (Fig. 7D). Similar results were observed in the Transwell invasion assay, with anti-VEGF, MK2206, and ABT-737 significantly inhibiting cell invasion (Fig. 7E). Next, we examined the expression of effector proteins related to proliferation and invasion, and the results showed that the activity of effector proteins was inhibited under the intervention of anti-VEGF, MK2206, and ABT-737 (Fig. 7F). Furthermore, apoptosis also showed similar changes (Fig. 7G). Summarily, lncRNA PCAT1 and RBP DKC1 regulated NSCLC cell proliferation, invasion, and apoptosis via the VEGF/AKT/Bcl-2/caspase 9 pathway.

#### *lncRNA PCAT1 Promotes Tumorigenesis in Nude Rats In Vivo*

The A549 cells stably transfected with empty vector (pcDNA3.1) and PCAT1 overexpression vector (pcDNA-PCAT1) were intraperitoneally injected into nude rats to establish a tumor-bearing nude rat model. As shown in Fig. 8A, nude rats overexpressing PCAT1 were more likely





**Fig. 6.** Knockdown of DKC1 inhibits NSCLC cell proliferation and invasion and promotes cell apoptosis. A549 cells were transfected with siDKC1 (10 nM or 30 nM) alone or together with siPCAT1 (30 nM) or scramble for 24 h, respectively. (A) Relative expression of DKC1 was detected by Western blotting. (B) Cell proliferation was analyzed by cell counting kit-8 assay. (C) Cell invasion was detected by the Transwell invasion assay. (D) Apoptosis of A549 cells was detected by flow cytometry. (E) Western blotting was used to detect the expression of apoptosis-related proteins. Glyceraldehyde 3-phosphate dehydrogenase was used as the loading control. siPCAT1 + siDKC1: co-transfection of siPCAT1 (30 nM) and siDKC1 (30 nM). Statistical significance was assessed by using one-way variation analysis or Student's *t*-test. Values are expressed as mean  $\pm$  SEM,  $n = 3$  for each group. \* $P < 0.05$ , \*\* $P < 0.01$ , # $P < 0.05$  versus 10 nM DKC1 group, & $P < 0.05$  versus 30 nM DKC1 group. DKC1: dyskerin pseudouridine synthase I; NSCLC: nonsmall cell lung cancer; PCAT1: prostate cancer associated transcript 1.

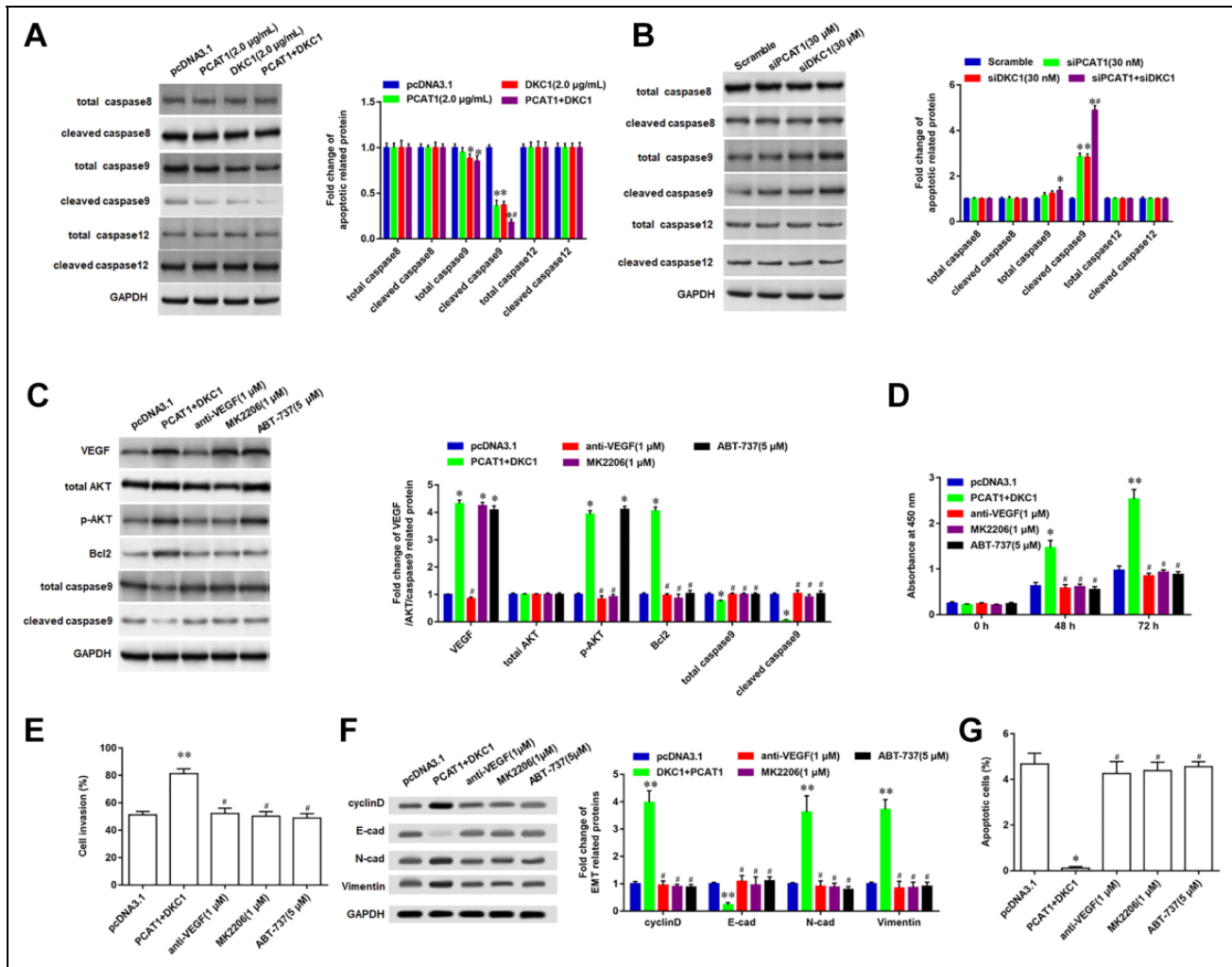
to form tumor than nude rats in the control group. In addition, we observed that the tumor volume of nude rats overexpressing PCAT1 was significantly larger than that of nude rats in the control group (Fig. 8B). The weight of the tumor from nude rats overexpressing PCAT1 was significantly greater than that from nude rats in the control group (Fig. 8C).

## Discussion

PCAT1 has been shown to promote malignant phenotypes in several human cancers, such as hepatocellular carcinoma<sup>22</sup>, extrahepatic cholangiocarcinoma<sup>23</sup>, and esophageal squamous cell carcinoma<sup>12</sup>. Here, we found that PCAT1 promoted NSCLC cell proliferation and invasion and inhibited apoptosis, while silencing PCAT1 inhibited cell proliferation and invasion, and promoted cell apoptosis. Furthermore, we found that PCAT1 can bind directly to DKC1 and confirmed

that there are 2 DKC1 binding sites in the PCAT1 sequence. Moreover, DKC1, a binding protein of PCAT1, promoted NSCLC cell proliferation and invasion and inhibited apoptosis. Then we explored the pathway through which PCAT1 and DKC1 impacted on. We confirmed that PCAT1 and DKC1 regulated NSCLC cell proliferation, invasion, and apoptosis via the VEGF/AKT/Bcl-2/caspase 9 pathway. Most importantly, the results from the intraperitoneal injection of A549 cells stably overexpressing PCAT1 into nude rats indicated the tumorigenic ability of PCAT1 *in vivo*. Recent studies showed that both PCAT1 and DKC1 could activate the AKT signaling pathway<sup>14,24</sup>, which supports our conclusions.

Studies have reported that lncRNA PCAT1 is upregulated in tumor tissues and plays an oncogenic role. For instance, in colorectal cancer, downregulation of PCAT1 inhibited cell proliferation and induced cell cycle arrest *in vitro*<sup>25</sup>. In osteosarcoma cells, silencing PCAT1 caused an increase in

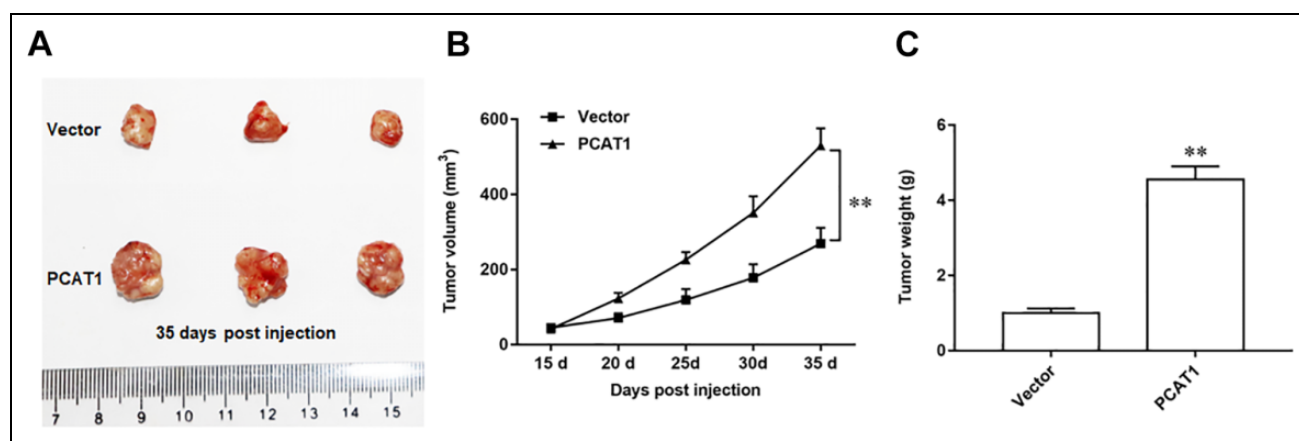


**Fig. 7.** PCAT1 and DKC1 regulate NSCLC cell proliferation, invasion, and apoptosis via the VEGF/AKT pathway. (A) A549 cells were transfected with pcDNA-DKC1, pcDNA-PCAT1 (2.0 µg/ml) alone or together with pcDNA-DKC1 and pcDNA-PCAT1 (2.0 µg/ml) for 24 h, respectively. Relative protein expression was detected by Western blotting. (B) A549 cells were transfected with siDKC1, siPCAT1 (30 µM) alone or together with siDKC1 and siPCAT1 (30 µM) for 24 h, respectively. Relative protein expression was detected by Western blotting. (C) A549 cells were transfected together with pcDNA-DKC1 and pcDNA-PCAT1 for 24 h and then incubated with anti-VEGF, AKT, and Bcl-2 inhibitors. Western blotting was used to detect the expression of AKT pathway-related proteins. (D) Cell proliferation was analyzed by cell counting kit-8 assay. (E) Cell invasion was detected by the Transwell invasion assay. (F) Western blotting was used to detect the expression of proliferous-related and invasive-related proteins. (G) Apoptosis of A549 cells was detected by flow cytometry. Glycerol-aldehyde 3-phosphate dehydrogenase was used as an internal reference. Statistical significance was assessed by using one-way variation analysis or Student's *t*-test. Values are expressed as mean  $\pm$  SEM,  $n = 3$  for each group. \* $P < 0.05$ , \*\* $P < 0.01$ , # $P < 0.05$  versus 2.0 µg/ml PCAT1 + DKC1 group or 30 nM DKC1 group. AKT: protein kinase B; DKC1: dyskerin pseudouridine synthase I; NSCLC: nonsmall cell lung cancer; PCAT1: prostate cancer associated transcript I; VEGF: vascular endothelial growth factor.

the cell population at the G0/G1 phase and a decrease in the S phase<sup>26</sup>. Overexpression of PCAT1 inhibited the chemosensitivity of esophageal cancer cells to cisplatin<sup>27</sup>. PCAT1 inhibited the radiation sensitivity of glioma stem cells<sup>28</sup>. Upregulation of PCAT1 expression promoted tumor cell migration and inhibited apoptosis<sup>29–31</sup>.

Furthermore, PCAT1 can interact with a large number of factors and pathways involved in cancer development and progression. Evidence suggested that in prostate cancer, PCAT1 inhibited HR activity by mediating post-transcriptional

inhibition of BRCA2 via reducing BRCA2 mRNA stability<sup>32</sup>. Huang et al. discovered that PCAT1 interacted with EZH2 and inhibited the expression of p21 in osteosarcoma cells<sup>31</sup>. PCAT1 is located at Chr8q24, which is located only 725 kb upstream of the oncogene MYC, and regulates c-Myc in a post-transcriptional manner through 3'-untranslated region activation in prostate cancer cells<sup>33</sup>. And PCAT1 could act as a molecular sponge or competitive endogenous RNA to sponge miRNAs. Studies have shown that PCAT1 functions as a competitive endogenous RNA for miR-145-5p and regulates the



**Fig. 8.** LncRNA PCAT1 promotes tumorigenesis in nude rats. The A549 cells stably transfected with empty vector (pcDNA3.1) and PCAT1 overexpression vector (pcDNA-PCAT1) were intraperitoneally injected into nude rats. The rats were sacrificed on days 15, 20, 25, 30, and 35 after injection, respectively, and their tumors were obtained. (A) Representative tumor pictures at day 35 postinjection. (B) Tumor volume. (C) Tumor weight. Statistical significance was assessed by using one-way variation analysis or Student's *t*-test. Values are expressed as mean  $\pm$  SEM,  $n = 3$  for each group. \*\* $P < 0.01$ . LncRNA: long noncoding RNA; PCAT1: prostate cancer associated transcript 1.

expression of fascin-1 in prostate cancer progression<sup>34</sup>. In gastric cancer, PCAT1 could confer cisplatin resistance to gastric cancer by sponging miR-128<sup>35</sup>.

DKC1 has been reported to be associated with congenita dyskeratosis and increase cancer susceptibility<sup>18,19</sup>. As an oncogene, DKC1 was correlated with other cancers and promoted cancer progression<sup>36,37</sup> and predicted poor prognosis in patient<sup>38</sup>. Expression of DKC1 was abnormally increased in hepatocellular carcinoma cells and correlated with MYC and MKI67 expression<sup>39,40</sup>. In NSCLC, we also observed that DKC1 was positively correlated with PCAT1 and promoted the proliferation and invasion of NSCLC cells. Importantly, we found that DKC1 could directly bind to PCAT1 and regulated apoptosis of human NSCLC cells via the VEGF/AKT pathway, thereby affecting the EMT pathway. In summary, we found abnormal expression of PCAT1 and DKC1 in human NSCLC tissues and cell lines and described a new mechanism that PCAT1 bounds to DKC1 to promote NSCLC progression.

### Ethical Approval

This study was approved by the Ethics Committee at the Second Affiliated Hospital of Xi'an Jiaotong University.

### Statement of Human and Animal Rights

All procedures in this study were conducted in accordance with the Second Affiliated Hospital of Xi'an Jiaotong University of Ethics Committee's (Approval No. 2020136) approved protocols.

### Statement of Informed Consent

Written informed consent was obtained from the patients for their anonymized information to be published in this article.


### Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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### References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394–424.
- Tsim S, O'Dowd CA, Milroy R, Davidson S. Staging of non-small cell lung cancer (NSCLC): a review. *Respir Med.* 2010; 104(12):1767–1774.
- Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin.* 2007;57(1):43–66.
- Zhang Q, Wei Y, Yan Z, Wu C, Chang Z, Zhu Y, Li K, Xu Y. The characteristic landscape of lncRNAs classified by RBP-lncRNA interactions across 10 cancers. *Mol Biosyst.* 2017; 13(6):1142–1151.
- Evans JR, Feng FY, Chinnaiyan AM. The bright side of dark matter: lncRNAs in cancer. *J Clin Invest.* 2016;126(8): 2775–2782.
- Yu W, Sun Z, Yang L, Han Y, Yue L, Deng L, Yao R. lncRNA PTAR promotes NSCLC cell proliferation, migration and invasion by sponging microRNA101. *Mol Med Rep.* 2019;20(5): 4168–4174.

7. Jia YC, Wang JY, Liu YY, Li B, Guo H, Zang AM. LncRNA MAFG-AS1 facilitates the migration and invasion of NSCLC cell via sponging miR-339-5p from MMP15. *Cell Biol Int*. 2019;43(4):384–393.
8. Prensner JR, Iyer MK, Balbin OA, Dhanasekaran SM, Cao Q, Brenner JC, Laxman B, Asangani IA, Grasso CS, Kominsky HD, Cao X, et al. Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression. *Nat Biotechnol*. 2011; 29(8):742–749.
9. Yang ML, Huang Z, Wu LN, Wu R, Ding HX, Wang BG. LncRNA-PCAT1 rs2632159 polymorphism could be a biomarker for colorectal cancer susceptibility. *Biosci Rep*. 2019; 39(7):BSR20190708.
10. Yuan Q, Chu H, Ge Y, Ma G, Du M, Wang M, Zhang Z, Zhang W. LncRNA PCAT1 and its genetic variant rs1902432 are associated with prostate cancer risk. *J Cancer*. 2018;9(8): 1414–1420.
11. Liu W, Xu J. LncRNA PCAT-1 in gastrointestinal cancers: a meta-analysis. *Medicine*. 2018;97(48):e13429.
12. Huang L, Wang Y, Chen J, Wang Y, Zhao Y, Wang Y, Ma Y, Chen X, Liu W, Li Z, Zhao L, et al. Long noncoding RNA PCAT1, a novel serum-based biomarker, enhances cell growth by sponging miR-326 in oesophageal squamous cell carcinoma. *Cell Death Dis*. 2019;10(7):513.
13. Zhao X, Fan Y, Lu C, Li H, Zhou N, Sun G, Fan H. PCAT1 is a poor prognostic factor in endometrial carcinoma and associated with cancer cell proliferation, migration and invasion. *Bosn J Basic Med Sci*. 2019;19(3):274–281.
14. Shang Z, Yu J, Sun L, Tian J, Zhu S, Zhang B, Dong Q, Jiang N, Flores-Morales A, Chang C, Niu Y. LncRNA PCAT1 activates AKT and NF-kappaB signaling in castration-resistant prostate cancer by regulating the PHLPP/FKBP51/IKKalpha complex. *Nucleic Acids Res*. 2019;47(8):4211–4225.
15. Zhao B, Hou X, Zhan H. Long non-coding RNA PCAT-1 overexpression promotes proliferation and metastasis in non-small cell lung cancer cells. *Int J Clin Exp Med*. 2015;8(10): 18482–18487.
16. Li J, Li Y, Wang B, Ma Y, Chen P. LncRNA-PCAT-1 promotes non-small cell lung cancer progression by regulating miR-149-5p/LRIG2 axis. *J Cell Biochem*. 2018.
17. Li JH, Liu S, Zhou H, Qu LH, Yang JH. starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res*. 2014;42:D92–D97.
18. Turano M, Angrisani A, De Rosa M, Izzo P, Furia M. Real-time PCR quantification of human DKC1 expression in colorectal cancer. *Acta Oncol*. 2008;47(Database issue): 1598–1599.
19. Sieron P, Hader C, Hatina J, Engers R, Wlazlinski A, Muller M, Schulz WA. DKC1 overexpression associated with prostate cancer progression. *Br J Cancer*. 2009;101(8):1410–1416.
20. Penzo M, Casoli L, Ceccarelli C, Trere D, Ludovini V, Crino L, Montanaro L. DKC1 gene mutations in human sporadic cancer. *Histol Histopathol*. 2013;28(3):365–372.
21. Sur S, Nakanishi H, Steele R, Ray RB. Depletion of PCAT-1 in head and neck cancer cells inhibits tumor growth and induces apoptosis by modulating c-Myc-AKT1-p38 MAPK signalling pathways. *BMC Cancer*. 2019;19(1):354.
22. Shen X, Zhang Y, Wu X, Guo Y, Shi W, Qi J, Cong H, Wang X, Wu X, Ju S. Upregulated lincRNA-PCAT1 is closely related to clinical diagnosis of multiple myeloma as a predictive biomarker in serum. *Cancer Biomark*. 2017;18(3):257–263.
23. Zhang F, Wan M, Xu Y, Li Z, Leng K, Kang P, Cui Y, Jiang X. Long noncoding RNA PCAT1 regulates extrahepatic cholangiocarcinoma progression via the Wnt/beta-catenin-signaling pathway. *Biomed Pharmacother*. 2017;94:55–62.
24. Watanabe A, Tagawa H, Yamashita J, Teshima K, Nara M, Iwamoto K, Kume M, Kameoka Y, Takahashi N, Nakagawa T, Shimizu N, et al. The role of microRNA-150 as a tumor suppressor in malignant lymphoma. *Leukemia*. 2011;25(8): 1324–1334.
25. Qiao L, Liu X, Tang Y, Zhao Z, Zhang J, Feng Y. Down regulation of the long non-coding RNA PCAT-1 induced growth arrest and apoptosis of colorectal cancer cells. *Life Sci*. 2017;188:37–44.
26. Zhang X, Zhang Y, Mao Y, Ma X. The lincRNA PCAT1 is correlated with poor prognosis and promotes cell proliferation, invasion, migration and EMT in osteosarcoma. *Onco Targets Ther*. 2018;11:629–638.
27. Zhen Q, Gao LN, Wang RF, Chu WW, Zhang YX, Zhao XJ, Lv BL, Liu JB. LncRNA PCAT-1 promotes tumour growth and chemoresistance of oesophageal cancer to cisplatin. *Cell Biochem Funct*. 2018;36(1):27–33.
28. Zhang P, Liu Y, Fu C, Wang C, Duan X, Zou W, Zhao T. Knockdown of long non-coding RNA PCAT1 in glioma stem cells promotes radiation sensitivity. *Med Mol Morphol*. 2019; 52(2):114–122.
29. Wen J, Xu J, Sun Q, Xing C, Yin W. Upregulation of long non coding RNA PCAT-1 contributes to cell proliferation, migration and apoptosis in hepatocellular carcinoma. *Mol Med Rep*. 2016;13(5):4481–4486.
30. Cui WC, Wu YF, Qu HM. Up-regulation of long non-coding RNA PCAT-1 correlates with tumor progression and poor prognosis in gastric cancer. *Eur Rev Med Pharmacol Sci*. 2017;21(13):3021–3027.
31. Huang J, Deng G, Liu T, Chen W, Zhou Y. Long noncoding RNA PCAT-1 acts as an oncogene in osteosarcoma by reducing p21 levels. *Biochem Biophys Res Commun*. 2018;495(4): 2622–2629.
32. Prensner JR, Chen W, Iyer MK, Cao Q, Ma T, Han S, Sahu A, Malik R, Wilder-Romans K, Navone N, Logothetis CJ, et al. PCAT-1, a long noncoding RNA, regulates BRCA2 and controls homologous recombination in cancer. *Cancer Res*. 2014; 74(6):1651–1660.
33. Prensner JR, Chen W, Han S, Iyer MK, Cao Q, Kothari V, Evans JR, Knudsen KE, Paulsen MT, Ljungman M, Lawrence TS, et al. The long non-coding RNA PCAT-1 promotes prostate cancer cell proliferation through cMyc. *Neoplasia*. 2014; 16(11):900–908.

34. Xu W, Chang J, Du X, Hou J. Long non-coding RNA PCAT-1 contributes to tumorigenesis by regulating FSCN1 via miR-145-5p in prostate cancer. *Biomed Pharmacother.* 2017;95:1112–1118.
35. Guo Y, Yue P, Wang Y, Chen G, Li Y. PCAT-1 contributes to cisplatin resistance in gastric cancer through miR-128/ZEB1 axis. *Biomed Pharmacother.* 2019;118:109255.
36. Donaires FS, Alves-Paiva RM, Gutierrez-Rodrigues F, da Silva FB, Tellechea MF, Moreira LF, Santana BA, Traina F, Dunbar CE, Winkler T, Calado RT. Telomere dynamics and hematopoietic differentiation of human DKC1-mutant induced pluripotent stem cells. *Stem Cell Res.* 2019;40:101540.
37. Miao FA, Chu K, Chen HR, Zhang M, Shi PC, Bai J, You YP. Increased DKC1 expression in glioma and its significance in tumor cell proliferation, migration and invasion. *Invest New Drugs.* 2019;37(6):1177–1186.
38. Zhang M, Pan Y, Jiang R, Hou P, Shan H, Chen F, Jiang T, Bai J, Zheng J. DKC1 serves as a potential prognostic biomarker for human clear cell renal cell carcinoma and promotes its proliferation, migration and invasion via the NFkappaB pathway. *Oncol Rep.* 2018;40(2):968–978.
39. Liu B, Zhang J, Huang C, Liu H. Dyskerin overexpression in human hepatocellular carcinoma is associated with advanced clinical stage and poor patient prognosis. *PLoS One.* 2012;7(8):e43147.
40. Alawi F, Lee MN. DKC1 is a direct and conserved transcriptional target of c-MYC. *Biochem Biophys Res Commun.* 2007;362(4):893–898.