Suppression of CDCA3 inhibits prostate cancer progression via NF-κB/cyclin D1 signaling inactivation and p21 accumulation

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Abstract. Dysregulation of the cell cycle contributes to tumor progression. Cell division cycle-associated 3 (CDCA3) is a known trigger of mitotic entry and has been demonstrated to be constitutively upregulated in tumors. It is therefore associated with carcinogenic properties reported in various cancers. However, the role of CDCA3 in prostate cancer is unclear. In the present study, western blotting and analysis of gene expression profiling datasets determined that CDCA3 expression was upregulated in prostate cancer and was associated with a poor prognosis. CDCA3 knockdown in DU145 and PC-3 cells led to decreased cell proliferation and increased apoptosis, with increased protein expression levels of cleaved-caspase3. Further experiments demonstrated that downregulated CDCA3 expression levels induced G0/G1 phase arrest, which was attributed to increased p21 protein expression levels and decreased cyclin D1 expression levels via the regulation of NF-κB signaling proteins (NFκB-p105/p50, IKKα/β, and pho-NFκB-p65). In conclusion, these results indicated that CDCA3 may serve a crucial role in prostate cancer and consequently, CDCA3 knockdown may be used as a potential therapeutic target.

Introduction

Prostate cancer is the most common type of malignant tumor in males and is the leading cause of cancer-related mortality in numerous countries (1,2). At present, in 2021 there have been ~248,530 new cases and 34,130 deaths as a result of prostate cancer in the United States alone (3). It has also previously been determined that the incidence rate of prostate cancer is significantly increased in the Asian population (4). Due to advances in medical oncology and improvements in surgical techniques, a variety of cancers initially respond to treatment, but tumor progression, recurrence and resistance to chemotherapy and radiotherapy may still occur (5). Patients with localized prostate cancer can be initially treated with surgery and radiotherapy, and subsequently treated using androgen deprivation therapy. However, in certain patients the disease eventually becomes metastatic and resistant to treatment, which is referred to as castration-resistant prostate cancer (CRPC).

Generally, the prognosis of patients with prostate cancer depends on the depth of tumor infiltration, as well as the appearance of lymph nodes and long-distance metastases, which can be assessed by pathological microscopy (6). The poor prognosis associated with prostate cancer has prompted the research and discovery of novel diagnostic markers. However, the molecular mechanisms governing the development and progression of prostate cancer remain unclear. It is therefore crucial to elucidate new molecular approaches to improve existing prognostics and supply relevant key clinical insights into prognosis determination in patients with prostate cancer.

Currently, researchers are focused on targeting the cell cycle to exploit and block the proliferative capacity of cancer cells. Progression through the phases of the cell cycle relies on a complex network of proteins. Previous studies have suggested that the abnormal expression of cell cycle regulatory proteins may contribute to the development of cancer (7,8). The cell division cycle-associated (CDCA) protein family (CDCA1-8) consists of a group of proteins that serve a crucial role in numerous biological processes, including the cell cycle. Furthermore, it has been demonstrated that CDCAs may contribute towards prostate cancer progression. A clinical trial that was carried out on patients with CRPC determined that CDCA1 peptide vaccination could induce peptide-specific cytotoxic T lymphocytes (CTLs) in patients with CRPC (9). It has also been demonstrated that CDCA2 is upregulated in patients with prostate cancer, which regulates cell proliferation and is a direct target of the hypoxia signaling pathway (10). Inhibition of CDCA6 induces prostate cancer cell death, indicating CDCA6 may potentially have use as a drug target in CRPC (11). Furthermore, bioinformatics analysis has revealed that CDCA8 may facilitate the tumorigenesis and progression

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of prostate cancer. CDCA8 could therefore be a novel therapeutic target and biomarker for the diagnosis and prognosis of prostate cancer (12). CDCA3 regulates cell cycle processes and serves a role in triggering mitosis entry. CDCA3 is frequently upregulated in tumor tissues and is associated with the oncogenic properties of a variety of cancers, including colorectal cancer (CRC) (13,14), gastric cancer (GC) (15), non-small cell lung cancer (NSCLC) (16), oral squamous cell carcinoma (OSCC) (17) and pancreatic cancer (PAC) (18). It has also been reported that CDCA3 acts as a downstream target gene of HOXB3, which promotes prostate cancer progression (19).

The aim of the present study was to determine, using microarray analysis, whether CDCA3 served as a hub gene in prostate cancer progression and was associated with patient prognosis. It was also investigated whether CDCA3 was differentially expressed in prostate cancer and paracancerous tissues and if CDCA3 was essential for cell proliferation, apoptosis and cell cycle arrest.

Materials and methods

Microarray analysis. Gene expression profiling datasets for prostate cancer were analyzed using the Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/geo2r/) database. In the GSE27616 dataset (20), four normal prostate and nine cancerous prostate samples were analyzed. In the GSE3325 dataset (21), six normal prostate and 13 cancerous prostate samples were analyzed. For microarray data analysis, differentially expressed genes (DEGs) were defined as genes whose expression differed between normal and cancerous samples. The overlapping DEGs in the GSE27616 and GSE3325 datasets were identified to determine the genes involved with tumorigenesis in prostate cancer. DEGs were screened using the P-value (P<0.01) and fold change (FC; log |FC| >1). FunRich software (version FunRich_3.1.3) was used to identify overlapping DEGs (22). The upregulated and downregulated genes were analyzed.

UALCAN (http://ualcan.path.uab.edu/analysis.html) is a web portal based on level 3 RNA-seq and clinical data from 31 cancer types in The Cancer Genome Atlas (TCGA) database (23). It helps analyze, integrate and discover cancer transcriptomic data, and was used to analyze TCGA gene expression information.

Identification of hub genes and survival analysis. Search Tool for the Retrieval of Interacting Genes (STRING) is a biological database (https://string-db.org) for constructing a protein-protein interaction (PPI) network, providing a system-wide view of interactions between each member. DEGs were used to construct a STRING database, whereby an interaction with a combined score of >0.4 was considered to be statistically significant. Subsequently, a PPI network was established using Cytoscape software (Cytoscape_v3.7.2) (22), which visually explores biomolecular interaction networks composed of proteins, genes and other molecules. CytoHubba in Cytoscape was applied to screen the hub genes ranked by the MCC method (24).

Disease-free survival (DFS) of patients with prostate cancer was analyzed using the Gene Expression Profiling Interactive Analysis (GEPIA; http://gepia.cancer-pku.cn/detail/) database. Patients with prostate cancer were divided into a low expression and high expression group according to their median value of gene expression. P<0.05 was set as the significance cut-off level.

Tissue samples. The present study was approved (approval no. xs2020ky014) by the Ethics Committee of Xishan People's Hospital of Wuxi City (Wuxi, China). In total, seven prostate cancer samples were collected to investigate CDCA3 protein expression. Both cancerous and paracancerous tissue samples were collected from each patient. All patients (mean age, 74 years; range, 68-79 years) were treated with radical prostatectomy between January 2021 and July 2021, and consents were obtained orally. The inclusion criteria were tissue samples collected from patients who had not undergone androgen deprivation therapy, chemotherapy, radiotherapy, or other auxiliary treatment prior to surgery. The exclusion criteria were tissue samples collected from patients who had other severe comorbidities.

Cell culture and in vitro transfection. The human prostate cancer DU145 (serial cat. no. TCHu222) and PC-3 (serial cat. no. TCHu158) cell lines were purchased from The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences. CDCA3 protein levels were highly expressed in DU145 and PC-3 cell lines as revealed by analyzing the Harmonizome (https://maayanlab.cloud/Harmonizome/) database, which was the reason for selecting these two cell lines for subsequent analysis, and the cell line expression image is presented in Fig. S1. Cells were cultured in RPMI-1640 medium containing 10% FBS (both from Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere at 37˚C with 5% CO_2. To establish prostate cancer cell lines that persistently repressed CDCA3 expression, CDCA3 short hairpin (sh)RNA lentiviral constructs were purchased from Shanghai GeneChem Co. Ltd. The CDCA3 shRNA nucleotide sequence was as follows: 5'-GCACGGACACCTATG AAGA-3', which was confirmed the validation by a previous study (14). The negative control was a double-stranded shRNA without sequence homology to any known human genes, and the sequence was as follows: 5'-UUCUCCGAACGUGUC ACGUTT-3'. sh-CDCA3 and sh-negative control (NC) were transduced as previously described (25).

Cell viability assay. DU145 and PC-3 cells were seeded at 200,000 cells/well into 6-well plates and cultured in RPMI-1640 medium containing 10% FBS at 37˚C with 5% CO_2 overnight for transfection. Prostate cancer cells were transduced with sh-CDCA3 or sh-NC at a multiplicity of infection of 50 and cultured with 6 μg/ml polybrene (GeneChem Co. Ltd) for 12 h in a humidified atmosphere at 37˚C with 5% CO_2. Subsequently, the cells were seeded at 2,000 cells/well in a 96-well plate. At 24-96 h post-transfection, cell viability was detected using a Cell Counting Kit-8 (CCK-8) assay kit (Beyotime Institute of Biotechnology). Cells were incubated with 10 μl CCK-8 solution/well for 1 h at 37˚C according to the manufacturer's protocol. The absorbance was measured at a wavelength of 450 nm. Cell viability was measured across five wells in each group. All independent treatments were carried out in three replicates.

Colony formation assay. DU145 and PC-3 cells in the logarithmic growth phase were collected and 1,000 cells/well were seeded into a 6-well plate. A total of 3 replicate wells were used for each group. Cells were cultured in RPMI-1640 medium containing 10% FBS at 37˚C with 5% CO_2 for 10 days and when macroscopic colonies appeared the culture solution was discarded. After washing with PBS, the cells were fixed in 4% paraformaldehyde for 15 min at room temperature and stained with 0.5% crystal violet solution for 15 min at room temperature. Colonies were observed using a light microscope and the number of colonies was counted by visual inspection. Images were captured using a Canon EOS600D digital camera (Canon, Inc.). The minimum number of cells per colony was 50.

Apoptosis assay and cell cycle analysis. The apoptotic rate and cell cycle of prostate cancer cells were investigated using flow cytometry. DU145 and PC-3 cells were trypsinized (without EDTA). For the cell cycle analysis, cells were washed with PBS and subsequently incubated for 30 min at 37˚C in the dark with 500 μl PI (Beyotime Institute of Biotechnology). Cells were then scanned using a CytoFLEX flow cytometer (Beckman Coulter, Inc.). Cells were counted and the percentages of prostate cancer cells in the three cycle phases were compared. For the cell apoptosis analysis, cells were washed with PBS and were subsequently cultured for 30 min at 37˚C after adding 5 μl Annexin V-phycoerythrin and 10 μl 7-aminoactinomycin D (Hangzhou Multi Sciences Biotech Co., Ltd.) to identify apoptotic and necroptotic cells. Stained cells and their apoptotic rates were quantified using the software CytExpert 2.4.0.28 (Beckman Coulter, Inc.).

Western blotting. Total protein was extracted using RIPA lysis buffer (Beyotime Institute of Biotechnology). Protein concentration was determined using a BCA assay (Beyotime Institute of Biotechnology). Total protein (30 μg/lane) was separated using SDS-PAGE on a 6-12% gel (Beyotime Institute of Biotechnology) and transferred onto a PVDF membrane (MilliporeSigma). The membranes were blocked with 5% skimmed milk at room temperature for 1 h and incubated overnight at 4˚C with diluted primary antibodies. Subsequently, the membranes were washed using TBS with 0.1% Tween-20 (TBST) three times and incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. After being washed with TBST, the membranes were visualized using electrochemiluminescence (ECL) kit (Beyotime Institute of Biotechnology). GAPDH was used as the loading control. The following antibodies were used: Mouse monoclonal anti-GAPDH (1:2,000; cat. no. 33033M; BIOSS), rabbit polyclonal anti-CDCA3 (1:1,000; cat. no. YT0819), rabbit polyclonal anti-cleaved caspase-3 (1:1,000; cat. no. YC0006), rabbit polyclonal anti-pro-caspase-3 (1:1,000; cat. no. YT6113), and rabbit polyclonal anti-cyclin-dependent kinase inhibitor 1 (p21; 1:1,000; cat. no. YT3497; all from Immunoway Biotechnology Company), rabbit polyclonal anti-cyclin D1 (1:1,000; cat. no. 0623R; BIOSS), mouse monoclonal anti-NFκB-p65 (1:1,000; cat. no. YM311), rabbit polyclonal anti-phosphorylated (p)-NFκB-p65 (1:1,000; cat. no. YP0192), rabbit polyclonal anti-IKKα/β (1:1,000; cat. no. YT2302), and rabbit polyclonal anti-NFκB-p105/p50 (1:1,000; cat. no. YT3101; all from Immunoway Biotechnology Company), HRP-labeled goat anti-rabbit secondary antibody (1:5,000; cat. no. 40295G-HRP; BIOSS) and HRP-labeled goat anti-mouse secondary antibody (1:5,000; cat. no. 0368G-HRP; BIOSS).

Statistical analysis. The softwares SPSS 17.0 (SPSS, Inc.) and ImageJ 1.8.0 (National Institutes of Health) were used to carry out statistical analysis. Each value was acquired from at least three independent experiments. Data are presented as the mean ± SD. A two-tailed unpaired Student's t-test was used to analyze statistical differences between two groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Hub gene screening and survival analysis. In total, 1,119 and 1,690 DEGs were identified via the analysis of normal prostate vs. cancer tissues in the GSE27616 and GSE3325 datasets, respectively. A comparison of these two sets of genes revealed 231 overlapping genes, including 60 upregulated and 171 downregulated DEGs (Fig. 1A and B). Subsequently the STRING database was used for investigating and integrating the interactions between proteins. The PPI network of overlapped DEGs between normal vs. cancer tissues was constructed and data were exported for further analysis in Cytoscape. The top 20 genes were identified as hub genes with a score $\geq 1.22 \times 10^{17}$, according to the MCC method. The PPI network of these hub genes is presented in Fig. 1C. The identified hub genes were as follows: Baculoviral IAP repeat containing 5 (BIRC5), DLG associated protein 5 (DLGAP5), non-SMC condensing I complex subunit G (NCAPG), cyclin A2 (CCNA2), cyclin B2 (CCNB2), maternal embryonic leucine zipper kinase, targeting protein for Xklp2 (MELK), spindle and kinetochore associated complex subunit 3 (SKA3), epithelial cell transforming 2 (ECT2), hyaluronan mediated motility receptor, centromere protein F (HMMR), protein regulator of cytokinesis 1 (PRC1), nucleolar and spindle-associated protein 1 (NUSAP1), DNA topoisomerase II alpha (TOP2A), PDZ binding kinase (PBK), centromere protein A (CENPA), kinesin family member 20A (KIF20A), BUB1 mitotic checkpoint serine/threonine kinase (BUB1), family with sequence similarity 64 member A (FAM64A) and CDCA3. All 20 hub genes were expressed at elevated levels in prostate cancer compared with normal prostate tissues and were associated with the DFS of patients with prostate cancer, with the exception of ECT2. The DFS curve (produced in GEPIA) for the gene of interest in the present study, CDCA3, is presented in Fig. 1D. From these data it was indicated that prostate cancer patients with low CDCA3 expression had a longer DFS and a better prognosis than those with high CDCA3 expression ($P<0.001$).

CDCA3 overexpression in prostate cancer tissues. CDCA3 mRNA was demonstrated to be upregulated in prostate cancer compared with normal prostate tissues, both in GSE27616 and GSE3325 datasets. These results were also supported by TCGA database. In total, 497 prostate cancer tissues and 52 normal prostate samples were identified. CDCA3 was determined to

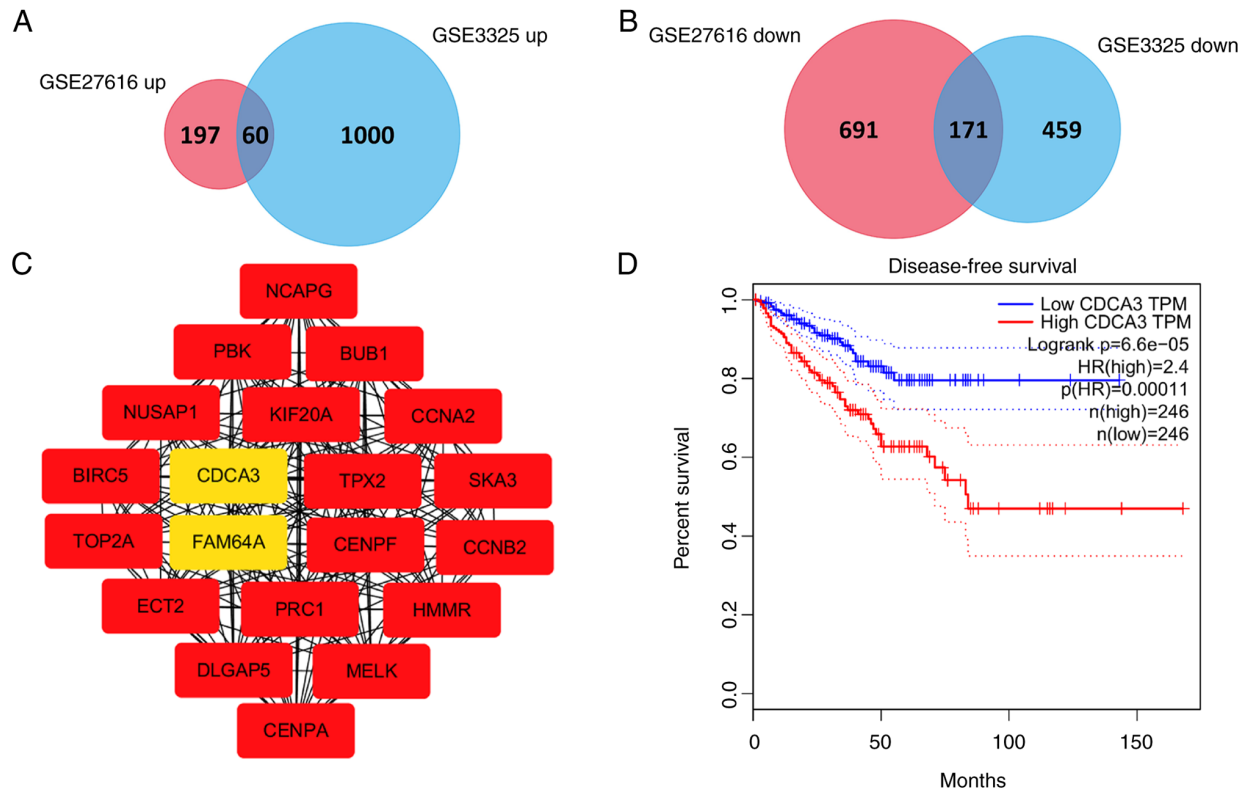

Figure 1. Hub gene screening and survival analysis. (A) In total, 257 and 1,060 DEGs were upregulated in prostate cancer tissues in the GSE27616 and GSE3325 datasets, respectively, and 60 overlapping genes were identified. (B) In total, 862 and 630 DEGs were downregulated in prostate cancer tissues in the GSE27616 and GSE3325 datasets, respectively, and 171 overlapping genes were identified. (C) A protein-protein interaction network of hub genes was constructed using Cytoscape. (D) Disease-free survival analysis of CDCA3 in prostate cancer patients was performed using the Gene Expression Profiling Interactive Analysis database. DEG, differentially expressed gene; CDCA3, cell division cycle-associated 3.

be significantly upregulated in prostate cancer compared with normal tissues (median 2.372 vs. 0.856 transcripts per million; P<0.001; Fig. 2A).

To verify the aforementioned results, prostate cancer and paracancerous tissues were collected from seven patients with prostate cancer at the Xishan People's Hospital of Wuxi city. Proteins were extracted from the tissues and the CDCA3 protein expression level was examined. The western blotting results revealed that CDCA3 protein expression levels were significantly higher in the prostate cancer tissue samples compared with the paracancerous tissue samples (P<0.01; Fig. 2B).

CDCA3 knockdown inhibits cell proliferation and induces apoptosis. Western blotting was used to detect the inhibition of CDCA3 protein expression following transduction with sh-CDCA3. CDCA3 protein expression levels were suppressed by 61.9% in DU145 cells and 66.3% in PC-3 cells transduced with sh-CDCA3 compared with the sh-NC transduced cells (P<0.001; Fig. 3). The results also demonstrated that CDCA3 knockdown increased the protein expression levels of cleaved caspase-3 by 2.1-fold in DU145 cells and 2.2-fold in PC-3 cells (P<0.001).

Subsequently, the effect of CDCA3 knockdown on DU145 and PC-3 cell proliferation was investigated using the CCK-8 and colony formation assays. The CCK-8 assay results demonstrated that knockdown of CDCA3 inhibited cell viability by 2.7% after 48 h (P=0.28), 26.2% after 72 h (P<0.001), 29.7% after 96 h (P<0.001) in DU145 cells, and 13.7% after 48 h (P<0.001), 37.0% after 72 h (P<0.001) and 34.7% after 96 h (P<0.001) in PC-3 cells, compared with the sh-NC cell group (Fig. 4A). The downregulation of CDCA3 also inhibited cell colony formation by 36.1% in DU145 cells (P<0.005) and 31.0% in PC-3 cells (P<0.001; Fig. 4B).

The mechanism of CDCA3 in cell apoptosis regulation was assessed using flow cytometry. The results demonstrated that CDCA3 knockdown promoted early apoptosis both in DU145 (45.3% in sh-CDCA3 vs. 5.1% in sh-NC; P<0.001) and PC-3 cells (37.8% in sh-CDCA3 vs. 4.9% in sh-NC; P<0.001). The late apoptotic rate was also increased in DU145 (7.1% in sh-CDCA3 vs. 5.3% in sh-NC; P<0.005) and PC-3 cells (13.8% in sh-CDCA3 vs. 8.9% in sh-NC; P<0.005; Fig. 4C).

CDCA3 knockdown induces G0/G1 phase arrest. The role of CDCA3 in modulating the prostate cancer cell cycle was analyzed using flow cytometry. The results demonstrated that CDCA3 knockdown induced G0/G1 phase arrest. The percentage of cells in the G0/G1 phase in the sh-CDCA3 group was higher compared with the sh-NC group, both in DU145 (35.06% in sh-CDCA3 vs. 30.91% in sh-NC; P<0.001) and PC-3 cells (36.61% in sh-CDCA3 vs. 33.06% in sh-NC; P<0.01; Fig. 5A).

Cyclin D1, a regulator of G1 phase progression, serves a crucial role in carcinogenesis and cancer progression (26). p21 also promotes cell cycle arrest in response to a variety

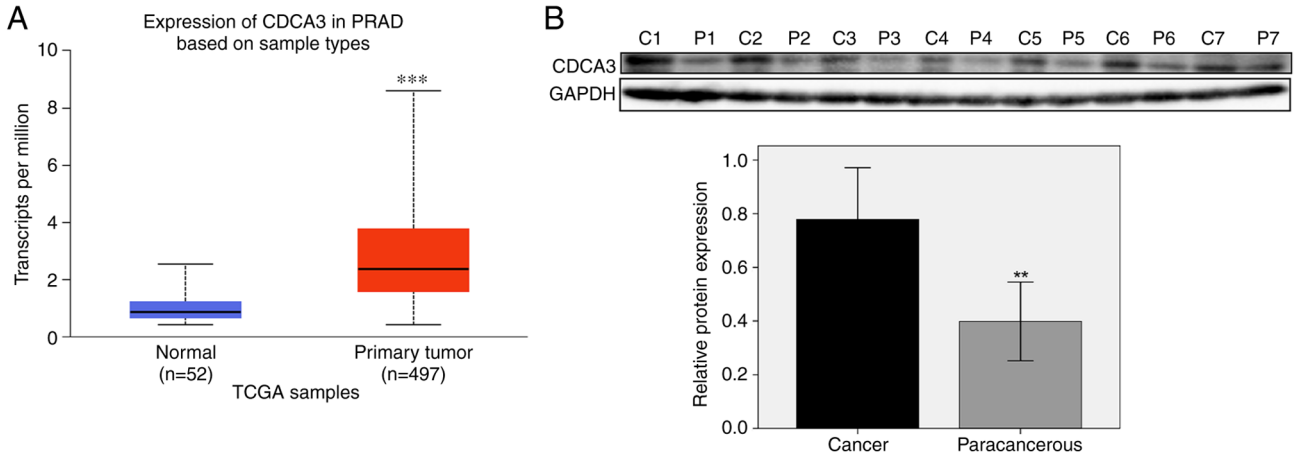

Figure 2. CDCA3 overexpression in prostate cancer tissues. (A) Transcriptomic levels of CDCA3 in prostate cancer and normal prostate tissues (The Cancer Genome Atlas database). (B) Western blotting was performed to determine CDCA3 protein expression levels in seven prostate cancer tissue samples compared with paracancerous tissue samples. The serial number of each case is displayed at the top of the figure. Data are presented as the mean ± SD. **P<0.01 and ***P<0.001. CDCA3, cell division cycle-associated 3; C, cancer tissue; P, paracancerous tissue.

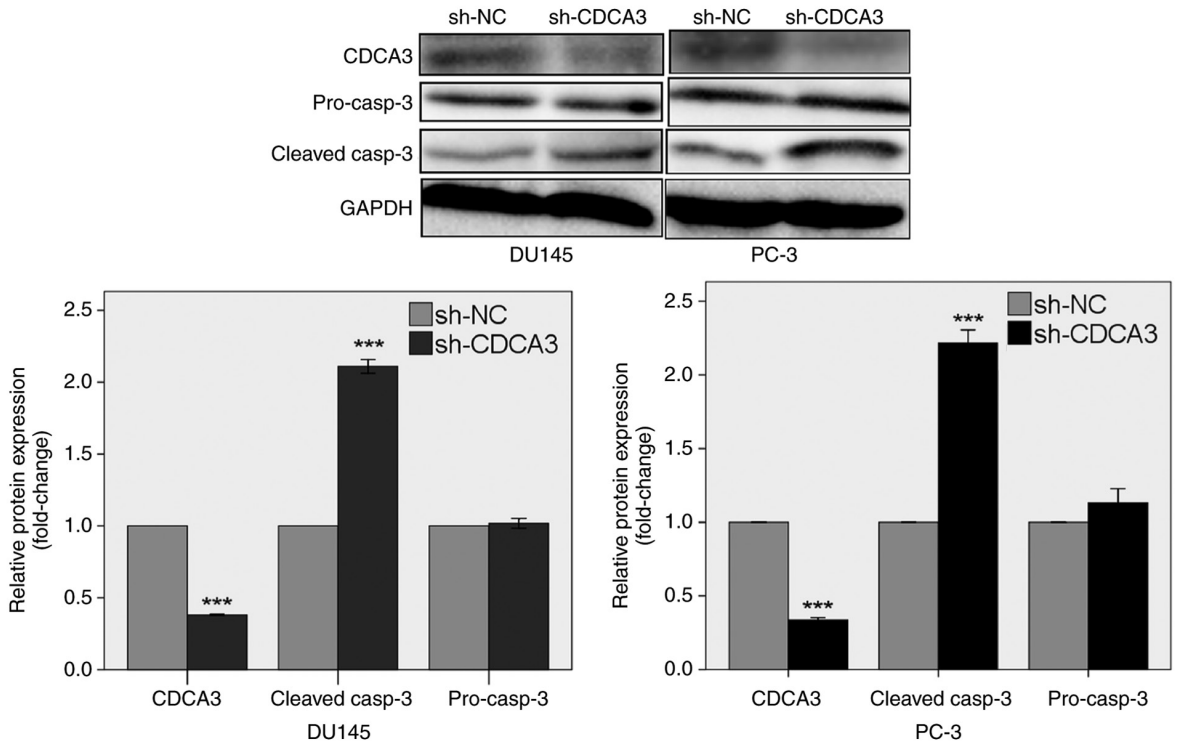

Figure 3. CDCA3 protein expression levels were suppressed in DU145 and PC-3 cells infected with sh-CDCA3 lentivirus. Protein expression levels of apoptosis-associated proteins, including pro-caspase-3 and cleaved caspase-3, were analyzed via western blotting. GAPDH was used as a loading control. Data are presented as the mean ± SD of three independent experiments. ***P<0.001. CDCA3, cell division cycle-associated 3; sh-, short hairpin; NC, negative control.

of stimuli (27). The results of the present study demonstrated that CDCA3 knockdown in DU145 and PC-3 cells reduced the protein expression levels of cyclin D1 and enhanced the protein expression levels of p21 (P<0.01; Fig. 5B).

CDCA3 knockdown inhibits the NF-κB signaling pathway. NF-κB activation is related to tumor initiation, progression and metastasis in prostate cancer (28). A previous study reported that NF-κB is constitutively activated in certain tumors, including in prostate, breast, ovarian and pancreatic cancers (29). Overexpression of NF-κB in the nucleus of prostate cancer cells is associated with a more aggressive cancer phenotype, chemoresistance and metastasis (28,29).

To discover the role of CDCA3 knockdown in the regulation of the NF-κB signaling pathway, western blotting was performed. The results demonstrated that CDCA3 knockdown suppressed the protein expression levels of NFκB-p105/p50, IKKα/β and p-NFκB-p65, both in DU145 and PC-3 cells (Fig. 6). CDCA3 knockdown had no effect on the protein expression levels of NFκB-p65. These results indicated that CDCA3 may potentially regulate the NF-κB signaling pathway in prostate cancer DU145 and PC-3 cells.

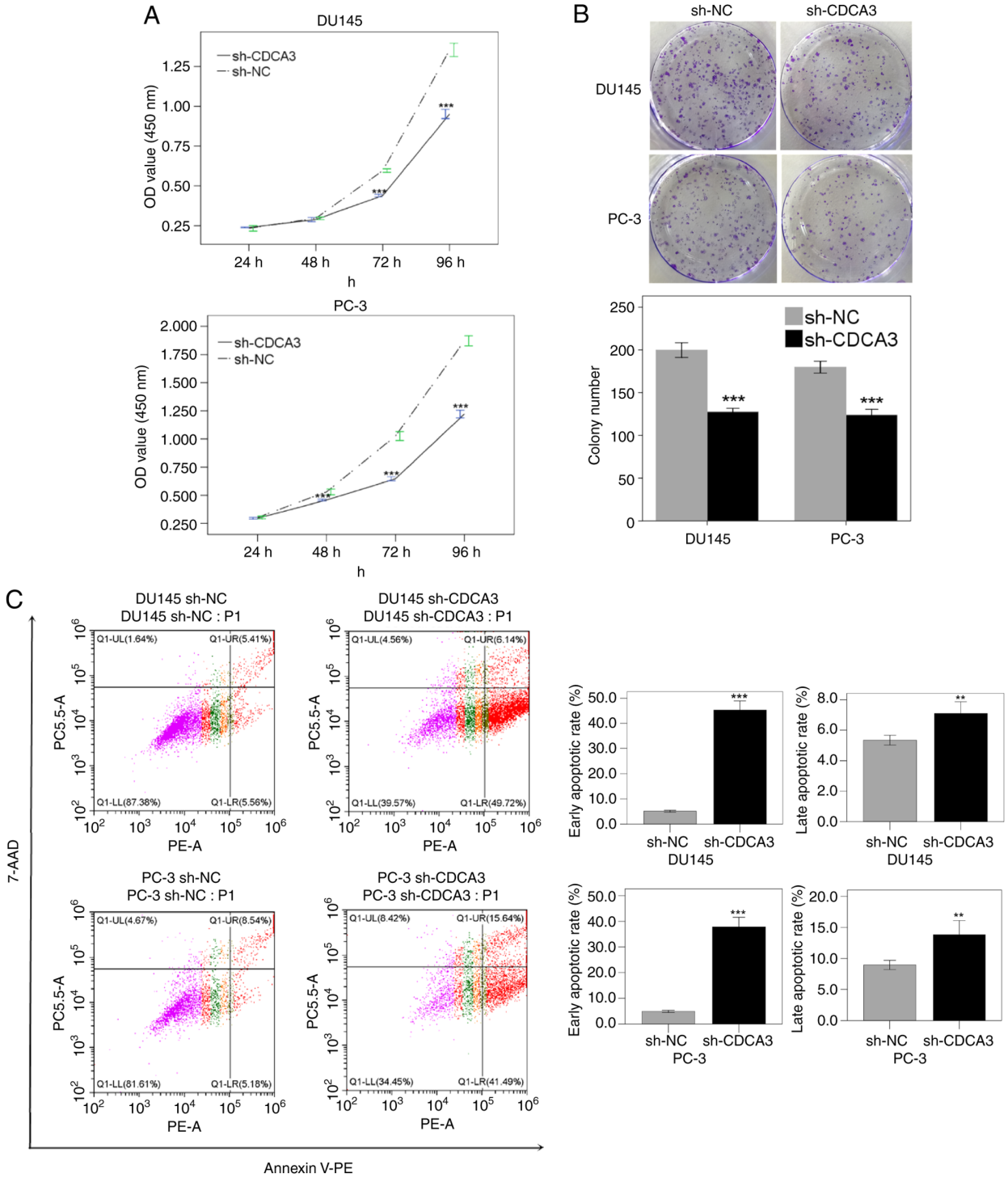

Figure 4. Knockdown of CDCA3 inhibits DU145 and PC-3 cell progression. (A) Effects of CDCA3 on cell proliferation were determined using the Cell Counting Kit-8 assay. Data are presented as the mean ± SD of five samples. (B) Effects of CDCA3 on cell proliferation were determined using the colony formation assay. (C) Flow cytometry determined that the downregulation of CDCA3 promoted early and late apoptosis. Data are presented as the mean ± SD of three samples. **P<0.01 and ***P<0.001. CDCA3, cell division cycle-associated 3; sh-, short hairpin; NC, negative control.

Discussion

Abnormal cell division can lead to cancer. Disturbance of cell cycle regulation is an important biological feature exhibited in malignant tumors and can lead to reduced apoptosis, unlimited proliferation and metastasis in malignant cells (30). CDCA3 belongs to a family of cell division-associated proteins, which function as part of the SKP1-Cullin-RING-F-box ubiquitin ligase complex that mediates the destruction of the mitosis-inhibitory kinase wee1 (14,16).

Previous studies have determined that CDCA3 expression levels are increased in tumor tissues and associated with a poor patient prognosis in CRC, GC, NSCLC, OSCC and PAC (13-18). In CRC, CDCA3 regulates E2F transcription factor 1, thereby inhibiting the expression of p21. Knockdown of CDCA3 results in G1/S-phase transition arrest via a significant increase in

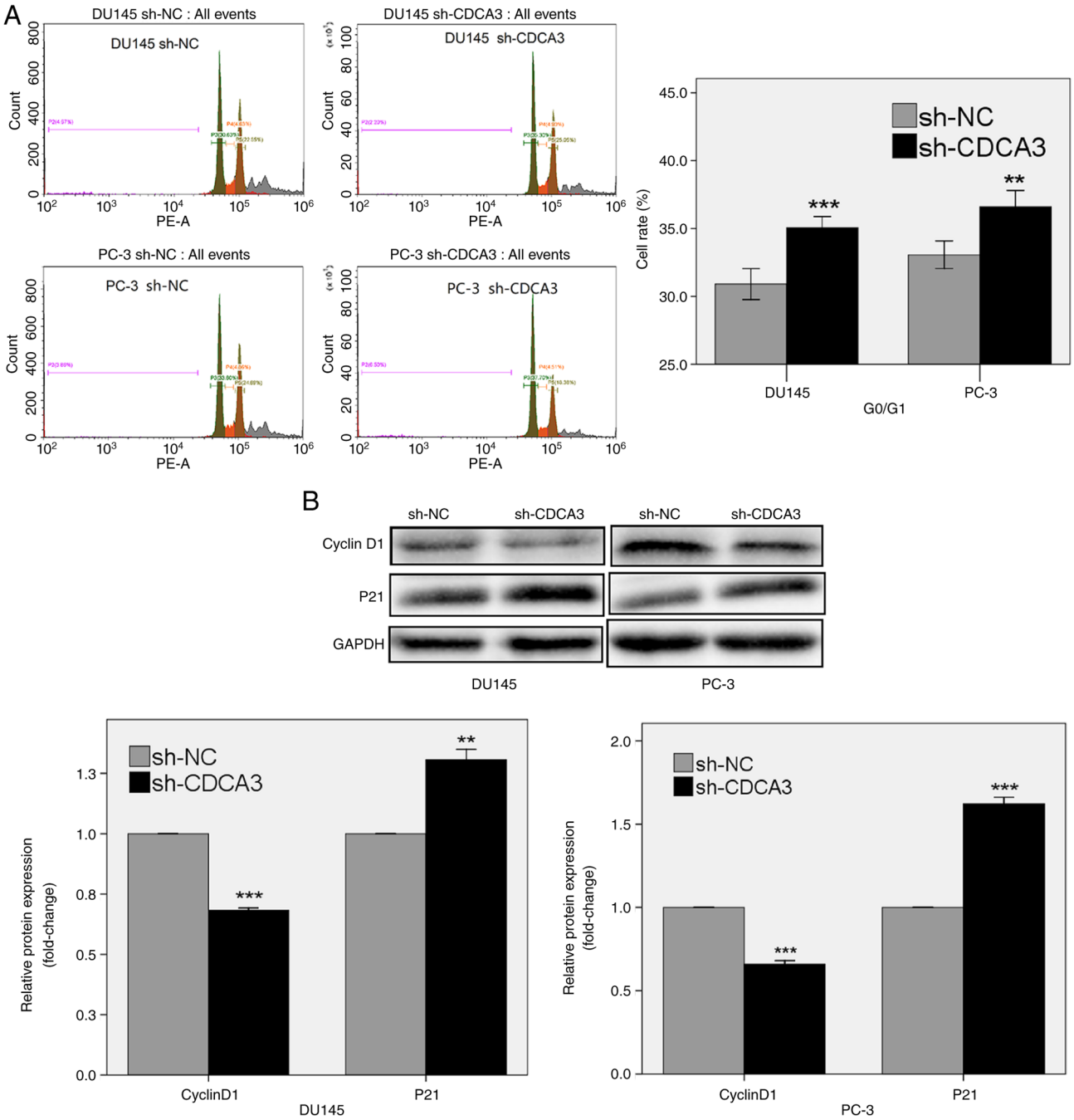

Figure 5. Knockdown of CDCA3 induces G0/G1-phase arrest in DU145 and PC-3 cells. (A) Flow cytometry was used to analyze the cell cycle following CDCA3 knockdown in DU145 and PC-3 cells. (B) Cell cycle-associated protein levels, including cyclin D1 and p21, were analyzed via western blotting. GAPDH was used as a loading control. Data are presented as the mean ± SD of three independent experiments. **P<0.01 and ***P<0.001. CDCA3, cell division cycle-associated 3; sh-, short hairpin; NC, negative control.

p21 levels in SW480 cells (13). Furthermore, CDCA3 knockdown not only induces cell cycle arrest but also promotes apoptosis via the NF-κB signaling pathway, interacting with TNF receptor-associated factor 2 (TRAF2) in CRC cells (14). In GC, CDCA3 knockdown inhibits cell proliferation and induces cell cycle arrest in the G0/G1 phase via mediating the Ras/ERK/MAPK axis (15). In NSCLC, depletion of CDCA3 expression reduces cell proliferation and causes abnormal G2/M-phase cell cycle progression, exhibiting an upregulation in p21 expression, independent of p53 (16). In OSCC, CDCA3 may be closely associated with cancer progression by preventing a pause of cell-cycle progression in the G1 phase via decreased expression of cyclin-dependent kinase inhibitors (17). In PAC, downregulation of CDCA3 inhibits cell proliferation, promotes cell apoptosis and suppresses *in vivo* tumor growth (18). In addition, CDCA3, as a downstream target gene of numerous genes and non-coding RNAs, promotes the progression of various tumors. In bladder cancer (BC), kinesin family member 4A promotes the development of BC via the transcriptional activation of CDCA3 expression (31). In prostate cancer, HOXB3 binds to the CDCA3 promoter region and transactivates CDCA3 expression, which

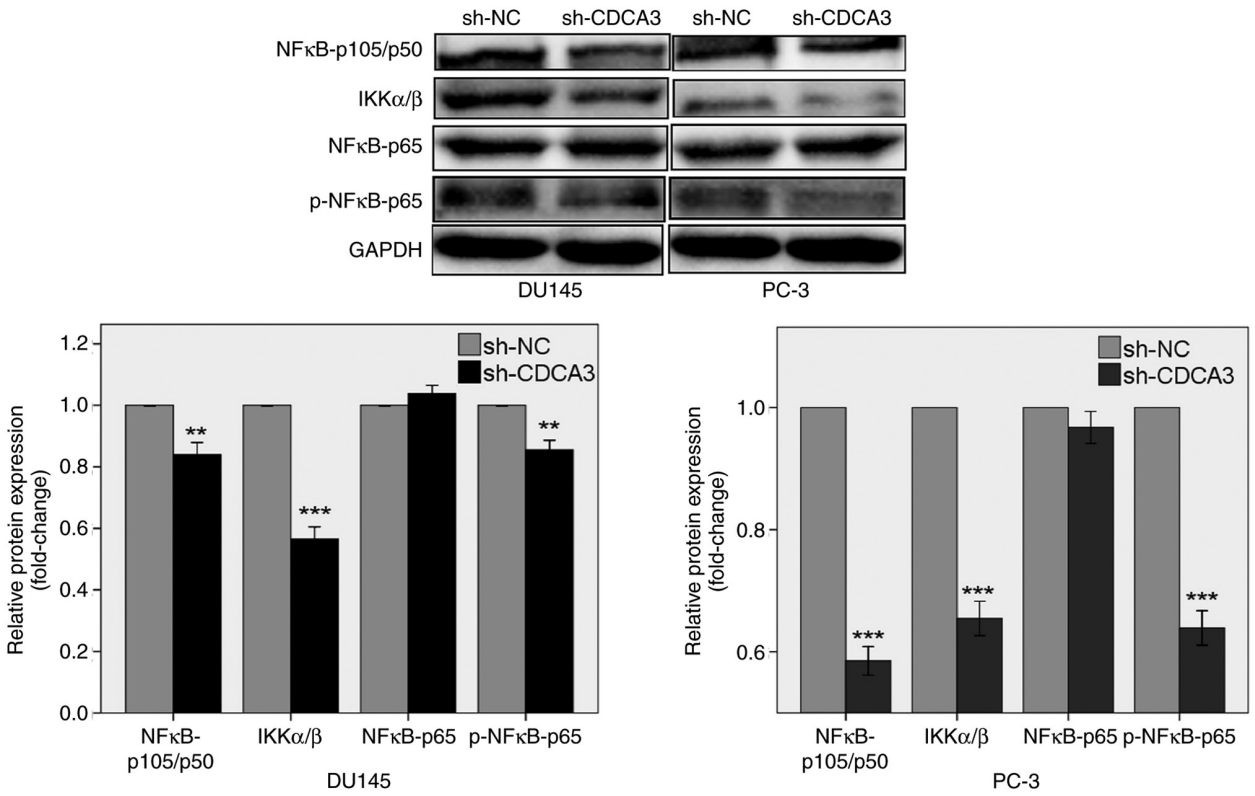

Figure 6. CDCA3 knockdown exerts an inhibitory effect on the NF-κB signaling pathway. Protein expression levels of NFκB-p105/p50, IKKα/β, NFκB-p65 and p-NFκB-p65 were determined via western blotting. GAPDH was used as the loading control. Data are presented as the mean ± SD of three independent experiments. **P<0.01 and ***P<0.001. CDCA3, cell division cycle-associated 3; p-, phosphorylated; sh-, short hairpin; NC, negative control.

upregulates CDCA3 expression and promotes prostate cancer progression (19). Knockdown of HOXB3 reduces the expression of CDCA3 in acute myeloid leukemia, which decreases cell proliferation (32). In renal cell carcinoma, long noncoding RNA, small nucleolar RNA host gene 12, promotes cell proliferation, migration, invasion and sunitinib resistance via CDCA3 (33).

Using the GEO database, the present study identified 20 hub genes that were expressed at elevated levels in prostate cancer compared with normal prostate tissues. The transcriptomic levels of CDCA3 were also significantly upregulated in prostate cancer compared with normal tissues in TCGA database. It was further verified by western blotting that CDCA3 protein expression levels were upregulated in prostate cancer. Using the GEPIA database, it was determined that high CDCA3 expression levels were associated with a poor prognosis, as discussed for the numerous other aforementioned tumors. The results also demonstrated that knockdown of CDCA3 in DU145 and PC-3 cells inhibited cell proliferation and facilitated early and late apoptosis with an increase in the protein expression levels of cleaved caspase-3.

Regarding the molecular mechanism of CDCA3 in the cell cycle, the NF-κB signaling pathway was further investigated. NF-κB is a major regulator of numerous important cell processes, such as inflammation, proliferation and apoptosis (34). Recent studies have demonstrated that the NF-κB signaling pathway is important for prostate cancer cell proliferation, invasion and the development of treatment resistance (35,36). Activated NF-κB is translocated to the nucleus and regulates gene transcription to activate a variety of downstream targets such as cyclin D1, which is a cell cycle regulatory protein. The activated NF-κB protein binds directly to specific sequences in the cyclin D1 promoter, inducing the upregulation of cyclin D1 expression levels (37). The results of the present study have demonstrated that CDCA3 knockdown influenced cell cycle arrest in the G0/G1 phase by decreasing cyclin D1 protein expression levels. Furthermore, the protein expression levels of NFκB-p105/p50, IKKα/β and p-NFκB-p65 were also reduced, while not interacting with TRAF2 (data not shown). p21 is a crucial regulator of cell cycle progression and serves an important role in tumorigenesis and is regarded as a tumor suppressive protein. Numerous studies have reported that the downregulation of p21 is involved in a number of human cancers and is associated with cell proliferation (38,39). p21 may also act independently or with other cell cycle regulators, such as the CDK4/6 complex and p53 (13). The present study indicated that CDCA3 knockdown may upregulate p21 expression in the cell cycle.

However, there are still many topics that remain to be explored. Firstly, the effects of CDCA3 on the NF-κB signaling pathway and the mechanisms involved need further investigation. RNA-seq analysis will be performed to identify DEGs between sh-CDCA3 and sh-NC in response to CDCA3 knockdown in DU145 and PC-3 cell lines, and the mechanisms of CDCA3 interacting with DEGs will be investigated. Secondly, to further confirm the effects of CDCA3 *in vivo*, a tumor xenograft nude mouse model will be used in a future study. Thirdly, a prostate cancer cell line with CDCA3 overexpression will be established to clarify whether CDCA3 overexpression has a consistent impact on S and G2/M phases,

and more experiments such as BrdU assay will be performed to aid in the analysis of the cell cycle. Fourthly, more prostate cancer tissue samples will be collected, and correlation between CDCA3 expression and clinicopathological features in patients with prostate cancer will be analyzed.

In conclusion, these data demonstrated that CDCA3 was upregulated in prostate cancer tissues and was associated with a poor prognosis. The results indicated that knockdown of CDCA3 potentially suppresses prostate cancer progression via the significant accumulation of p21 and via inhibiting the expression of cyclin D1 by regulating the NF-κB signaling pathway.

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Availability of data and materials

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

Authors' contributions

DY, XH, and PG designed the study. PG and MZ carried out the study, including data collection and data analysis. JZ performed data analysis. PG wrote the manuscript. PG and MZ confirm the authenticity of the raw data. All the authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. xs2020ky014) by the Ethics Committee of Xishan People's Hospital of Wuxi City (Wuxi, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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