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VASN promotes proliferation of prostate cancer through the YAP/TAZ axis

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Abstract. – OBJECTIVE: The purpose of this study was to uncover the role of VASN in regulating proliferative ability of prostate cancer (PCa) cells through the yes-associated protein/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) axis, thus influencing the progression of PCa.

PATIENTS AND METHODS: VASN, YAP, and TAZ levels in PCa tissues or in the serum were detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). The diagnostic value of VASN in PCa was assessed by introducing receiver operating characteristic (ROC) curves. Besides, the regulatory effects of VASN on viability, clonality, and expression levels of YAP/TAZ were evaluated by cell counting kit-8 (CCK-8), colony formation, and Western blot, respectively. Finally, rescue experiments were conducted to uncover the involvement of YAP in VASN-regulated proliferation of PCa.

RESULTS: Results manifested that VASN, YA, and TAZ were upregulated in PCa patients, and VASN presented a certain diagnostic value. Knockdown of VASN in LNCaP and C4-2 cells suppressed viability and clonality, and downregulated protein levels of YAP and TAZ. Notably, overexpression of YAP abolished the attenuated viability and clonality in PCa cells with VASN knockdown.

CONCLUSIONS: VASN promotes proliferative ability in PCa via regulating the YAP/TAZ axis, thus aggravating the progression of the disease.

Key Words:
Prostate cancer, VASN, YAP/TAZ.

Introduction

Prostate cancer (PCa) is a common malignancy in male urogenital system. The incidence of PCa varies a lot in different races and areas. In Western countries, the incidence of PCa ranks first in male malignancies, and the mortality ranks third[1]. In recent years, PCa severely affects aging men in China[2]. Therefore, it is urgent to clarify molecular mechanisms underlying the occurrence, progression, and metastasis of PCa.

VASN, also known as Slit-Like2, is a classic type I transmembrane protein. VASN contains LRR domain, EGF domain, and FN3 domain[3]. Secretory form of VASN is formed by shearing and exfoliating the extracellular domain of VASN protein through ADAM17[4]. VASN is abundantly expressed in vascular smooth muscle cells of aorta, which is extremely lowly expressed in brain, heart, and liver[3]. VASN is upregulated in breast cancer and hepatocellular cancer, presenting a close link in tumor progression[4,5].

Yes-associated protein (YAP), a transcriptional coactivator of the Hippo pathway located on chromosome 11q22, is considered as a candidate oncogene[6]. By enhancing activities of transcription factors, YAP expression is upregulated. There are two subtypes of YAP, namely YAP1 and YAP2. Transcriptional coactivator with PDZ-binding motif (TAZ) is homologous with YAP, presenting similar functions with those of YAP. Elevation of YAP/TAZ activity triggers tumor cell migration. Hall et al[7] pointed out that YAP level is closely linked to survival in ovarian cancer patients. Particularly, about 50% ovarian cancer patients with a high level of nuclear YAP and a low level of phosphorylated cytoplasmic YAP experience shorter than 5 years of overall survival. In addition, VASN is able to affect the progression of thyroid cancer by stimulating the expressions of YAP/TAZ[8]. In this paper, the role of VASN in promoting the progression of PCa through the YAP/TAZ axis was mainly investigated.

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Patients and Methods

Sample Collection

A total of 50 paired adjacent normal tissues and PCa tissues were collected in Affiliated People's Hospital of Jiangsu University from June 2016 to December 2018. None of enrolled PCa patients had medical history of preoperative anti-tumor therapy. Tissues were placed in liquid nitrogen within 30 min *ex vivo* and stored at –80°C. Patients and their families have been fully informed. This investigation was approved by the Ethics Committee of Affiliated People's Hospital of Jiangsu University.

Cell Culture and Transfection

PCa cell lines (LNCaP and C4-2) provided by Cell Bank, Shanghai, were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640; HyClone, South Logan, UT, USA) containing 10% fetal bovine serum (FBS; HyClone, South Logan, UT, USA) and 1% penicillin-streptomycin. Cell passage was conducted every 2-3 days at a ratio of 1:3.

Cells in logarithmic growth period were cultured to 70-80% confluence, and transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Si-VASN 1#, forward 5′-GCAUGAAAUCACCAAUGAGTT-3′, reverse 5′-CUCAUUGGUGAUUUCAUGCTT-3′; Si-VASN 2#, forward 5′-CUGGAUGUGAGCAACCUATT-3′, reverse 5′-UAGGUUGCUCACAUCCAGCTT-3′.

RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol (Invitrogen, Carlsbad, CA, USA) was applied for isolating cellular RNA, which was quantified using a spectrometer. RNA was reversely transcribed into complementary deoxyribose nucleic acid (cDNA) using the PrimeScript RT reagent Kit (TaKaRa, Otsu, Shiga, Japan), and SYBR Premix Ex Taq™ (TaKaRa, Otsu, Shiga, Japan) was utilized for qRT-PCR. Primer sequences are listed as follows: VASN, forward: 5′-CCACCTGCCCTTTGTCCTG-3′ and reverse: 5′-CAACCTGCCGCTCCTCATT-3′; YAP, forward: 5′- TAGCCCTGCGTAGCCAGTTA-3′ and reverse: 5′-TCATGCTTAGTCCACTGTCTGT-3′; TAZ, forward: 5′- CACCGTGTCCAATCACCAGTC -3′ and reverse: 5′- TCCAACGCATCAACTTCAGGT-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH), forward: 5′-CAATGACCCCTTCATTGACC-3′ and reverse: 5′-GACAAGCTTCCCGTTCTCAG-3′.

Cell Counting Kit-8 (CCK-8)

Cells were inoculated in the 96-well plate (3×10^3 cells per well) and reacted with 10 μL CCK-8 (Dojindo Laboratories, Kumamoto, Japan) reagent per well for 4 h culture. Then, absorbance (A) at 450 nm was measured by a microplate reader.

Colony Formation Assay

Cells were inoculated in 12-well plates and cultured for 14 days. Afterwards, colonies were fixed in methanol and dyed in 1% crystal violet, and visible colonies were captured.

Western Blot

Cellular protein was isolated using the bicinchoninic acid (BCA) method (Pierce, Rockford, IL, USA). After preparation of protein samples, 40 μg protein per sample was electrophoresed and loaded on polyvinylidene difluoride (PVDF) membranes (Roche, Basel, Switzerland). Subsequently, non-specific antigens were blocked in 5% skim milk for 2 h. Membranes were reacted with primary and secondary antibodies for indicated time. Band exposure and analyses were finally conducted.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 22.0 (IBM, Armonk, NY, USA) was used for statistical analyses. Data were expressed as mean ± SD (standard deviation). Differences between two groups were analyzed by the *t*-test. Receiver operating characteristic (ROC) curves were introduced for assessing the diagnostic potential of VASN in PCa. $p<0.05$ indicated the significant difference.

Results

Upregulation of VASN in PCa

Compared with adjacent normal tissues, VASN was found to be upregulated in 50 PCa tissues (Figure 1A, 1B), suggesting a potential promotive effect on the malignant progression of PCa.

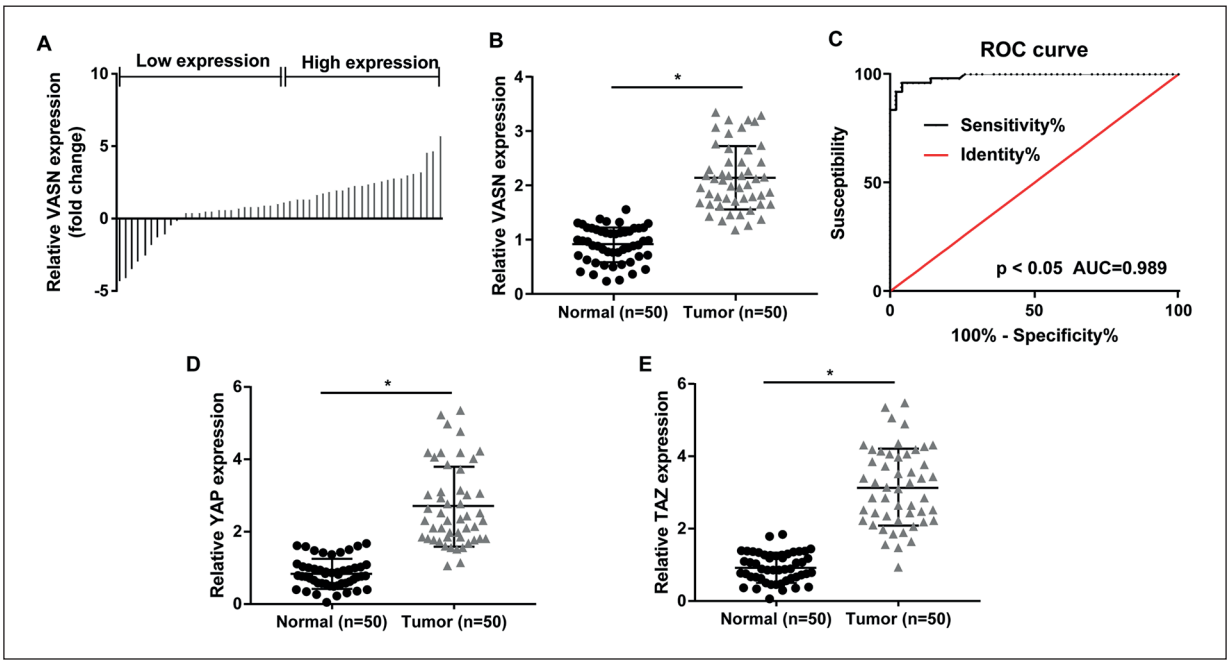

Figure 1. Upregulation of VASN in PCa. **A, B,** VASN levels in 50 paired adjacent normal tissues and prostate cancer tissues detected by qRT-PCR. **C,** ROC curves depicted the diagnostic value of VASN in prostate cancer. **C-E**, VASN, YAP and TAZ levels in 50 paired adjacent normal serum and prostate cancer serum analyzed by qRT-PCR.

Besides, diagnostic value of VASN in PCa was assessed by the ROC method. As the curves depicted, AUC was 0.989, indicating a certain diagnostic value of VASN in PCa (p<0.05, Figure 1C). In addition, the expression was tested in the serum of PCa patients, and it was found that the expressions of VASN, YAP, and TAZ were significantly increased (p<0.05, Figure 1D, 1E and 1F), suggesting that VASN can be a potential hallmark for PCa.

Overexpression of VASN Stimulated Proliferative Ability in PCa

Two VASN siRNAs were constructed and transfected in PCa cells. QRT-PCR data showed that transfection of either si-VASN 1# or si-VASN 2# markedly downregulated VASN in LNCaP and C4-2 cells (Figure 2A). Protein level of VASN was also markedly downregulated after transfection of si-VASN 1# or si-VASN 2# (Figure 2B). Moreover, cell viabilities in LNCaP and C4-2 cells were remarkably reduced after knockdown of VASN (Figure 2C). Similarly, relative colony number was declined in PCa cells with VASN knockdown (Figure 2D). It is indicated that VASN is able to stimulate PCa to proliferate.

Knockdown of VASN Downregulated YAP/TAZ

The knockdown of VASN markedly downregulated protein levels of YAP and TAZ in LNCaP and C4-2 cells, suggesting that VASN positively regulated YAP/TAZ in PCa cells (Figure 3).

Overexpression of YAP Reversed the Inhibitory Effect of Downregulated VASN on Proliferative Ability in PCa

Transfection of si-YAP #1 significantly reduced viability in PCa cells (Figure 4A), which was partially reversed by co-transfection of pcDNA-YAP (Figure 4B). Similarly, overexpression of YAP abolished the inhibited clonality in PCa cells transfected with si-VASN #1 (Figure 4C). As a result, VASN aggravated the progression of PCa by positively regulating the YAP/TAZ axis.

Discussion

PCa has become the third leading cancer in male urogenital system following bladder cancer and kidney neoplasm. Effective strategies for PCa are still lacked[9]. Most of PCa patients are

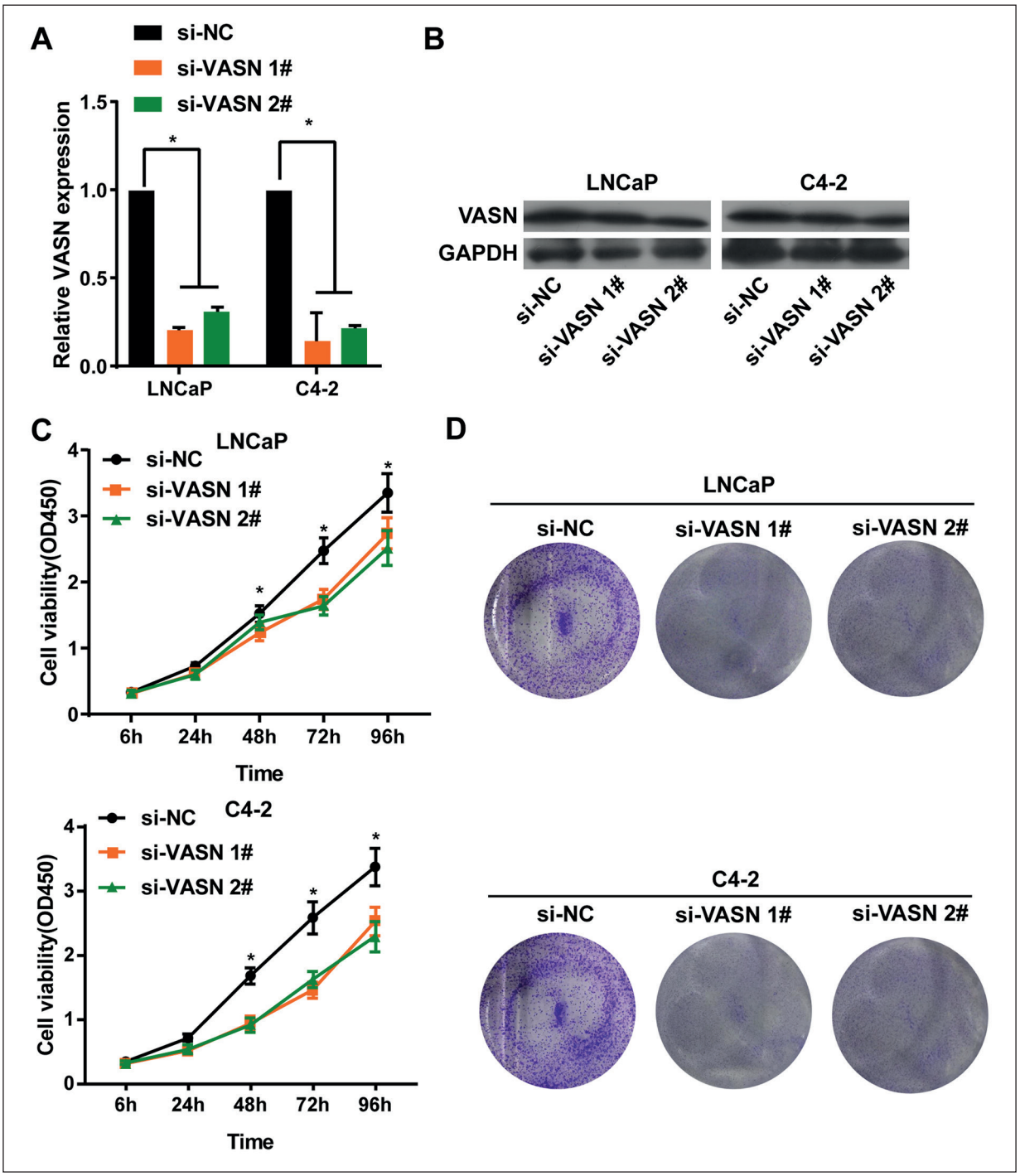

Figure 2. Overexpression of VASN stimulates proliferative ability in PCa. **A, B,** The mRNA (**A**) and protein (**B**) levels of VASN in LNCaP and C4-2 cells transfected with si-NC, si-VASN #1 or si-VASN #2. **C,** Viability in LNCaP and C4-2 cells transfected with si-NC, si-VASN #1 or si-VASN #2. **D,** Clonality in LNCaP and C4-2 cells transfected with si-NC, si-VASN #1 or si-VASN #2 (magnification 10×).

diagnosed in advanced stage because of insidious onset, atypical symptoms, and slow progression. So far, determination of prostate specific antigen (PSA) level and transrectal ultrasound-guided prostate biopsy are the most preferred diagnostic approaches for PCa. Nevertheless, sensitivity and specificity of these approaches are unsatisfied, leading to misdiagnosis or overdiagnosis

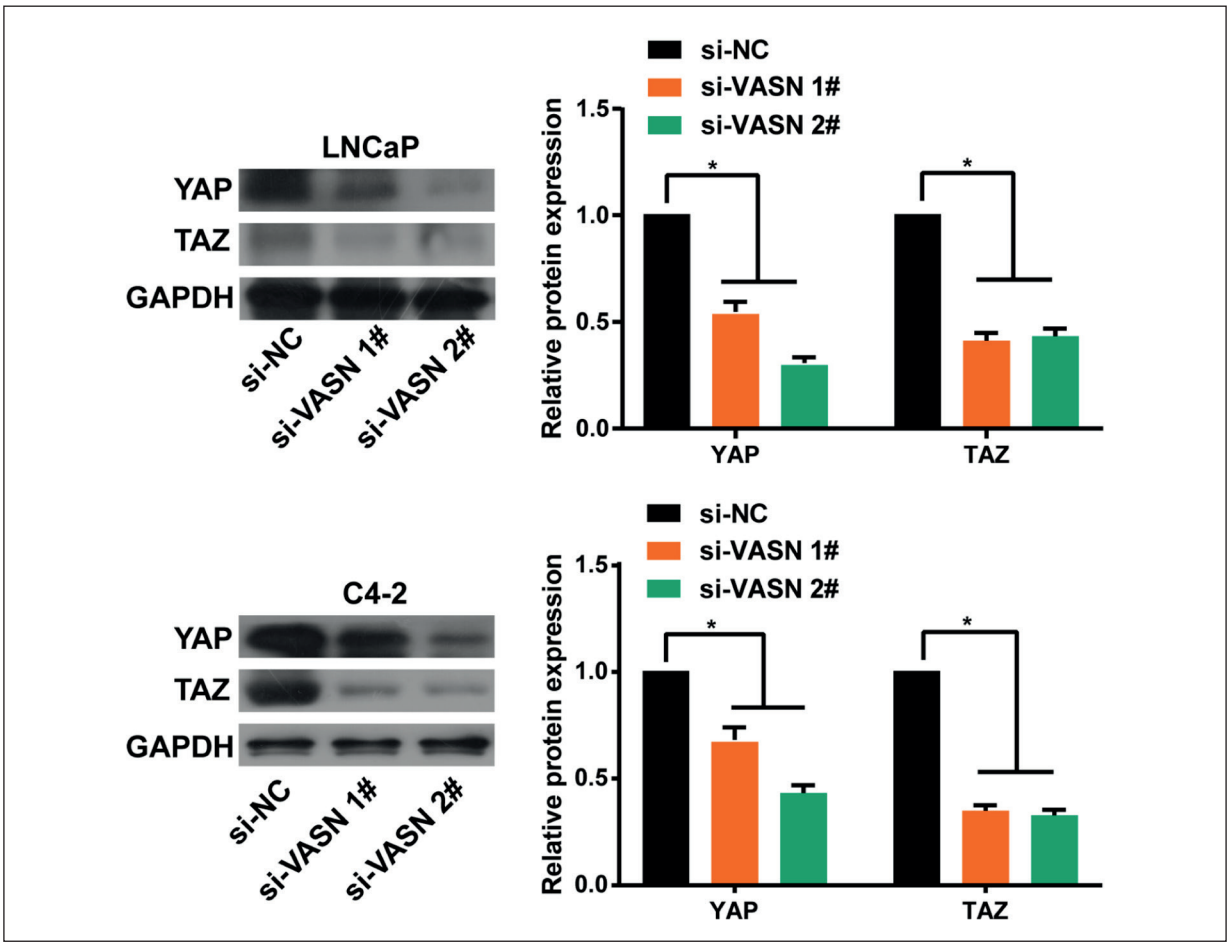

Figure 3. Knockdown of VASN downregulates YAP/TAZ. Protein levels of YAP and TAZ in LNCaP and C4-2 cells transfected with si-NC, si-VASN #1 or si-VASN #2.

of PCa[10]. It is urgent to develop novel hallmarks that could effectively and precisely diagnose and monitor PCa.

Human VASN is a newly discovered membrane protein, which is distributed in the form of membrane type and secretion type. VASN is involved in the regulation of vascular endothelial cells, EMT, oxidative stress, and other life activities[11-14]. VASN is upregulated in some types of tumor cells, serving as a potential tumor hallmark[5,15,16]. Consistently, our findings illustrated that VASN was upregulated in PCa tissues, presenting a certain diagnostic value in PCa patients.

Upregulation of YAP and TAZ in multiple types of malignancies gives them possibilities to become potential diagnostic and anti-tumor targets[17-19]. Diep et al[20] uncovered that positive expression of YAP is upregulated in nuclei of pancreatic cancer cells BX-PC-3 and PANC-1 cells, while YAP is dose-dependently downregulated in HPDE6 cells alongside the increased cell density. Silence of YAP great inhibits proliferative abilities in BX-PC-3 and PANC-1 cells, indicating that YAP is of significance during the progression of pancreatic cancer. Wang et al[21] demonstrated that TAZ is upregulated in lung adenocarcinoma cell line A549. Knockdown of TAZ downregulates Cyclin A and CTGF in A549 cells. Moreover, knockdown of TAZ also inhibits proliferative progression, arrests cell cycle in G_0-G_1 phase, and stimulates Paclitaxel-induced apoptosis in A549 cells. Opposite trends are observed after overexpression of TAZ. It is reported that expressions of YAP/TAZ are regulated by VASN[8]. In this paper, protein levels of YAP and TAZ were downregulated in PCa cells with VASN knockdown. Notably, overexpression of YAP could abolish regulatory effects of VASN on proliferative abil-

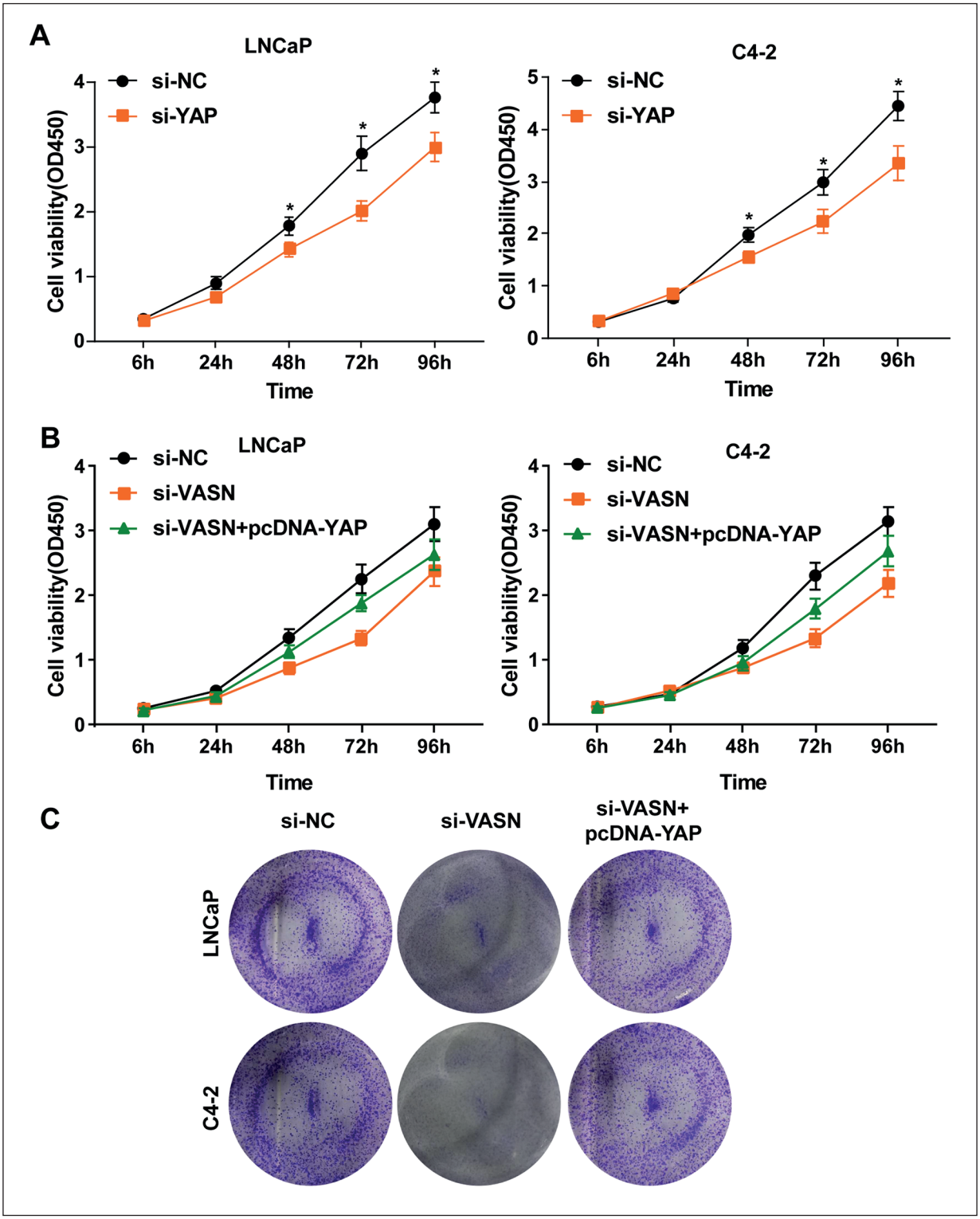

Figure 4. Overexpression of YAP reverses the inhibitory effect of downregulated VASN on proliferative ability in PCa. LNCaP and C4-2 cells are transfected with si-NC, si-YAP. **A**, Cell viability. LNCaP and C4-2 cells are transfected with si-NC, si-VASN #1 or si-VASN #1+pcDNA-YAP. **B,** Cell viability. **C,** Relative colony numbers (magnification 10×).

ity in PCa, suggesting that VASN regulated the progression of PCa *via* the YAP/TAZ axis. The findings of this study may provide novel directions in the clinical treatment of PCa.

Conclusions

This study demonstrated that VASN promotes proliferative ability in prostate cancer *via* regulating the YAP/TAZ axis, thus aggravating the progression of prostate cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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