Article

Discovery Medicine 2024; 36(180): 61–70
https://doi.org/10.24976/Discov.Med.202436180.5

ALKBH5 Suppresses Autophagy in Prostate Cancer Cells via Inhibiting m6A-Modification of *TSPAN1* mRNA

Xudong Zhao[1,*], Zhiqiang Zhao[1], Honglin Xie[1], Wenjun Gao[1], Chunhua Ye[1]

[1]Department of Urology, Affiliated Hospital of Hangzhou Normal University, 310015 Hangzhou, Zhejiang, China
*Correspondence: zhaoxudonghz@163.com (Xudong Zhao)
Published: 20 January 2024

Background: Activating autophagy promotes the invasion and progression of prostate cancer (PCa). Tetraspanin 1 (*TSPAN1*) has been found to promote autophagy flux and its up-regulation can enhance the migration of PCa cells. In addition, there is a binding relationship between *TSPAN1* and the N6-methyladenosine (m6A) demethylase AlkB homolog 5 (*ALKBH5*). Therefore, we wanted to know whether *ALKBH5* could affect autophagy by regulating *TSPAN1* expression, and thereby participate in PCa malignant progression.

Methods: The expression of *ALKBH5* and *TSPAN1* in PCa was examined by quantitative real-time polymerase chain reaction (qRT-PCR), and the functional tests included cell counting kit-8 and 5-ethynyl-2′-deoxyuridine (EdU) staining assays. The expression of autophagy-related proteins was confirmed by western blot. Detection of the m6A level of *TSPAN1* was performed using methylated RNA immunoprecipitation sequencing (MeRIP)-qPCR.

Results: *ALKBH5* was significantly downregulated in PCa cells (LNCaP, DU145 and PC3 cells; $p < 0.001$). Overexpression of *ALKBH5* inhibited cell viability and the number of EdU-positive cells ($p < 0.01$, $p < 0.001$), decreased the ratio of microtubule-associated protein light chain 3B (LC3B)-II/LC3B-I, and promoted P62 protein expression in LNCaP and DU145 cells ($p < 0.001$). The m6A level of *TSPAN1* was high in LNCaP and DU145 cells, but was inhibited by the overexpression of *ALKBH5* ($p < 0.001$). *TSPAN1* overexpression promoted cell viability ($p < 0.001$), increased EdU-positive cells and the LC3B-II/LC3B-I ratio ($p < 0.001$, $p < 0.05$), reduced P62 protein expression ($p < 0.05$, $p < 0.001$), and reversed the regulation of *ALKBH5* overexpression in LNCaP and DU145 cells ($p < 0.01$, $p < 0.001$).

Conclusions: Promoting *ALKBH5* expression may inhibit PCa autophagy by reducing the m6A level of *TSPAN1*.

Keywords: prostate cancer; *ALKBH5*; *TSPAN1*; autophagy; m6A modification

Introduction

Prostate cancer (PCa) is a common malignancy of the urinary tract [1,2]. There have been clear trends in PCa incidence and mortality in recent years [1]. Most patients with early PCa diagnosis have a good prognosis and low mortality [3]. Androgen deprivation therapy (ADT) is the preferred approach in the treatment of PCa; however, there are still some patients who develop drug resistance and become desmoresistant (castration-resistant prostate cancer [CRPC]), resulting in shorter survival time [4,5]. New diagnostic and therapeutic approaches based on the biological and molecular mechanisms of PCa progression are needed.

Autophagy is a biological process in which organelles and unfolded proteins, etc., that need to be degraded in the cell are catabolized in the lysosome [6,7]. Autophagy is associated with multiple biological processes in PCa, and studies have shown that autophagy can promote the invasion and progression of PCa and simultaneously resist radiation-induced damage [6,8,9]. The cooperative effects of multiple cancer suppressor genes and oncogenes are involved in the development and metastasis of PCa [10,11]. Autophagy-related genes, including family with sequence similarity 215 member A (*FAM215A*), *FDD*, MYC proto-oncogene (*MYC*), Ras homolog enriched in brain (*RHEB*), and autophagy related 16-like 1 (*ATG16L1*) have been analyzed as predictive factors for prognostic assessment of PCa [12]. We screened the aberrantly expressed genes in PCa using The Cancer Genome Atlas (TCGA) database, then reviewed the literature to verify their relationship with autophagy. Finally, Tetraspanin 1 (TSPAN1), a new member of the Tetraspanins protein family, was identified and selected to be further studied in our paper [13,14]. It has been reported that in gastric, colon, and cervical cancers, *TSPAN1* expression levels significantly increased in tumor tissues, and that these elevated levels were able to promote tumor cell proliferation and invasion at the *in vitro* cellular level [15–17]. Importantly, studies suggest that *TSPAN1* promotes autophagic flux; specifically, *TSPAN1* promotes autophagy maturation through direct binding of two conserved LC3-interacting region (LIR) motifs to microtubule-associated protein light chain 3 (LC3) [14]. Although previous studies have reported that *TSPAN1* is under es-

trogenic control, upregulation can enhance PCa migration [18]. However, our study sought to determine whether it could promote PCa progression through the autophagic pathway.

N6-methyladenosine (m6A) methylation, a methylation modification on RNA molecules, is one of the most prevalent post-transcriptional modifications [19]. The m6A regulatory pattern is also widespread in PCa. Liu Z *et al.* [20] and Wang J *et al.* [21], for example, revealed that m6A regulators *MRTTL14* and YTH N6-methyladenosine RNA binding protein F2 (*YTHDF2*) can be used as prognostic markers in PCa. Using m6ATarget2 prediction, AlkB homolog 5 (*ALKBH5*) was found to bind to *TSPAN1* mRNA, and *ALKBH5* expression significantly decreased in PCa [22]. The study suggested that *ALKBH5* could increase the degradation of mRNA [23]. Therefore, we speculated that promoting *ALKBH5* expression could inhibit PCa cell autophagy by decreasing *TSPAN1* stability. In our study, we detected the effects of *ALKBH5* overexpression on cell proliferation and autophagy, and then performed additional transfection of overexpressed *TSPAN1* to explore whether *ALKBH5* could affect autophagy by regulating *TSPAN1* expression.

Materials and Methods

Cells and Grouping

RWPE-1 (CRL-11609), DU145 (HTB-81), and PC3 cells (CRL-1435) were obtained from ATCC (Rockefeller, Maryland, USA), and LNCaP cells (ACC-256) were purchased from DSMZ (Konstanz, Germany). All cells were incubated in 10%–20% fetal bovine serum (FBS; 30-2021, ATCC, USA) supplemented with RPMI 1640 (SH30096.02, Cytiva, Shanghai, China) at 37 °C, 5% CO_2. All cells were routinely tested for short tandem repeat (STR) identification and mycoplasma contamination, and were confirmed to be mycoplasma-free.

ALKBH5 and *TSPAN1* were amplified by PCR and then cloned into the pEX-3 (C05003) vector from GenePharma (Shanghai, China) to construct *ALKBH5* and *TSPAN1* overexpression plasmids, and the cell transfection was performed with Lipofectamine 2000 (11668500, Invitrogen, Carlsbad, CA, USA). In short, *ALKBH5*/*TSPAN1* overexpression plasmid and Lipofectamine 2000 were separately diluted in the cell medium, and the two dilutions were then mixed and cultured for 20 min at 25 ℃, after which the complex was incubated with LNCaP and DU145 cells for 24 h before examination of transfection efficiency through quantitative real-time polymerase chain reaction (qRT-PCR).

In the first part, PCa, LNCaP, and DU145 cells were each separated into three groups (Control, NC, and *ALKBH5*). Control was the normal culture group and NC was the negative control group whose cells were transfected with empty vector. Cells in the *ALKBH5* group were transfected with *ALKBH5* overexpression plasmid. In the second part, LNCaP and DU145 cells were also separately divided into three groups (Control, NC, and *TSPAN1*). The cells in this part were transfected with *TSPAN1* overexpression plasmid (*TSPAN1* group) or empty vector (NC group), or had no treatment (Control group). In the third part, PCa cells were divided into five groups: NC, *TSPAN1*, *ALKBH5*, *ALKBH5* + Rapamycin, and *ALKBH5* + *TSPAN1*. Cells in the NC group were treated as before, while cells in the *TSPAN1* and *ALKBH5* groups were separately transfected with *TSPAN1* or *ALKBH5* overexpression plasmids. In addition, cells in the *ALKBH5* + Rapamycin group were transfected with *ALKBH5* and then treated with rapamycin (250 nM, dissolved in DMSO; T1537, TargetMol, Shanghai, China) for 1 h [24], and cells in the *ALKBH5* + *TSPAN1* group were transfected with *ALKBH5* and *TSPAN1* overexpression plasmid.

qRT-PCR

The qRT-PCR assays were conducted for determination of *TSPAN1* and *ALKBH5* expression, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference. Total RNA was extracted from LNCaP and DU145 cells by TRIzol solution (R0016, Beyotime, Shanghai, China). First strand cDNA synthesis was conducted by Universal cDNA Master (05893151001, Roche, Basel, Switzerland). After purification, cDNA was harvested to perform qRT-PCR, using a real-time PCR instrument (MA-6000, Yarui, Zhangjiagang, China) and SuperReal PreMix Plus (SYBR Green) (FP205-02, TIANGEN, Beijing, China). Primers were as follows (5′-3′): *ALKBH5*: CGGCGAAGGCTACACTTACG, CCACCAGCTTTTGGATCACCA; *TSPAN1*: CATGCAGTTTGTCAACGTGGG, CACTTGCTCTCAGTCTTAGCAC; *GAPDH*: ACAACTTTGGTATCGTGGAAGG, GCCATCACGCCACAGTTTC.

Cell Counting Kit-8 (CCK-8)

A CCK-8 kit (A311-01), purchased from Vazyme (Nanjing, China), was used for cell viability analysis. LNCaP and DU145 cells (2×10^4) were mixed with CCK-8 for 4 hours. Absorbance was determined at 450 nm using a Multiskan FC Enzyme Analyzer (1410101, Thermo Fisher, Boston, MA, USA).

5-Ethynyl-2′-Deoxyuridine (EdU) Staining

A BeyoClick EdU Cell Proliferation Kit with Alexa Fluor 594 (C0078L) was purchased from Beyotime (China). LNCaP and DU145 cells were cultured in 6-well plates and incubated for 2 hours with 2× EdU working solution (20 μM) pre-warmed at 37 °C. After EdU labeling of the cells was completed, 1 mL of fixative solution (P0098, Beyotime, China) was added and cells were fixed for 15 min at room temperature. Cells were incubated with perme-

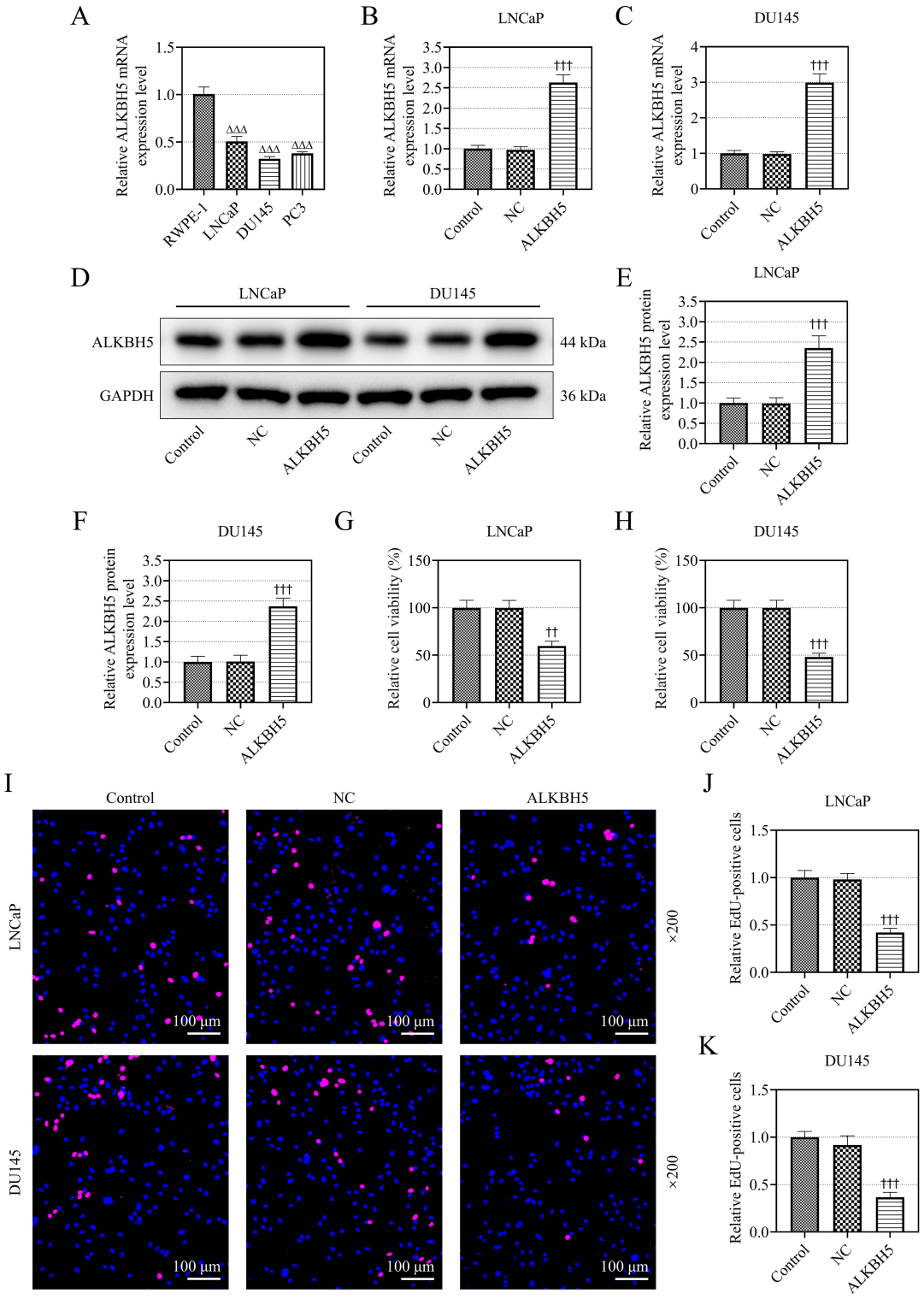

Fig. 1. AlkB homolog 5 (*ALKBH5*) overexpression inhibited prostate cancer (PCa) cell viability and proliferation. (A) Expression of *ALKBH5* in PCa cells (LNCaP, DU145, PC3) or normal cells (RWPE-1) was detected using quantitative real-time polymerase chain reaction (qRT-PCR). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference. (B–F) *ALKBH5* overexpression plasmids were transfected into LNCaP and DU145 cells to overexpress *ALKBH5*. (G,H) The effect of *ALKBH5* overexpression on LNCaP and DU145 cell viability was detected using cell counting kit-8 (CCK-8). (I–K) Cell proliferation in each group was assessed using 5-ethynyl-2′-deoxyuridine (EdU) staining (EdU: red; Hoechst 33342: blue; Magnification ×200). $^{\Delta\Delta\Delta}p < 0.001$ vs. RWPE-1; $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs. NC (negative control). n = 3.

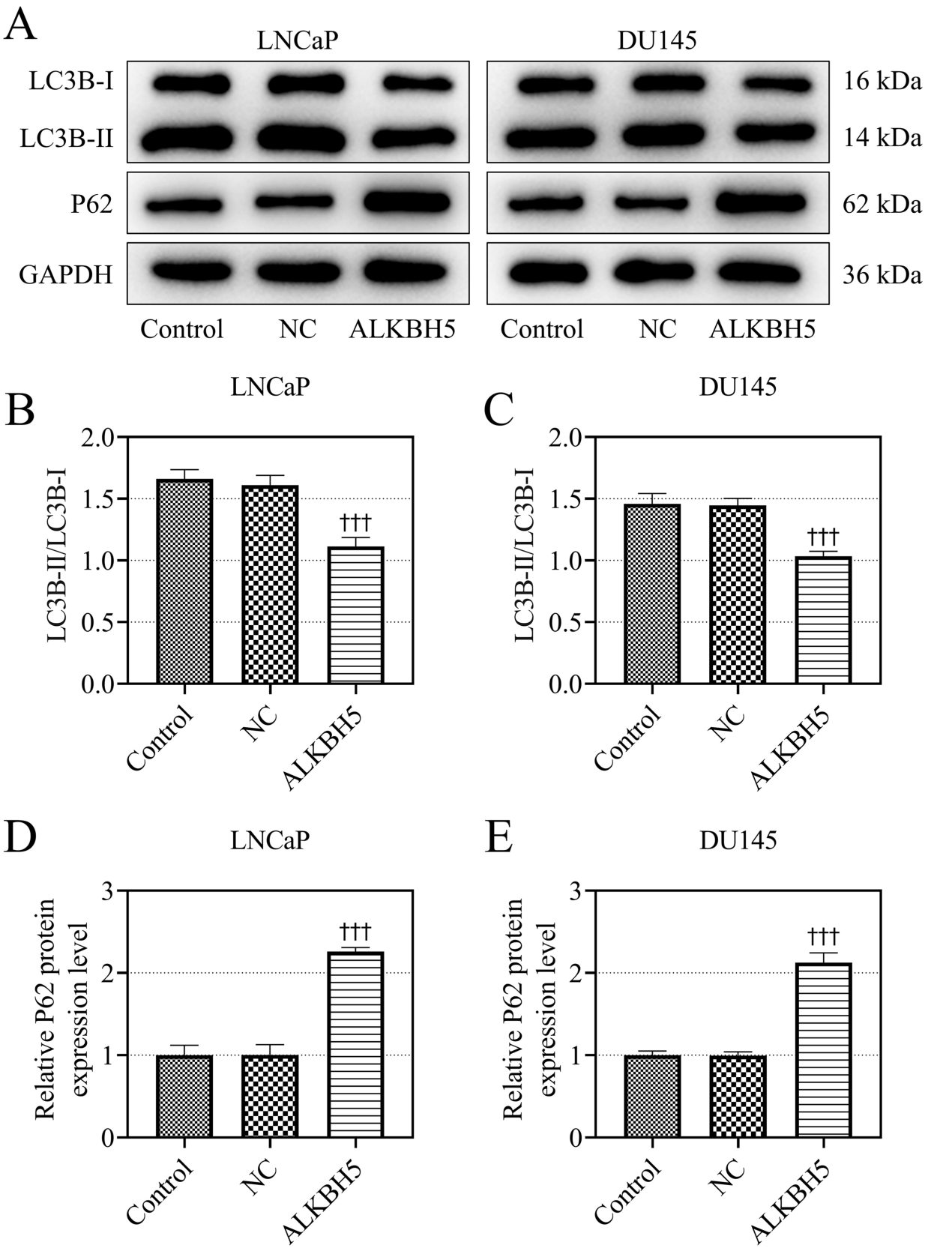

Fig. 2. *ALKBH5* overexpression suppressed autophagy-associated proteins in PCa cells. (A–E) Autophagy-associated proteins microtubule-associated protein light chain 3B (LC3B)-I, LC3B-II, and P62 protein levels were detected using western blot with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal reference [(A) Blots; (B–E) Protein quantification histogram]. $^{\dagger\dagger\dagger}p < 0.001$ vs. NC. n = 3.

abilization solution (P0097, Beyotime, China) for 10 min at room temperature, followed by the addition of 0.5 mL click reaction solution for 30 min at room temperature in the dark. Nuclei staining was performed using Hoechst 33342. EVOS M5000 (AMF5000, Invitrogen, USA) was used to observe EdU-positive cells.

Western Blot Assay

LNCaP and DU145 cells were treated with radioimmunoprecipitation assay (RIPA) lysis buffer (KGP702, KeyGEN BioTECH, Nanjing, China) to collect proteins. A bicinchoninic acid (BCA) Protein Assay Kit (ZJ101, EpiZyme, Shanghai, China) was used to measure protein concentrations. Proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes (WJ001, WJ002, EpiZyme, China). After being blocked by 5% fat-free milk, membranes were then incubated overnight with LC3B (1/2000, ab192890, 14/16 kDa, Abcam, Cambridge, London, UK), P62 (1/10,000, ab109012, 62 kDa, Abcam, UK), GAPDH (1/10,000, ab181602, 36 kDa, Abcam, UK), followed by incubation with Goat Anti-Rabbit IgG (ab205718, 1/4000, Abcam) at 37 °C for 2 hours. Immunoreactivity was detected with electrochemiluminescence (ECL) Western Blot Kit (CW0049M, CWBIO, Taizhou, China), followed by visualization with the ImageQuant LAS 4000MINI Ultra-Sensitive Chemiluminescence Imager (Sinopharm Chemical Reagent Co. Ltd, Shanghai, China), and the protein band intensity was detected using ImageJ software (3.0 version, National Institutes of Health, Bethesda, MA, USA), with GAPDH used as the loading control.

m6A Methylation Level Determination

To detect the level of m6A methylation of *TSPAN1* mRNA in PCa cells, m6A antibodies (ab151230, Abcam, UK) and Dynabeads (10015D, Invitrogen, USA) were used for methylated RNA immunoprecipitation sequencing (MeRIP)-qPCR. In the first part, RWPE-1, LNCaP, DU145, and PC3 cells were used. In the second part, LNCaP and DU145 cells were transfected with NC or *ALKBH5* overexpression plasmids. RNAs were incubated with m6A antibodies, or with IgG (ab172730, Abcam, UK) as a control. The m6A RNA was extracted and subsequently reverse transcribed for qRT-PCR assay.

Statistics

Statistical analyses of the data were performed using one-way analysis of variance or independent samples *t* test, and the post hoc comparison was performed with the Tukey test. Graphpad 8.0 (GraphPad Software, San Diego, CA, USA) was used for analysis, and data were shown as mean $\pm$ standard deviation. $p < 0.05$ was considered to be statistically significant.

Results

ALKBH5 Overexpression Inhibited PCa Cell Viability, Proliferation and Autophagy

Fig. 1A illustrates low expression of *ALKBH5* in PCa cells, suggesting that it may be a potential diagnostic marker ($p < 0.001$, Fig. 1A). The two PCa cell lines with the highest and lowest *ALKBH5* expression, LNCaP and DU145, were selected and transfected with *ALKBH5* overexpression plasmid, pending subsequent functional validation ($p < 0.001$, Fig. 1B–F). We observed that *ALKBH5* overexpression inhibited cell viability and significantly reduced the number of EdU-positive cells ($p < 0.01$ and $p < 0.001$, Fig. 1G–K). Previous studies have shown that autophagy promotes PCa invasion and progression [12]. Therefore, we examined the effect of *ALKBH5* on autophagy-related proteins, and found that *ALKBH5* overexpression decreased the LC3B-II/LC3B-I protein ratio and increased P62 expression ($p < 0.001$, Fig. 2A–E).

ALKBH5 Overexpression Reduced the m6A Level of TSPAN1 in PCa Cells

It has been suggested that *TSPAN1* can promote autophagic flux [14], and pre-prediction revealed its binding to *ALKBH5*. Fig. 3A shows that the m6A level of *TSPAN1* was higher in PCa cells than in RWPE-1 cells ($p < 0.001$, Fig. 3A). The mRNA level of *TSPAN1* was reduced by *ALKBH5* overexpression ($p < 0.001$, Fig. 3B,C). In addition, *ALKBH5* overexpression also suppressed the m6A level of *TSPAN1* ($p < 0.001$, Fig. 3D,E).

TSPAN1 Upregulation Reversed the Inhibiting Effect of ALKBH5 Overexpression on the Viability, Proliferation, and Autophagy of PCa Cells

Next, *TSPAN1* overexpression plasmids were transfected into LNCaP and DU145 cells. In both cell lines, we observed that *TSPAN1* expression in the *TSPAN1* group was markedly higher than that in the NC group ($p < 0.001$, Fig. 3F,G). CCK-8 assays showed that *TSPAN1* overexpression greatly promoted cell viability, which was further inhibited by *ALKBH5* overexpression ($p < 0.01$ and $p < 0.001$, Fig. 3H,I). Furthermore, *TSPAN1* overexpression increased the number of EdU-positive cells, while *ALKBH5* overexpression reversed the effect of *TSPAN1* on cell proliferation ($p < 0.001$, Fig. 4A–C). Mechanistically, *TSPAN1* upregulation increased the LC3B-II/ LC3B-I protein ratio and decreased P62 expression, whereas *ALKBH5* overexpression reversed the regulation of autophagy-related proteins by *TSPAN1* ($p < 0.05$ and $p < 0.001$, Fig. 5A–E). In addition, we observed that the inhibiting effect of *ALKBH5* overexpression was offset by autophagy activator Rapamycin ($p < 0.01$ and $p < 0.001$, Fig. 5A–E). To sum up, *ALKBH5* overexpression restrained the autophagy of PCa cells by reducing the m6A level of *TSPAN1*.

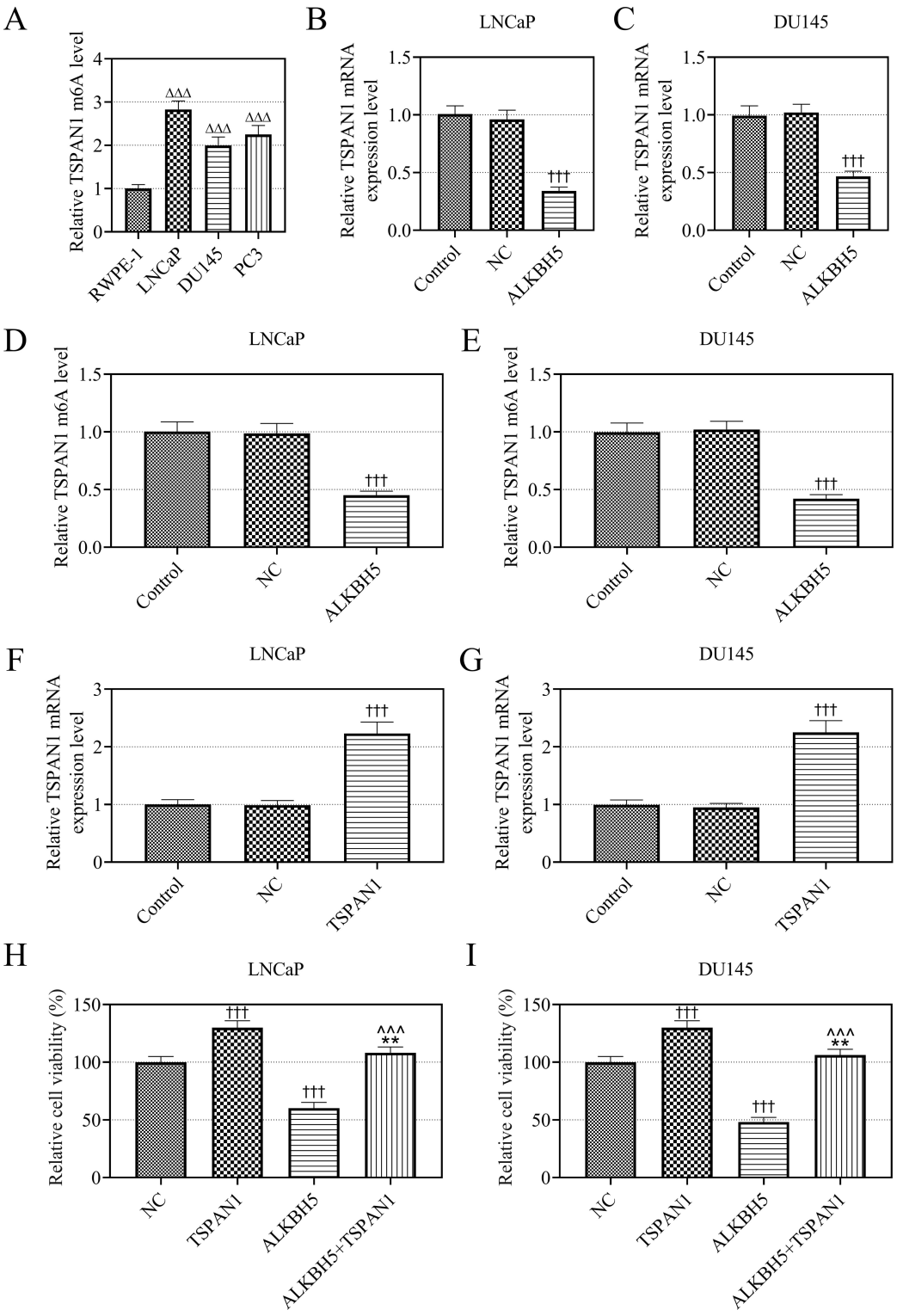

Fig. 3. *ALKBH5* reduced the N6-methyladenosine (m6A) level of Tetraspanin 1 (*TSPAN1*) to affect PCa cell viability. (A) Methylated RNA immunoprecipitation sequencing (MeRIP)-qPCR was performed to detect the m6A levels of *TSPAN1* in different PCa cells. (B,C) The effect of *ALKBH5* overexpression on mRNA of *TSPAN1* was detected using qRT-PCR. (D,E) MeRIP-qPCR revealed that *ALKBH5* overexpression inhibited m6A modification of *TSPAN1*. (F,G) *TSPAN1* overexpression plasmids were transfected into LNCaP and DU145 cells. (H,I) The effect of *ALKBH5* and/or *TSPAN1* overexpression on PCa cell viability was detected using CCK-8. *TSPAN1* overexpression greatly promoted cell viability which was further inhibited by *ALKBH5* overexpression. $^{\Delta\Delta\Delta}p < 0.001$ vs. RWPE-1; $^{\dagger\dagger\dagger}p < 0.001$ vs. NC; $^{**}p < 0.01$ vs. *TSPAN1*; $^{\wedge\wedge\wedge}p < 0.001$ vs. *ALKBH5*. n = 3.

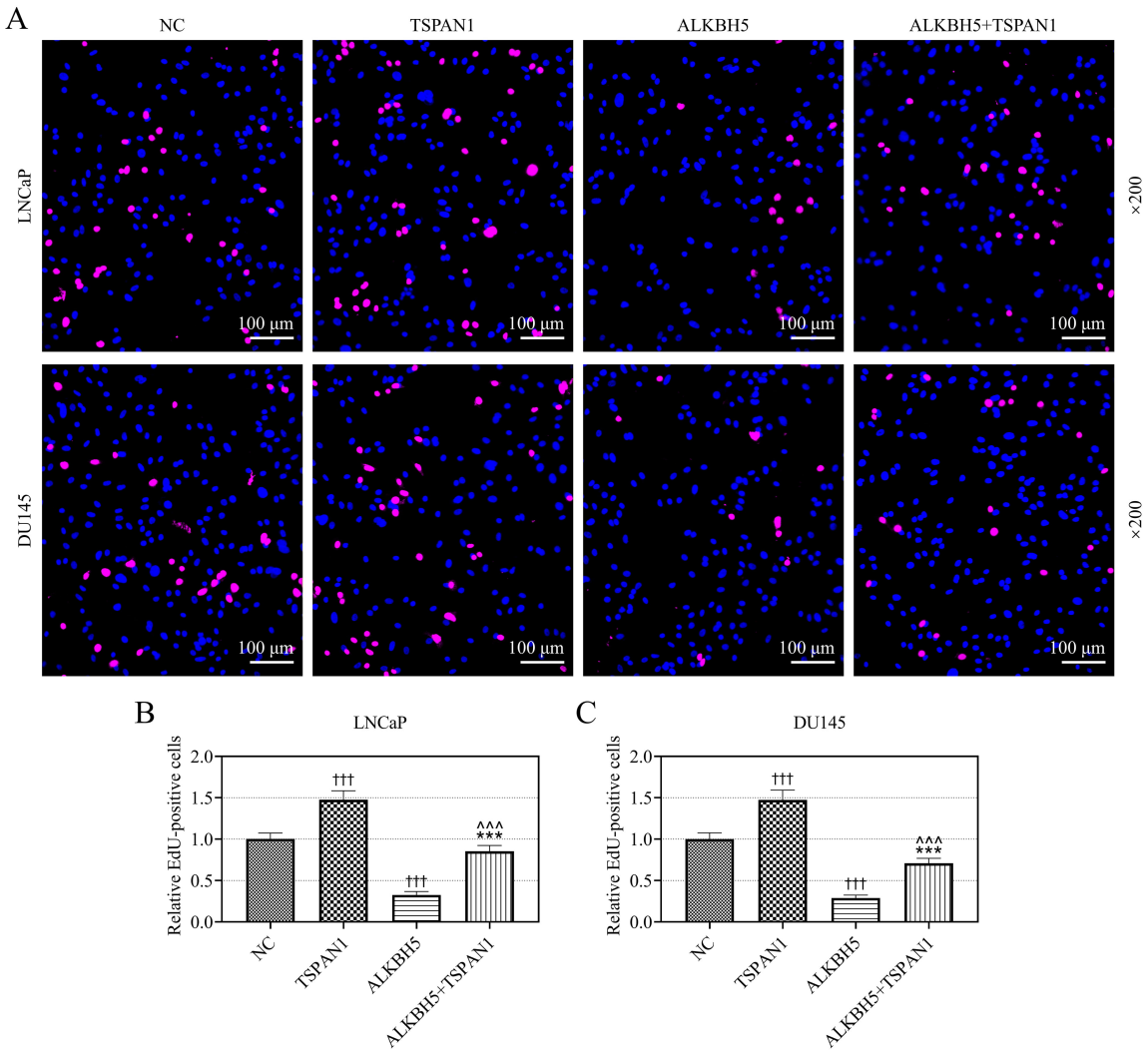

Fig. 4. The effect of *ALKBH5* and/or *TSPAN1* overexpression on the proliferation of LNCaP and DU145 cells was assessed using EdU staining (EdU: red; Hoechst 33342: blue). (A–C) *TSPAN1* overexpression promoted the number of EdU-positive cells, while *ALKBH5* overexpression reversed the effect of *TSPAN1* on cell proliferation. Magnification: ×200, scale bar = 100 μm. $^{\dagger\dagger\dagger}p < 0.001$ vs. NC; $^{***}p < 0.001$ vs. *TSPAN1*; $^{\wedge\wedge\wedge}p < 0.001$ vs. *ALKBH5*. n = 3.

Discussion

PCa is the predominant malignant tumor in men globally [2]. Tumor recurrence after radical surgery is one of the difficulties in clinical treatment of PCa, and there is no absolutely effective treatment method [3]. Many studies have been conducted to demonstrate that the prognosis of patients with limited PCa can be accessed by combining multiple gene tests, which can guide clinical treatment [25]. In this study, we found that *ALKBH5* was lowly expressed in PCa and its upregulation inhibited PCa cell viability, proliferation, and autophagy, in part by suppressing the m6A level of *TSPAN1*. Our study adds a new theoretical basis for the role of *ALKBH5* and *TSPAN1* in PCa.

The m6A regulatory mode is a reversible dynamic RNA modification that maintains normal biological processes in the organism [21]. m6A modifications are mediated by *ALKBH5*, a well-recognized m6A demethylase, in a variety of diseases [26,27]. Previous reviews have indicated that *ALKBH5*-mediated m6A demethylation affects cancer progression by influencing multiple events in RNA metabolism, and thus may serve as a potential target in cancer therapy [28]. *ALKBH5* silencing also sensitizes melanoma, colorectal cancer, and potentially other cancers to cancer immunotherapy [29]. *ALKBH5*-mediated m6A demethylation leads to post-transcriptional repression of LYPD1, which attenuates the oncogenic behavior of tumors [30]. *ALKBH5* has also been analyzed to a small extent in PCa studies, but these mostly focused on the expression level and were not in-depth investigations [31–33]. Regardless, the potential of *ALKBH5* as a biomarker in PCa has been identified. Here, we revealed that *ALKBH5* overexpression has the ability to inhibit PCa cell viability and proliferation.

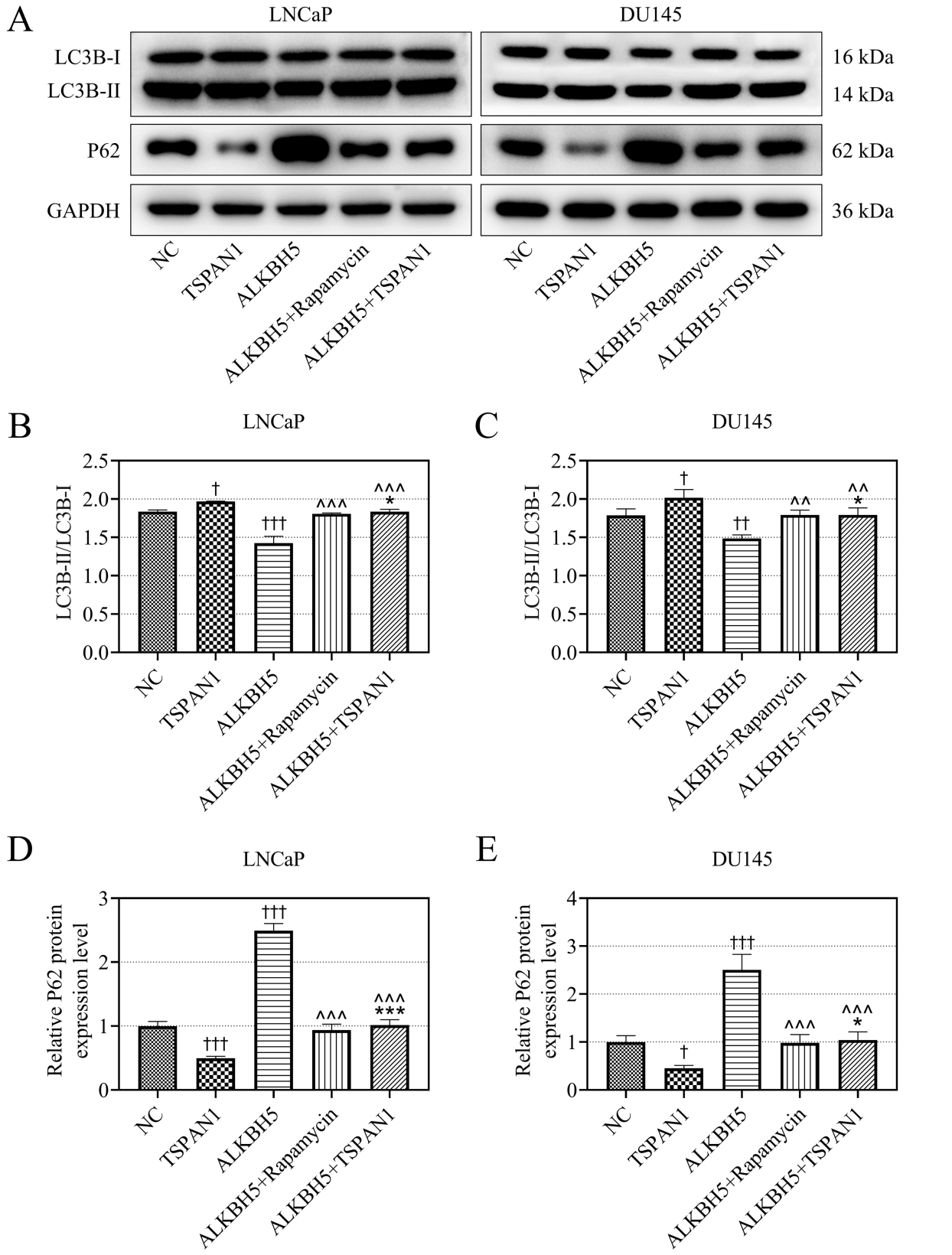

Fig. 5. Overexpression of *ALKBH5* and/or *TSPAN1* influenced autophagy-related protein expression in PCa cells. (A–E) LC3B-I, LC3B-II, and P62 protein levels were detected using western blot. Upregulation of *TSPAN1* increased the LC3B-II/LC3B-I protein ratio and decreased P62 expression, whereas *ALKBH5* overexpression reversed the effects of *TSPAN1*. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs. NC; $^{*}p < 0.05$, $^{***}p < 0.001$ vs. *TSPAN1*; $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ vs. *ALKBH5*. n = 3.

TSPAN1 is associated with cell survival, proliferation, and invasion in tumors including PCa and involved in chemoresistance [18,34]. Stinnesbeck M *et al.* [13] have suggested that *TSPAN1* is promising as an independent prognostic indicator for PCa. Currently, no scholars have focused on the m6A modification of *TSPAN1*. Here we found high m6A levels of *TSPAN1* in PCa cells. m6A is a widespread base modification behavior on mRNA, and m6A is a hot spot in the direction of research in recent years [20,35]. We found that *ALKBH5* overexpression suppressed the m6A level of *TSPAN1*, and thus reduced *TSPAN1* expression. *TSPAN1* overexpression promoted PCa cell viability and proliferation, which is consistent with previous studies [18]. Moreover, *TSPAN1* overexpression reversed the anticancer effect of *ALKBH5* in PCa cells, further suggesting that *ALKBH5* could affect PCa progression by regulating *TSPAN1* m6A modification.

The importance of autophagy in PCa disease was mentioned in the introduction, and activation of autophagy exacerbates PCa malignant behavior. Zhu H *et al.* [36] have reported that *ALKBH5* inhibits autophagy in epithelial ovarian cancer. *ALKBH5* is downregulated in primary Sjögren's syndrome and may regulate the autophagic pathway [37]. Deletion of *TSPAN1* reduces autophagy in head and neck squamous cell carcinoma and pancreatic cancer [14,38]. P62 is an important autophagy receptor and its expression is negatively correlated with autophagy, and P62 downregulation can lead to the impairment of autophagic flux. LC3 is a classical autophagy marker; it is esterified to form LC3-II type II protein upon autophagy activation, and the magnitude of the LC3-II/I ratio can estimate the level of autophagy [39,40]. Our study clearly showed that *ALKBH5* promoted P62 and decreased the LC3-II/I ratio in PCa cells, but these effects were reversed by *TSPAN1* overexpression or autophagy activator Rapamycin; this also indicated that *ALKBH5* inhibited the autophagic process by reducing the m6A level of *TSPAN1*, thereby inhibiting PCa cell progression.

Our study confirms a partial mechanism of *ALKBH5* and *TSPAN1* in PCa cell progression, adding to the scientific evidence for its future clinical diagnosis and treatment in PCa. Nevertheless, our study has some limitations. For example, our conclusions were obtained based on *in vitro* experiments and further *in vivo* validation and clinical research may be needed.

Conclusions

In summary, the results of this study showed that overexpression of the *ALKBH5* gene in PCa cells LNCaP and DU145 inhibited the activation of autophagy, and the mechanism may be related to the downregulation of the m6A level of *TSPAN1*. Our research provides a new perspective on the molecular mechanism of PCa, and identifies potential targets for early diagnosis and prognosis of PCa. Future studies will further analyze and verify the specific translation regulation mechanism of *ALKBH5*.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

XDZ designed the research study; ZQZ, HLX, WJG and CHY performed the research; ZQZ, HLX, WJG and CHY collected and analyzed the data. All authors have been involved in drafting the manuscript and all authors have been involved in revising it critically for important intellectual content. All authors give final approval of the version to be published. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

References

[1] Ko YH, Kim BH. Should Contemporary Western Guidelines Based on Studies Conducted in the 2000s Be Adopted for the Prostate-Specific Antigen Screening Policy for Asian Men in the 2020s? The World Journal of Men's Health. 2022; 40: 543–550.

[2] Wang G, Zhao D, Spring DJ, DePinho RA. Genetics and biology of prostate cancer. Genes & Development. 2018; 32: 1105–1140.

[3] Schatten H. Brief Overview of Prostate Cancer Statistics, Grading, Diagnosis and Treatment Strategies. Advances in Experimental Medicine and Biology. 2018; 1095: 1–14.

[4] Desai K, McManus JM, Sharifi N. Hormonal Therapy for Prostate Cancer. Endocrine Reviews. 2021; 42: 354–373.

[5] Shafi AA, Yen AE, Weigel NL. Androgen receptors in hormone-dependent and castration-resistant prostate cancer. Pharmacology & Therapeutics. 2013; 140: 223–238.

[6] Wang Y, Wu N, Jiang N. Autophagy provides a conceptual therapeutic framework for bone metastasis from prostate cancer. Cell Death & Disease. 2021; 12: 909.

[7] Levy JMM, Towers CG, Thorburn A. Targeting autophagy in cancer. Nature Reviews. Cancer. 2017; 17: 528–542.

[8] Hu D, Jiang L, Luo S, Zhao X, Hu H, Zhao G, *et al*. Development of an autophagy-related gene expression signature for prognosis prediction in prostate cancer patients. Journal of Translational Medicine. 2020; 18: 160.

[9] Mukha A, Kahya U, Dubrovska A. Targeting glutamine metabolism and autophagy: the combination for prostate cancer radiosensitization. Autophagy. 2021; 17: 3879–3881.

[10] Man YG, Mannion C, Jewett A, Hsiao YH, Liu A, Semczuk A, *et al*. The most effective but largely ignored target for prostate cancer early detection and intervention. Journal of Cancer. 2022; 13: 3463–3475.

[11] Datta D, Aftabuddin M, Gupta DK, Raha S, Sen P. Human Prostate Cancer Hallmarks Map. Scientific Reports. 2016; 6: 30691.

[12] Jiang L, Ren L, Chen H, Pan J, Zhang Z, Kuang X, *et al*. NCAPG confers trastuzumab resistance via activating SRC/STAT3 signaling pathway in HER2-positive breast cancer. Cell Death & Disease. 2020; 11: 547.

[13] Stinnesbeck M, Kristiansen A, Ellinger J, Hauser S, Egevad L, Tolkach Y, *et al*. Prognostic role of TSPAN1, KIAA1324 and ESRP1 in prostate cancer. APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica. 2021; 129: 204–212.

[14] Zhou C, Liang Y, Zhou L, Yan Y, Liu N, Zhang R, *et al*. TSPAN1 promotes autophagy flux and mediates cooperation between WNT-CTNNB1 signaling and autophagy via the *MIR454*-FAM83A-TSPAN1 axis in pancreatic cancer. Autophagy. 2021; 17: 3175–3195.

[15] Lu Z, Luo T, Nie M, Pang T, Zhang X, Shen X, *et al*. TSPAN1 functions as an oncogene in gastric cancer and is downregulated by miR-573. FEBS Letters. 2015; 589: 1988–1994.

[16] Lee CH, Im EJ, Moon PG, Baek MC. Discovery of a diagnostic biomarker for colon cancer through proteomic profiling of small extracellular vesicles. BMC Cancer. 2018; 18: 1058.

[17] Hölters S, Anacker J, Jansen L, Beer-Grondke K, Dürst M, Rubio I. Tetraspanin 1 promotes invasiveness of cervical cancer cells. International Journal of Oncology. 2013; 43: 503–512.

[18] Munkley J, McClurg UL, Livermore KE, Ehrmann I, Knight B, Mccullagh P, *et al*. The cancer-associated cell migration protein TSPAN1 is under control of androgens and its upregulation increases prostate cancer cell migration. Scientific Reports. 2017; 7: 5249.

[19] He L, Li H, Wu A, Peng Y, Shu G, Yin G. Functions of N6-methyladenosine and its role in cancer. Molecular Cancer. 2019; 18: 176.

[20] Liu Z, Zhong J, Zeng J, Duan X, Lu J, Sun X, *et al*. Characterization of the m6A-Associated Tumor Immune Microenvironment in Prostate Cancer to Aid Immunotherapy. Frontiers in Immunology. 2021; 12: 735170.

[21] Wang J, Lin H, Zhou M, Xiang Q, Deng Y, Luo L, *et al*. The m6A methylation regulator-based signature for predicting the prognosis of prostate cancer. Future Oncology (London, England). 2020; 16: 2421–2432.

[22] Wu Q, Xie X, Huang Y, Meng S, Li Y, Wang H, *et al*. N6-methyladenosine RNA methylation regulators contribute to the progression of prostate cancer. Journal of Cancer. 2021; 12: 682–692.

[23] Li Z, Wang P, Li J, Xie Z, Cen S, Li M, *et al*. The N^6-methyladenosine demethylase ALKBH5 negatively regulates the osteogenic differentiation of mesenchymal stem cells through PRMT6. Cell Death & Disease. 2021; 12: 578.

[24] Huang D, Zhang LL, Zhou B, Luo ZZ, Zhang J, Tang B, *et al*. Rapamycin inhibits LOC102553434-mediated pyroptosis to improve lung injury induced by limb ischemia-reperfusion. 3 Biotech. 2021; 11: 335.

[25] Ye Y, Li SL, Wang SY. Construction and analysis of mRNA, miRNA, lncRNA, and TF regulatory networks reveal the key genes associated with prostate cancer. PloS One. 2018; 13: e0198055.

[26] Guo X, Li K, Jiang W, Hu Y, Xiao W, Huang Y, *et al*. RNA demethylase ALKBH5 prevents pancreatic cancer progression by posttranscriptional activation of PER1 in an m6A-YTHDF2-dependent manner. Molecular Cancer. 2020; 19: 91.

[27] Nie S, Zhang L, Liu J, Wan Y, Jiang Y, Yang J, *et al*. ALKBH5-HOXA10 loop-mediated JAK2 m6A demethylation and cisplatin resistance in epithelial ovarian cancer. Journal of Experimental & Clinical Cancer Research: CR. 2021; 40: 284.

[28] Qu J, Yan H, Hou Y, Cao W, Liu Y, Zhang E, *et al*. RNA demethylase ALKBH5 in cancer: from mechanisms to therapeutic potential. Journal of Hematology & Oncology. 2022; 15: 8.

[29] Li N, Kang Y, Wang L, Huff S, Tang R, Hui H, *et al*. ALKBH5 regulates anti-PD-1 therapy response by modulating lactate and suppressive immune cell accumulation in tumor microenvironment. Proceedings of the National Academy of Sciences of the United States of America. 2020; 117: 20159–20170.

[30] Chen Y, Zhao Y, Chen J, Peng C, Zhang Y, Tong R, *et al*. ALKBH5 suppresses malignancy of hepatocellular carcinoma via m^6A-guided epigenetic inhibition of LYPD1. Molecular Cancer. 2020; 19: 123.

[31] Ji G, Huang C, He S, Gong Y, Song G, Li X, *et al*. Comprehensive analysis of m6A regulators prognostic value in prostate cancer. Aging. 2020; 12: 14863–14884.

[32] Ding D, Liu G, Gao J, Cao M. Unveiling the m6A Methylation Regulator Links between Prostate Cancer and Periodontitis by Transcriptomic Analysis. Disease Markers. 2022; 2022: 4030046.

[33] Zhao S, Geybels MS, Leonardson A, Rubicz R, Kolb S, Yan Q, *et al*. Epigenome-Wide Tumor DNA Methylation Profiling Identifies Novel Prognostic Biomarkers of Metastatic-Lethal Progression in Men Diagnosed with Clinically Localized Prostate Cancer. Clinical Cancer Research: an Official Journal of the American Association for Cancer Research. 2017; 23: 311–319.

[34] Garcia-Mayea Y, Mir C, Carballo L, Sánchez-García A, Bataller M, LLeonart ME. TSPAN1, a novel tetraspanin member highly involved in carcinogenesis and chemoresistance. Biochimica et Biophysica Acta. Reviews on Cancer. 2022; 1877: 188674.

[35] Su H, Wang Y, Li H. RNA m6A Methylation Regulators Multi-Omics Analysis in Prostate Cancer. Frontiers in Genetics. 2021; 12: 768041.

[36] Zhu H, Gan X, Jiang X, Diao S, Wu H, Hu J. ALKBH5 inhibited autophagy of epithelial ovarian cancer through miR-7 and BCL-2. Journal of Experimental & Clinical Cancer Research: CR. 2019; 38: 163.

[37] Cheng L, Li H, Zhan H, Liu Y, Li X, Huang Y, *et al*. Alterations of m6A RNA methylation regulators contribute to autophagy and immune infiltration in primary Sjögren's syndrome. Frontiers in Immunology. 2022; 13: 949206.

[38] Garcia-Mayea Y, Mir C, Carballo L, Castellvi J, Temprana-Salvador J, Lorente J, *et al*. TSPAN1: A Novel Protein Involved in Head and Neck Squamous Cell Carcinoma Chemoresistance. Cancers. 2020; 12: 3269.

[39] Elbialy A. In vivo autophagy quantification: Measuring LC3 and P62 puncta in 3D image system from zebrafish larvae. Journal of Cellular Biochemistry. 2021; 122: 1435–1444.

[40] Jiang P, Mizushima N. LC3- and p62-based biochemical methods for the analysis of autophagy progression in mammalian cells. Methods (San Diego, Calif.). 2015; 75: 13–18.