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Exploring the clinical and biological significance of the cell cycle-related gene CHMP4C in prostate cancer

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Abstract

Background Prostate cancer (PCa) stands as the second most prevalent malignancy impacting male health, and the disease's evolutionary course presents formidable challenges in the context of patient treatment and prognostic management. Charged multivesicular body protein 4 C (CHMP4C) participates in the development of several cancers by regulating cell cycle functions. However, the role of CHMP4C in prostate cancer remains unclear.

Methods In terms of bioinformatics, multiple PCa datasets were employed to scrutinize the expression of CHMP4C. Survival analysis coupled with a nomogram approach was employed to probe into the prognostic significance of CHMP4C. Gene set enrichment analysis (GSEA) was conducted to interrogate the functional implications of CHMP4C. In terms of cellular experimentation, the verification of RNA and protein expression levels was executed through the utilization of qRT-PCR and Western blotting. Upon the establishment of a cell line featuring stable CHMP4C knockdown, a battery of assays, including Cell Counting Kit-8 (CCK-8), wound healing, Transwell, and flow cytometry, were employed to discern the impact of CHMP4C on the proliferation, migration, invasion, and cell cycle function of PCa cells.

Results The expression of CHMP4C exhibited upregulation in both PCa cells and tissues, and patients demonstrating elevated CHMP4C expression levels experienced a notably inferior prognosis. The nomogram, constructed using CHMP4C along with clinicopathological features, demonstrated a commendable capacity for prognostic prediction. CHMP4C knockdown significantly inhibited the proliferation, migration, and invasion of PCa cells (LNcaP and PC3). CHMP4C could impact the advancement of the PCa cell cycle, and its expression might be regulated by berberine. Divergent CHMP4C expression among PCa patients could induce alterations in immune cell infiltration and gene mutation frequency.

Conclusions Our findings suggest that CHMP4C might be a prognostic biomarker in PCa, potentially offering novel perspectives for the advancement of precision therapy for PCa.

Keywords Prostate cancer, CHMP4C, Cell cycle, Prognosis, Biomarker

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Introduction

Prostate cancer (PCa) poses a growing burden on men's health. Among male malignancies, it stands as the second most frequently diagnosed cancer and the fifth leading cause of cancer-related mortality [1]. Currently, an escalating number of investigations are being conducted concerning the occurrence and progression of PCa. This malignancy entails a multifaceted and multipartite pathological progression, yet its precise pathogenic mechanisms remain incompletely elucidated [2]. Furthermore, despite substantial advancements in treatment modalities for PCa, encompassing the evolution of minimally invasive surgical techniques, the utilization of radiotherapy and chemotherapy, as well as the transition from monotherapies to combinatorial regimens, the challenge of tumor recurrence and metastasis persists as a significant quandary in the realm of PCa health management [3]. In recent years, there has been a significant focus on the development of precise and targeted therapy for tumors [4]. However, it is important to note that the field of targeted therapy for PCa is currently in its nascent phase [5]. Therefore, the exploration of novel biomarkers for PCa holds substantial significance in facilitating personalized precision therapies and improving the prognostic management of patients with PCa.

Charged multivesicular body protein 4 C (CHMP4C), belonging to the family of chromatin-modifying proteins/charged multivesicular body proteins, constitutes an essential constituent of the endosomal sorting complex required for transporter III (ESCRT-III) [6]. CHMP4C takes part in the shedding checkpoint and holds a crucial function in the cell cycle [7]. Its significant regulatory role in the occurrence and development of multiple cancers has been established. For example, Liu et al. demonstrated that CHMP4C regulates the initiation and progression of lung squamous carcinoma through the regulation of cell cycle pathways [8]. Lin et al. revealed CHMP4C's capability to promote the malignant progression of cervical cancer cells [9]. In the realm of PCa, Fujita et al. identified heightened CHMP4C expression in the prostatic fluid of individuals with PCa characterized by high Gleason scores [10]. Zhang et al. delineated CHMP4C's regulatory effect on the progression of PCa [11]. Our previous studies indicated the potential impact of CHMP4C on the prognosis of PCa patients [12]. However, the potential of CHMP4C as an emerging therapeutic target in PCa, as well as the elucidation of its molecular mechanisms and functional regulation in PCa, demands continued and comprehensive investigation.

Berberine, obtained from the medicinal herb Coptis chinensis, has an array of pharmacological roles, embracing antioxidative, anti-inflammatory, and organ-preserving qualities [13]. Furthermore, its anticancer impacts have been extensively investigated across diverse cancer types. In particular, berberine has demonstrated the capability to impede androgen receptor transduction in PCa [14]. Additionally, it has the potential to induce apoptosis in PCa cells [15], while also regulating the cell cycle of PCa cells to affect cell progression [16]. Interestingly, CHMP4C could emerge as a potential regulator of the cell cycle in PCa. Consequently, the inquiry into whether berberine modulates the expression of CHMP4C and consequentially impacts the progression of PCa cells warrants a comprehensive investigation.

In this study, we focused on primary PCa. First, we examined the expression of CHMP4C in both PCa and normal prostate samples by integrating several online databases and corresponding cellular experimentation. Subsequently, we analyzed the correlation between CHMP4C and the clinical prognosis of PCa patients, as well as the effect of CHMP4C on the proliferation, invasion, and regulation of cell cycle function of PCa cells. Additionally, we explored the potential of berberine to modulate CHMP4C expression in PCa. Our investigations aim to offer novel possibilities for individualized health management strategies for PCa.

Materials and methods

Data acquisition and organization

We acquired gene expression data, mutation data, and corresponding clinical data of PCa samples (TCGA-PRAD) from the TCGA database (https://portal.gdc.cancer.gov/). We procured expression data for 4 PCa datasets (GSE70768, GSE88808, GSE46602, and GSE69223) from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). We acquired immunohistochemical data for CHMP4C in both PCa and normal prostate tissues from the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/). Finally, we conducted data processing using the R software, employing the R packages "dplyr" [17], "tidyr" [18], "tibble" [19], and "limma" [20]. Information on the cohorts included can be found in Supplementary Table 1. The clinical information of the patients can be found in Supplementary Table 2.

Bioinformatics analysis

Using the R packages "ggpubr" [21] and "limma", we conducted an analysis of CHMP4C expression in both PCa and normal prostate samples across the five aforementioned cohorts. The results were visually presented through violin plots. Concurrently, we leveraged the immunohistochemical data available in the HPA database to assess disparities in CHMP4C protein expression within tissues. In the TCGA-PRAD cohort, we scrutinized the relationship between the CHMP4C expression levels and various clinicopathological features (age, T stage, N stage, PSA value, and Gleason score) of patients. Subsequently, we examined the correlation between

CHMP4C and the prognosis of PCa patients utilizing the R packages "survival" [22] and "survminer" [23]. Afterwards, we developed a prognostic nomogram involving CHMP4C and clinicopathological features, employing the R packages "rms" [24], "survival", and "regplot" [25]. The validity of the nomogram was assessed through ROC curves and calibration curves. Moreover, we used the CIBERSORT algorithm to calculate infiltration levels for 22 different immune cell types across all samples in the TCGA-PRAD cohort [26]. We then evaluated the association between CHMP4C expression and the infiltration levels of these immune cells using the Spearman method and presented the results using lollipop plots. Then, employing the R package "maftools" [27], we conducted an analysis of somatic mutation, single nucleotide variant (SNV), and gene mutation among patients categorized into the CHMP4C high and low expression groups. Additionally, we investigated the correlation between CHMP4C expression levels and patients' tumor mutation burden (TMB), while also assessing their potential impact on patient prognosis. Finally, we used the R packages "org.Hs.eg.db" [28] and "clusterProfiler" [29] to perform functional analysis of CHMP4C.

Cell culture

Three different types of PCa cells (LNCaP, PC3, and DU-145) were purchased from Servicebio (Wuhan, China), and normal prostate cells (RWPE-1) were purchased from the Shanghai Institute of Cell Research (Shanghai, China). These four cell lines were selected because they have been extensively used in previous studies to validate prostate cancer biomarkers experimentally [30, 31]. The product information for cell lines can be found in Supplementary Table 4. Our cell culture mediums were supplemented with 10% fetal bovine serum. Specifically, 1640 mediums (Gibco) were used to culture PCa cells, while RWPE-1 cells were cultured in DMEM mediums (Gibco). All cell lines were maintained at 37 °C in a 5% CO2 incubator.

Lentiviral packaging and cell transfection

Knockdown plasmids (sh-CHMP4C#1 and sh-CHMP4C#2) were purchased from Miaolingbio (Wuhan, China). Lentiviral generation involved the co-transfection of HEK293T cells with a combination of the expression plasmids and packaging plasmids (psPAX2 and pMD2.G). At the conclusion of a 48-hour incubation, the supernatant was gathered, cleared of debris through centrifugation, and concentrated using PEG8000. The resulting lentiviral concentrate was subsequently utilized for the purpose of infecting LNcaP and PC3 cells (In our results, the two cells had the highest expression of CHMP4C.). A concentration of 2 μg/ml puromycin was utilized for the purpose of selecting stably transfected cell lines. The sequence of the knockdown plasmid can be found in Supplementary Table 3.

qRT-PCR

For the isolation of total cellular RNA, we employed the TRIzol reagent (Accuratebio, AG21101, China). Subsequently, cDNA was synthesized using the appropriate reverse transcription kit (Accuratebio, AG11705, China). We then set up the reaction conditions for qRT-PCR using a Bio-Rad PCR machine, starting with an initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 5 s, and annealing/extension at 60 °C for 30 s. The relative expression of mRNA was obtained using the $2-\Delta\Delta CT$ method. The sequences of the primers can be found in Supplementary Table 3.

Western blotting

Both the extraction of total cellular proteins and the western blotting technique were executed in accordance with a previously established standard protocol [32]. The primary antibodies employed encompassed anti-CHMP4C (1:500, abcam, ab168205, UK) and anti-β-actin (1:20000, Proteintech, 66009-1-Ig, China). For secondary antibodies, we utilized HRP-conjugated goat anti-mouse antibody (1:5000, Proteintech, SA00001-1, China).

Cell proliferation assay

Stable transfected cells and control cells were respectively seeded into 96-well plates at a density of 2000 cells per well. Upon cells achieving adherent growth, a mixture of CCK-8 reagent (10 μl /well) and serum-free medium (90 μl/well) was introduced into the respective wells. Subsequent incubation was carried out at 37 °C for 1 h. Following the incubation period, cellular absorbance at 450 nm was measured using a BioTek microplate reader (Bertram, USA). The time points measured were at 0 h, 24 h, 48 h, 72 h, and 120 h.

Wound healing

Cells were seeded into individual wells of six-well plates at a density of 500,000 cells per well and cultured for 24 h according to the aforementioned culture conditions. Subsequently, employ a sterile 200 μl pipette tip to vertically generate a scratch across the cell layer. Thoroughly rinse away the suspended cells using PBS, and then introduce mediums with 2% FBS. Images capturing the wound sites were obtained through light microscopy at 0, 24, and 48 h following treatment, with the purpose of documenting the extent of cellular migration distances. We performed three independent experiments.

Cell migration and invasion assays

In the context of migration experiments, cells were suspended in serum-free medium at a concentration of

50,000 cells per well. These suspended cells were subsequently introduced into the upper chambers of transwell inserts (8.0 μm, Corning, USA). In the context of invasion experiments, a total of 150,000 cells per well were introduced into the upper chamber, which had been coated with a layer of 10% Matrigel (Corning, MA, USA). The lower chamber was supplemented with medium containing 20% FBS to induce cell migration or invasion. Once incubated for 48 h, cells that remained in the upper chamber were removed, while the cells that had migrated or invaded were immobilized using 4% paraformaldehyde and subsequently stained with 0.1% crystal violet. The quantification of migrated or invaded cells was conducted in three randomly selected microscopic fields.

Flow cytometry

For the detection of the cell cycle, we obtained appropriate cells, washed them with the PBS solution, and obtained cell pellets by centrifugation. Next, in accordance with the guidelines outlined in the Cell Cycle Staining Kit (MultiSciences, CCS012-01, China), 1 ml of DNA staining solution and 10 μl of permeabilization solution were added to the cells. The mixture was vortexed for 5–10 s to ensure proper mixing, followed by a 30-minute incubation at room temperature in the absence of light. Subsequently, cell cycle analysis was conducted using flow cytometry (BECKMAN, CytoFLEX, USA). Three independent flow cytometry experiments were performed.

Acquisition of berberine IC50

The cells were placed in a 96-well plate at 5000 per well and allowed to adhere. Subsequently, different concentrations of berberine (0μM, 100μM, 200μM, 300μM, 400μM, 500μM, 600μM, sourced from Macklin, B875003, China) in serum-free medium were administered. The cells were then cultured for a period of 24 h. Absorbance was measured by the CCK-8 assay. The IC50 of berberine was calculated using GraphPad Prism 9 software.

Statistical analysis

In this study, R software (version 4.3.1), GraphPad Prism (version 9.0), FlowJo (version 10.8.1) and ImageJ were used for statistical analysis. The Spearman correlation method was used to evaluate the interrelationships between variables. Disparities between groups were assessed using the Student's t-test and one-way analysis of variance (ANOVA). The log-rank test and the Kaplan-Meier method were used for survival analyses. A significance level of $p<0.05$ was considered indicative of statistically significant outcomes ($*p<0.05$, $**p<0.01$, $***p<0.001$, $****p<0.0001$).

Results

CHMP4C shows significant overexpression in both PCa cells and tissues

In our preliminary investigation utilizing the TCGA-PRAD dataset, it was determined that there was a noteworthy increase in CHMP4C expression in prostate tumor tissues compared to normal prostate tissues (Fig. 1a). This outcome was corroborated through validation in multiple GEO datasets, including GSE70768 (Fig. 1b), GSE88808 (Fig. 1c), GSE46602 (Fig. 1d), and GSE69223 (Fig. 1e). Through qRT-PCR, a higher RNA expression level of CHMP4C was detected in PCa lines

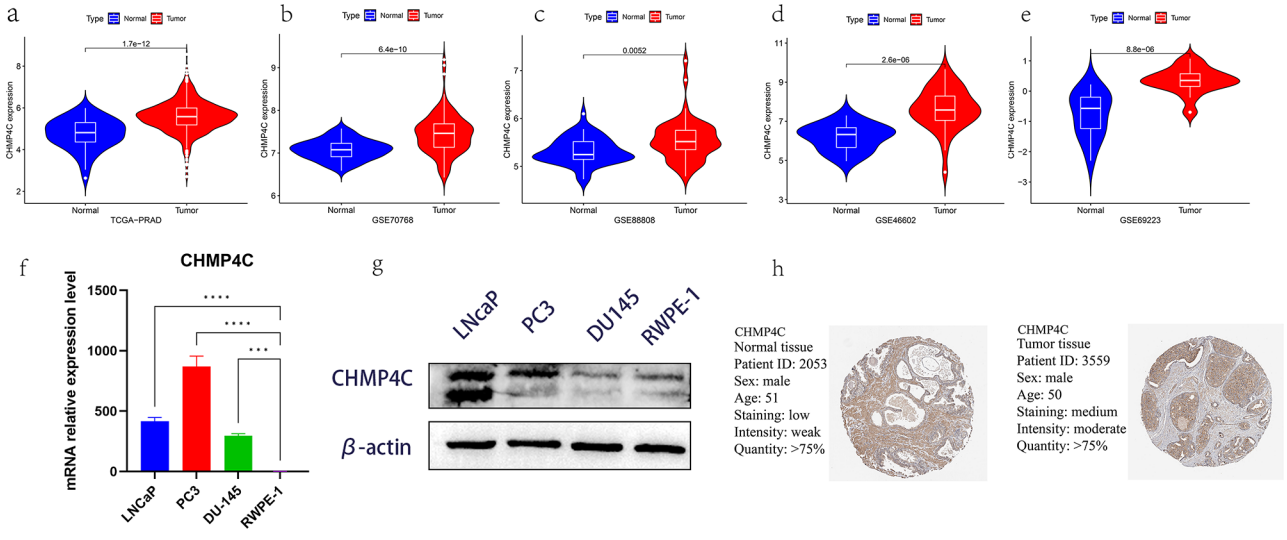

Fig. 1 The expression of CHMP4C in PCa. Differential expression of CHMP4C in PCa and normal prostate tissue in the TCGA-PRAD (**a**), GSE70768 (**b**), GSE88808 (**c**), GSE46602 (**d**), and GSE69223 (**e**) datasets. (**f**) The RNA expression level of CHMP4C in PCa cells and normal prostate cells. (**g**) CHMP4C protein expression levels in PCa cells and normal prostate cells. (**h**) Immunohistochemical data on CHMP4C expression in PCa from the HPA database

(LNCaP, PC3, and DU145) when compared to RWPE-1 cells (Fig. 1f). At the protein level, Western blotting results demonstrated a higher expression of CHMP4C in both LNCaP and PC3 cells (Fig. 1g). Immunohistochemical findings in the HPA database similarly revealed an increased expression of CHMP4C within tumor tissues (Fig. 1h).

CHMP4C facilitates proliferation, migration, and invasion of PCa cells

Based on the expression of CHMP4C in PCa cells, we chose to proceed with LNcaP and PC3 cell lines for subsequent experimental investigations. Initially, we established stable cell lines of LNcaP (sh1 and sh2) and PC3 (sh1 and sh2) with CHMP4C knockdown. Subsequently, we proceeded to confirm the knockdown efficiency through qRT-PCR and Western blotting. The obtained results demonstrated a significant reduction in CHMP4C expression in both LNcaP and PC3 cells within the sh1 and sh2 groups, as compared to the control group (Fig. 2a, b, c, and d). Then, the CCK-8 assay was employed to evaluate the impact of CHMP4C on the proliferation of PCa cells. The results underscored that the suppression of CHMP4C notably curbed the proliferation of both LNcaP and PC3 cells (Fig. 2e, f). In addition, the results of wound healing experiments showed that knockdown of CHMP4C significantly inhibited the motility of LNcaP and PC3 cells (Fig. 2g, h). Ultimately, the outcomes from the Transwell experiments demonstrated a noteworthy reduction in the migration (Fig. 2i) and invasion (Fig. 2j) capacities of LNcaP and PC3 cells following the down-regulation of CHMP4C expression. These data suggest that CHMP4C can promote the proliferation, migration, and invasion of PCa cells in vitro.

The correlation between CHMP4C and the prognosis of patients and the construction of clinical prognosis nomogram

By conducting a correlation analysis between the clinicopathological characteristics of patients in the TCGA-PRAD dataset and the expression levels of CHMP4C, the findings indicated a significant upregulation in CHMP4C expression among patients aged > 60 years (Fig. 3A), T3/4 stages (Fig. 3B), N1 stage (Fig. 3C), PSA levels ≥ 10 ug/ml (Fig. 3D), and Gleason score > 7 (Fig. 3E). In addition, patients with elevated CHMP4C expression have a significantly worse prognosis for progression free survival (PFS) (Fig. 3F). To delve deeper into the influence of CHMP4C on PCa prognosis, we integrated CHMP4C with the aforementioned clinicopathological characteristics to construct a prognostic nomogram (Fig. 3G). Using patient "TCGA-KK-A8IH" as an example, the disease progression probabilities for this specific patient at 1 year, 3 years, and 5 years were 4.2%, 12.6%, and 19.8%, respectively (Fig. 3G). The ROC curve (Fig. 3H) and calibration curve (Fig. 3I) indicated that this prognostic nomogram had high predictive power. Therefore, CHMP4C may be a prognostic biomarker in PCa.

CHMP4C regulates the cell cycle function of PCa cells, and berberine has the ability to modulate the expression of CHMP4C

Functional enrichment analysis using GSEA revealed a significant involvement of CHMP4C in the cell cycle function of PCa (Fig. 4a). Furthermore, results from our flow cytometry assay showed that in LNcaP cells, CHMP4C knockdown resulted in a significant increase in cells within the G1 phase (Fig. 4b). However, in PC3 cells, CHMP4C knockdown resulted in a significant increase in the S phase and G2 phase (Fig. 4c). It has been documented that LNcaP cells express the androgen receptor, whereas PC3 cells lack this receptor [33]. In addition, research has shown that LNcaP expresses p53, in contrast to PC3, which lacks p53 expression [34]. Given these differences between the two cell types, we hypothesized that the influence of CHMP4C on the cell cycle might vary due to these inherent differences. In addition, previous studies have shown that berberine also affects the functionality of the cell cycle in PCa. This influence leads to an accumulation of cells in the G1 phase in LNcaP cells [16]. Meanwhile, the role of berberine in PC3 cells is complex; an escalation in berberine dosage results in the accumulation of PC3 cells in the G2 phase [35, 36]. Therefore, we further investigated whether CHMP4C would be regulated by berberine. We first employed the CCK8 assay to determine the IC50 of berberine in LNcaP (399 μm) and PC3 (567.2 μm) cells after 24 h (Fig. 4d). Subsequent to subjecting the cells to the IC50 concentrations for a duration of 24 h, there was an observed decrease in both the RNA and protein expression levels of CHMP4C (Fig. 4e, f). We speculate that the function of CHMP4C, which affects the PCa cell cycle, may be regulated by berberine.

Correlation of CHMP4C with immune cell infiltration and tumor mutation burden in PCa

We explored the relationship between CHMP4C and the tumor microenvironment in PCa using data from the TCGA database. Initially, our data revealed a statistically significant positive correlation between the expression of CHMP4C and the infiltration levels of Macrophages M2, Macrophages M1, and naive B cells. Conversely, there was a significant negative correlation observed between CHMP4C expression and the infiltration levels of CD8 T cells, memory B cells, activated dendritic cells, and plasma cells (Fig. 5a). In addition, the mutation waterfall plot of the CHMP4C high and low expression groups showed that the mutation rate of the samples in the high expression group (65.32%) was significantly higher than

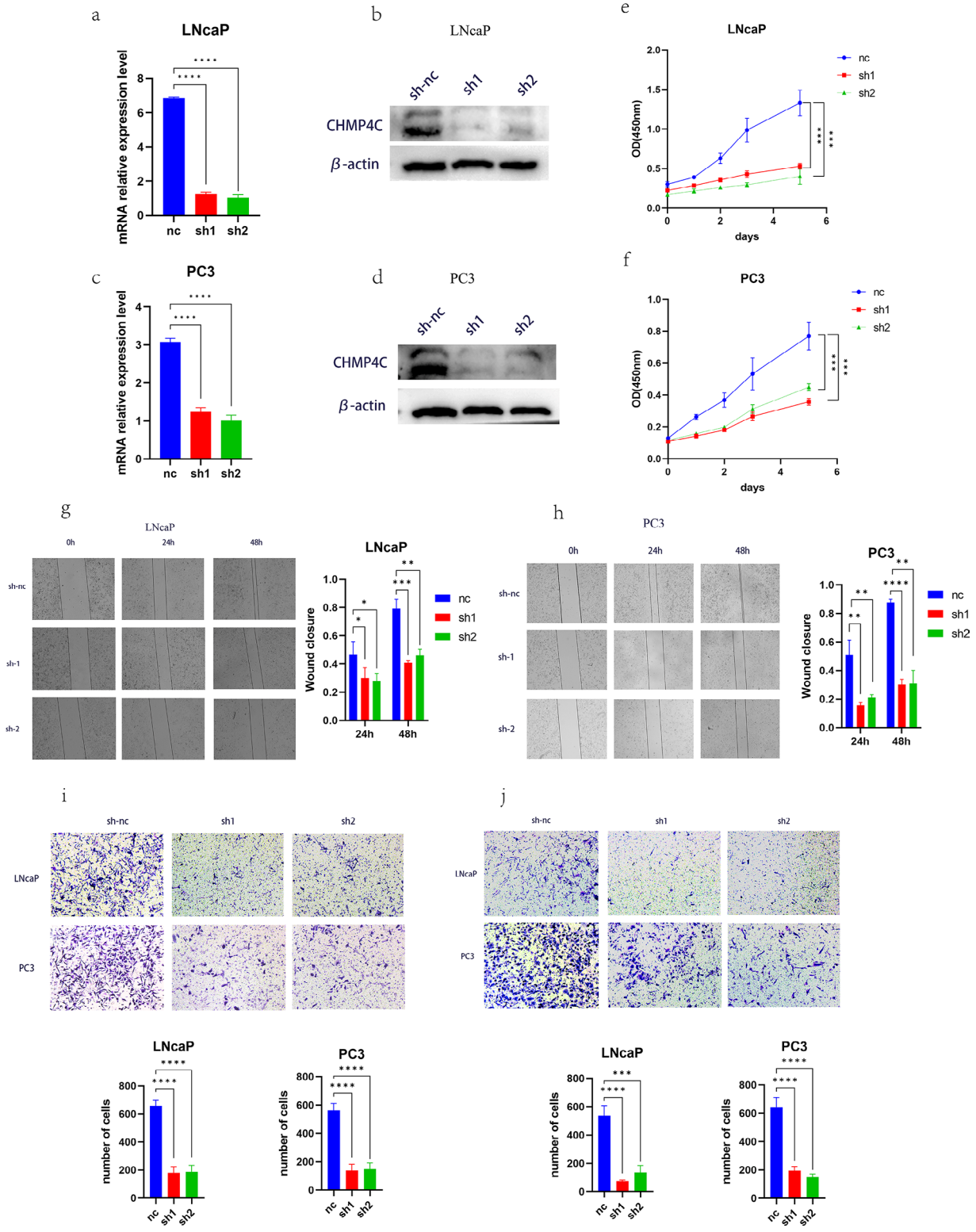

Fig. 2 Effects of CHMP4C on the proliferation, migration, and invasion abilities of PCa cells. (**a**, **b**) qRT-PCR and western blotting were used to analyze the mRNA and protein expression levels of CHMP4C after CHMP4C-sh1, CHMP4C-sh2, and CHMP4C-nc transfected LNcaP cells. (**c**, **d**) qRT-PCR and western blotting were used to analyze the mRNA and protein expression levels of CHMP4C after CHMP4C-sh1, CHMP4C-sh2, and CHMP4C-nc transfected PC3 cells. (**e**, **f**) The CCK-8 assay was used to evaluate the cellular proliferation of LNcaP and PC3 cells following CHMP4C knockdown. (**g**, **h**) The wound healing assay was employed to evaluate the migratory capability of LNcaP and PC3 cells subsequent to CHMP4C knockdown. (**i**, **j**) Transwell experiments were used to assess the migration and invasion capacities of LNcaP and PC3 cells following CHMP4C knockdown

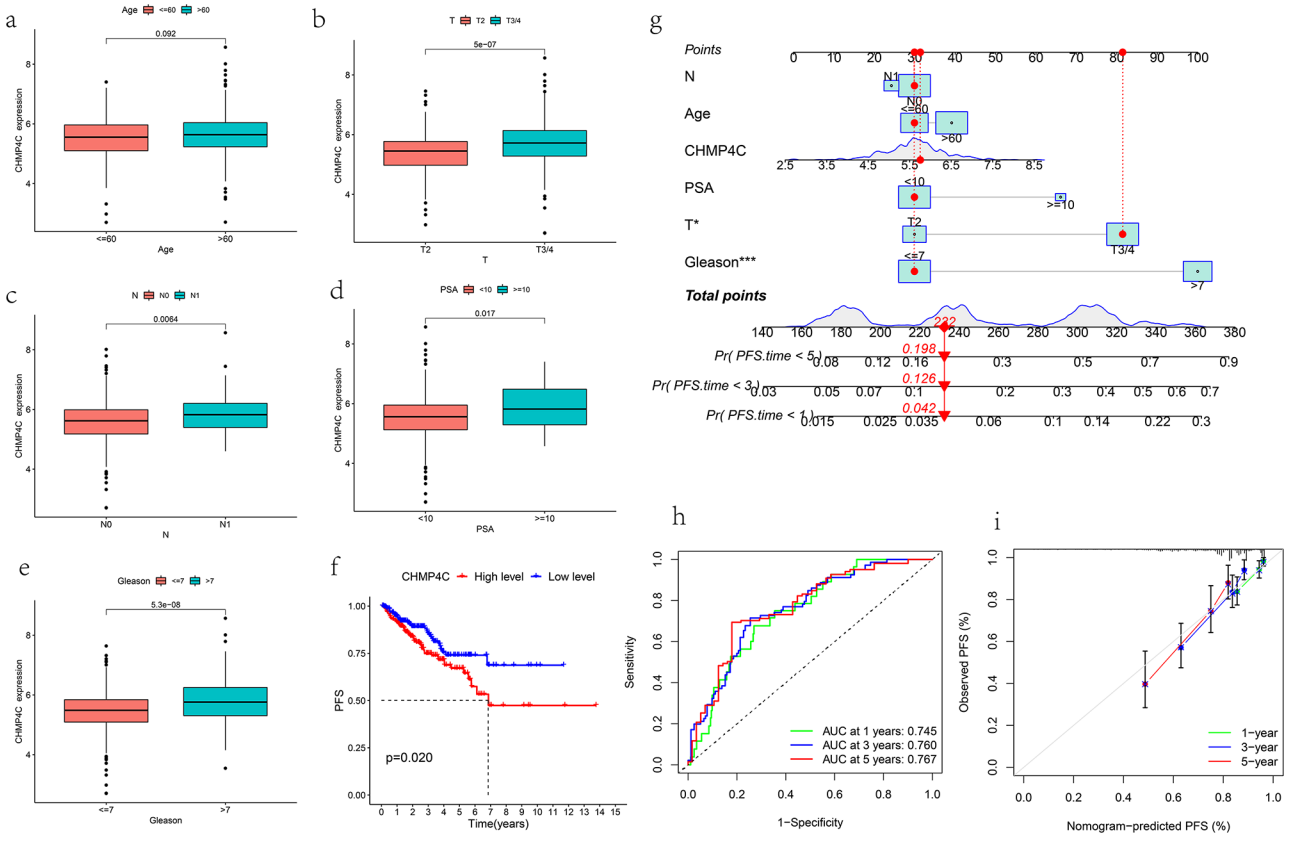

Fig. 3 Relationship between CHMP4C and clinicopathological features and prognosis of PCa patients. For patients aged > 60 years old (**a**), T3/4 stage (**b**), N1 stage (**c**), PSA > = 10 ug/ml (**d**), and Gleason score > 7 (**e**), the expression of CHMP4C was significantly up-regulated. (**f**) Kaplan-Meier survival analysis was carried out to evaluate the survival results of patients divided into high-expression and low-expression groups of CHMP4C. (**g**) A prognostic nomogram was developed through the integration of CHMP4C and clinicopathological characteristics. The ROC curve (**h**) and calibration curve (**i**) were used to assess the predictive accuracy of the prognostic nomogram

that in the low expression group (48.02%), and that the mutation frequency of genes in the high expression group was significantly higher than that in the low expression group, such as TP53, SPOP, and TTN (Fig. 5b, c). Simultaneously, the expression of CHMP4C was significantly positively correlated with the TMB score (Fig. 5d, e). Furthermore, survival analysis showed that patients with elevated TMB and patients with both high TMB and high CHMP4C expression had a less favorable prognosis (Fig. 5f, g). These findings suggest that variations in CHMP4C expression among PCa patients contribute to alterations in the tumor microenvironment, potentially offering valuable insights for the development of immunotherapeutic strategies targeting PCa.

Discussion

The global incidence and mortality rates of PCa are experiencing an annual increase in the male population worldwide [37]. The recurrence and progression of tumors pose significant challenges to the prognosis of PCa, especially given the incurability of the development of castration-resistant PCa [38]. In view of the speedy progress in genomics and bioinformatics, the utilization of both genomics and clinicopathology is becoming increasingly necessary for identifying biomarkers and predicting clinical outcomes for PCa [39, 40]. Research investigations have shown that CHMP4C exhibits involvement in the pyroptosis pathway and may become a new biomarker for various cancers, such as renal clear cell carcinoma [41], cervical cancer [42], bladder cancer [43], and skin cutaneous melanoma [44]. Furthermore, as a constituent of the ESCRT, CHMP4C also contributes to the modulation of cell cycle-associated functionalities [45]. In the context of PCa, prior research has suggested the possible impact of CHMP4C on PCa progression through cell cycle mechanisms. Nonetheless, a thorough examination of this topic has yet to be conducted. Therefore, it is essential to investigate the potential involvement of CHMP4C in PCa and the underlying mechanisms to enhance accurate treatment and prognostic management of PCa.

In our study, we first analyzed the expression levels of CHMP4C across PCa-associated cohorts, utilizing data from the TCGA and GEO databases. The

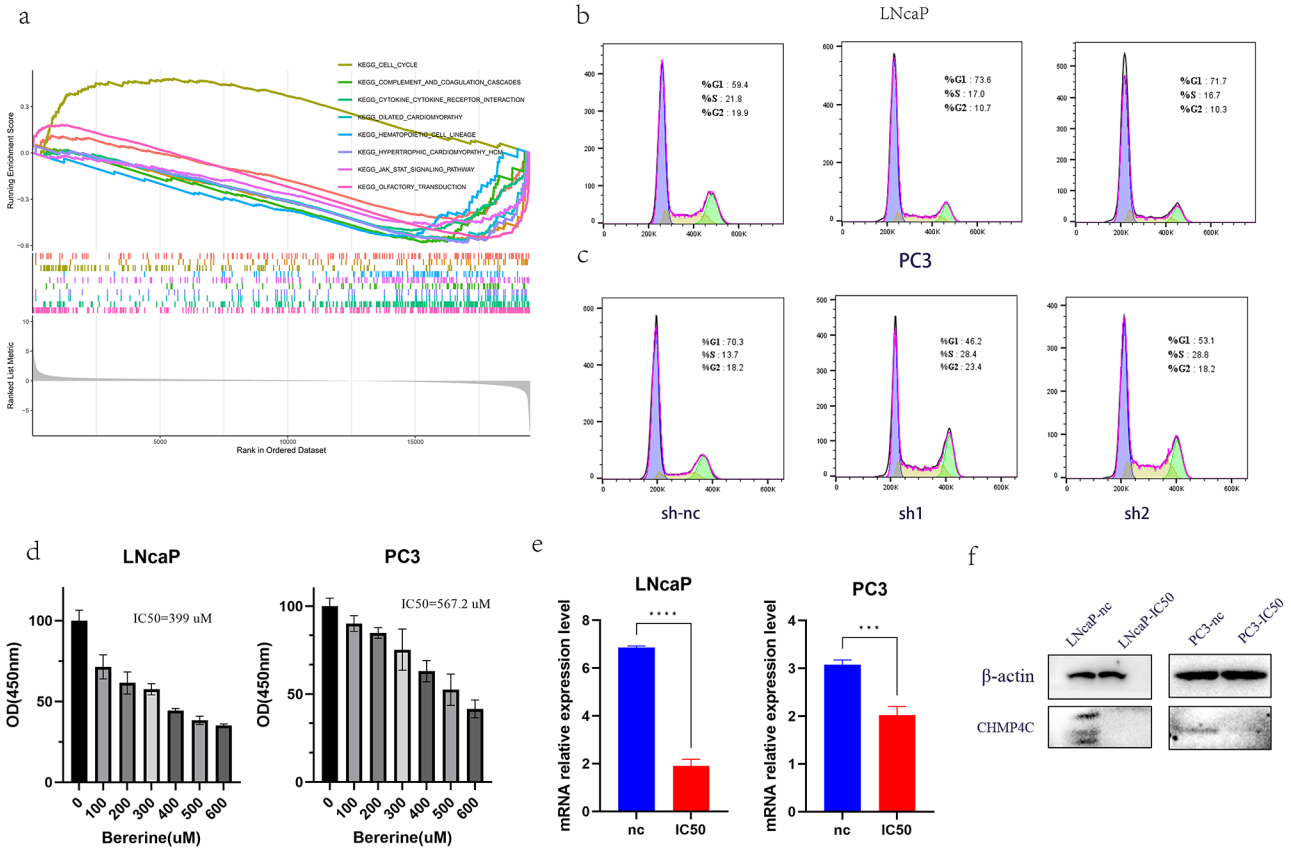

Fig. 4 The regulatory influence of CHMP4C on the cell cycle function in PCa cells. (**a**) GSEA functional analysis indicated the involvement of CHMP4C in the cell cycle of PCa. (**b**, **c**) Flow cytometry was utilized to assess alterations in the cell cycle of LNcaP and PC3 cells subsequent to CHMP4C knockdown. (**d**) The CCK-8 assay was used to determine the IC50 of LNcaP and PC3 cells treated with berberine for 24 h. (**e**, **f**) After treating LNcaP and PC3 cells with berberine at the IC50 concentration for a duration of 24 h, the mRNA and protein expression levels of CHMP4C were evaluated using qRT-PCR and Western blotting in LNcaP and PC3 cells

collective dataset from all cohorts consistently indicated an upregulation of CHMP4C in PCa. To substantiate its validity, we proceeded to validate the elevated expression of CHMP4C in comparison to RWPE-1 across PCa cell lines (LNCaP, PC3, and DU145) through qRT-PCR assays at the RNA level, and then we corroborated the increased CHMP4C expression in LNCaP and PC3 cells at the protein level using Western blotting experiments. Subsequently, immunohistochemistry data consistently demonstrated increased CHMP4C expression in PCa tissue relative to normal prostate tissue. To investigate the clinical significance, several studies have shown that patients with PSA<10 ng/ml and Gleason score<=7 are considered low-risk for PCa [46]. In addition, according to the recommendations of the American Joint Committee on Cancer pathological TNM classification of PCa, T2 stage tumors are limited to the prostate, while T3/4 stage tumors begin to invade tissues outside the prostate. Compared with stage N0 tumors, stage NI tumors have metastasized to lymph nodes. M1 stage tumors have distant metastasis relative to M0 stage tumors [47]. Therefore, the presence of stage T3/4, N1, and M1 PCa indicates tumor progression or metastasis, potentially leading to a poor prognosis for patients. Our results showed a significant up-regulation of CHMP4C expression in patients who were over 60 years old, those with stage T3/4 and stage N1, patients with a PSA level of >=10 ug/ml, and those with a Gleason score>7. Furthermore, patients with heightened CHMP4C expression exhibited a worse prognosis. These findings suggest that CHMP4C may play a crucial role in the progression and prognosis of PCa. Based on CHMP4C and the aforementioned clinicopathological characteristics, we constructed a prognostic nomogram for PCa that demonstrated commendable predictive efficacy. Moreover, our data revealed a substantial augmentation induced by CHMP4C in the proliferation, migration, and invasion capabilities of PCa cells (LNCaP and PC3). Through GSEA functional analysis, it was found that CHMP4C was related to the cell cycle of PCa. Additionally, flow cytometry indicated that CHMP4C could potentially regulate cellular processes by inducing changes within the G1/S/G2 phases of PCa cells. Additionally, when considering previous research, we observed similarities between the regulatory effects

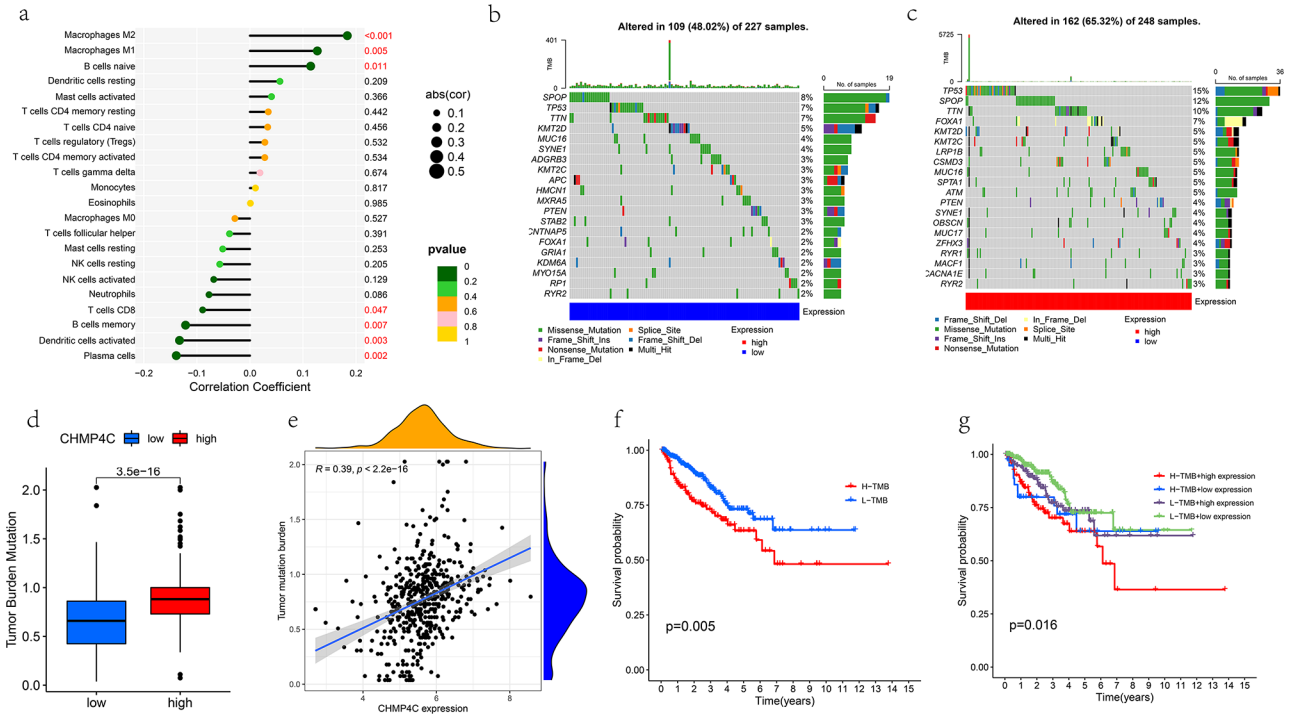

Fig. 5 The relationship between CHMP4C and the tumor microenvironment in PCa using the TCGA database. (**a**) The relationship between CHMP4C expression and the infiltration patterns of 22 different immune cell types in PCa. (**b**, **c**) The distribution of mutations in the top 20 genes with the most frequent mutations in the CHMP4C high-expression and low-expression groups. (**d**, **e**) The relationship between the expression of CHMP4C and the TMB score in PCa. (**f**) Kaplan-Meier survival analysis was performed on patients categorized into high and low TMB score groups. (**g**) Kaplan-Meier survival analysis was conducted by integrating both CHMP4C expression and TMB score

of berberine on PCa cells and those of CHMP4C. Consequently, this prompted us to undertake further confirmation of the potential regulatory role of berberine in modulating CHMP4C expression within the PCa context. These may bring new opportunities for the clinical treatment of PCa.

Nowadays, the exploration of the tumor microenvironment has garnered escalating attention within the scientific community. For exploring the relationship between CHMP4C and PCa tumor microenvironment, our investigation revealed that CHMP4C expression exhibits a marked positive correlation with infiltration of Macrophages M2, Macrophages M1, and naive B cells, while displaying a significant negative correlation with infiltration of CD8 T cells, memory B cells, activated dendritic cells, and plasma cells. Prior research has demonstrated that cancer stem cells may utilize M1 macrophages as a safeguarding barrier in the immune evasion mechanism [48]. M2 macrophages have the potential to facilitate the growth of tumors via immunosuppression, angiogenesis, and activation and restructuring of the matrix [49]. CD8 T cells are crucial in anti-tumor immune responses due to their effector memory T cell properties and metabolic regulatory functions [50]. Dendritic cells possess significant potential for facilitating efficacious anti-tumor immunity [51]. Tumor-infiltrating B lymphocytes comprise various subtypes, such as naive, activated, and memory B cells, germinal center B cells, plasma cells, and their transitional stages. These elements facilitate anti-tumor immunity through mechanisms including complement-dependent cytotoxicity, antibody-dependent cytotoxicity, and antibody-dependent cellular phagocytosis [52]. However, their potential to enhance tumor growth through immune complex formation, promotion of chronic inflammation, induction of angiogenesis, and immunosuppression cannot be overlooked [53]. Additionally, our results indicated an increased occurrence of genetic mutations in patients displaying high CHMP4C expression. Notably, there is a statistically significant positive correlation between the TMB score and the expression of CHMP4C. In the absence of immunotherapy, patients with high TMB and those with high TMB and high CHMP4C expression exhibited a poorer prognosis. Research suggests that an elevated TMB may increase the amount of immunogenic neoantigens, leading to a potential enhancement of the response of tumors to immunotherapy [54]. However, some studies demonstrate no positive correlation between neoantigen load in PCa and CD8 T cell infiltration, which could impact the predictive accuracy of predicting immune checkpoint blockade responses [55]. For the immunotherapy of PCa, it is important to take a comprehensive approach to the

tumor microenvironment and not rely on a single predictive indicator. Therefore, considering the correlation between CHMP4C and immune cell infiltration as well as tumor gene mutations, this could potentially bring new insights into the immunotherapy of PCa.

Our study aims to explore the influence of CHMP4C on the occurrence, development, and prognosis of PCa and to provide new insights into the health management of PCa. Nonetheless, our study does entail certain limitations. We require further clinical data, pathological specimens, and animal experiments to confirm the involvement of CHMP4C in the development and progression of PCa. Simultaneously, the study of the mechanism by which berberine regulates CHMP4C is obviously something we should continue in the next phase of research. In addition, although our results suggest that CHMP4C may promote PCa cell proliferation by regulating the cell cycle, more research is needed on cell cycle-related regulatory pathways such as the mTOR pathway. Finally, further investigation is required to determine the precise role of CHMP4C in PCa immunotherapy.

Conclusion

In summary, this study employed a combination of bioinformatics and in vitro experiments to scrutinize the expression of CHMP4C in PCa and to confirm that CHMP4C can promote the proliferation, migration, and invasion of PCa cells. Simultaneously, CHMP4C may affect the cell cycle in PCa, and the regulation of CHMP4C expression may be affected by berberine. Additionally, CHMP4C exhibits a favorable prognostic effect in PCa and may provide new insights into precision therapy and immunotherapy for PCa.

Abbreviations

PCa	Prostate cancer
CHMP4C	Charged multivesicular body protein 4 C
GSEA	Gene set enrichment analysis
qRT-PCR	Quantitative real-time polymerase chain reaction
CCK-8	Cell counting kit-8
ESCRT-III	Endosomal sorting complex required for transporter III
TCGA	The cancer genome atlas
GEO	Gene Expression Omnibus
HPA	Human Protein Atlas
ROC	Receiver Operating Characteristic
SNV	Single nucleotide variant
TMB	Tumor mutation burden

Supplementary Information

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Supplementary Material 1

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Not applicable.

Author contributions

The study's conception and design were performed by Xi Xiao and Jun Mi. Material preparation, data collection, and analysis were performed by Xi Xiao, Zonglin Li, Qingchao Li, Liangliang Qing, Yanan Wang, Fuxiang Ye, Yajia Dong and Xiaoyu Di. The first draft of the manuscript was written by Xi Xiao, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Xi Xiao, Zonglin Li, and Qingchao Li contributed equally to this article.

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Data availability

The datasets generated and/or analyzed during the current study are available in the TCGA database (https://portal.gdc.cancer.gov/), the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), and the Human Protein Atlas (HPA) database (https://www.proteinatlas.org/).

Declarations

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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