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Increased GPR35 expression is correlated with poor prognosis in prostate cancer

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Abstract

Background G-protein-coupled receptor 35 (GPR35) has been reported to be overexpressed in several types of human cancers, playing essential roles in tumorigenesis and development. However, its expression and prognostic value in Prostate cancer (PCa) remain unclear. This study aims to investigate the expression of GPR35 and its prognostic value in PCa.

Methods The expression of GPR35 was analyzed using the public database and validated by immunohistochemistry (IHC) in PCa tissues. Subsequently, the correlation between GPR35 expression and the clinical characteristics was evaluated using the Chi-squared test. Kaplan-Meier and Cox proportional hazards regression models were used to analyze the data. Hazard Ratios (HR) and 95% confidence intervals (CI) were calculated for each factor.

Results GPR35 messenger RNA (mRNA) and protein expression were confirmed to be overexpressed in PCa tissue samples. Furthermore, high GPR35 mRNA expression was correlated with clinical tumor stage (T stage) ($P<0.001$), lymph node metastasis ($P<0.001$), primary therapy outcome ($P=0.009$), residual tumor ($P<0.001$), prostate-specific antigen (PSA) levels ($P=0.004$), and Gleason score ($P<0.001$). IHC analysis also confirmed that GPR35 overexpression was associated with lymph node metastasis ($P=0.010$). Additionally, Kaplan-Meier analysis showed that PCa patients with high expression of GPR35 were associated with shorter overall survival (OS) (HR: 3.370, 95% CI: 1.085–10.470, $P=0.047$), progress free interval (PFI) (HR: 3.385, 95% CI: 2.234–5.131, $P<0.001$), and biochemical relapse time (BCR) (HR: 2.229, 95% CI: 1.308–3.801, $P=0.007$). Moreover, univariate Cox regression analyses suggested that T stage ($P<0.001$), lymph node involvement ($P=0.046$), serum PSA levels ($P=0.013$), Gleason score ($P<0.001$), and GPR35 expression ($P<0.001$) were unfavorable prognostic factors for PCA patients. Multivariate Cox regression analysis showed that GPR35 was an independent poor prognostic factor of PCa patients (HR: 1.915, 95%CI: 1.368–2.682).

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Conclusion Overexpression of GPR35 is associated with poor clinical prognosis, suggesting that GPR35 may serve as a potential prognostic biomarker for PCa.

Clinical trial number Not applicable.

Keywords GPR35, Prostate cancer, Prognosis, Biomarkers, Prostate

Introduction

Prostate cancer (PCa) is one of the most common malignant tumors of the male genitourinary system. The 2020 incidence of PCa was 1,414,259, and mortality was 375,304 [1]. These trends continue to rise, as there were 1,276,106 cases and 358,989 deaths reported in 2018 [2]. In China, the aging population and rapid demographic growth have contributed to a significant rise in new prostate cancer cases, with the incidence rate increasing from 7.10 per 100,000 in 2011 to 18.61 per 100,000 in 2022 [3, 4]. Despite the significant progress of clinical treatments such as Androgen Deprivation Therapy and Androgen Receptor Signalling Inhibitors [5], the prognosis for advanced PCa remains poor [6–8], causing substantial harm to patients. Prostate-specific antigen (PSA) remains the most widely used tumor marker for early screening and diagnosis of PCa. However, PSA has significant limitations in assessing the clinical prognosis of patients [9]. Therefore, there is a critical need to identify novel biomarkers for evaluating disease prognosis, dynamically monitoring tumor progression, and guiding effective, individualized treatment for PCa, in order to overcome the limitations of PSA testing.

Given this unmet clinical need, the identification of novel biomarkers that regulate tumor progression is crucial. Among the potential candidates, G protein-coupled receptors (GPCRs)—the most prominent family of transmembrane protein receptors in the human body—have emerged as promising therapeutic targets due to their central role in cell signaling [10]. Notably, G-protein-coupled receptor 35 (GPR35), a member of the GPCR family, is particularly recognized for its oncogenic roles. It is highly expressed in various cancer tissues, where it plays a key role in tumor cell proliferation and metastasis [11, 12]. Mackiewicz et al. [13, 14] found that GPR35 is highly expressed in colon cancer, and its elevated expression is associated with poorer survival prognosis. Similar studies have shown that the CXCL17-CXCR8 (GPR35) axis plays a critical role in breast cancer, where elevated expression of CXCL17 is linked to poor survival prognosis and promotes the proliferation and migration of breast cancer cells [15]. Furthermore, Yue et al. [16] discovered through a mouse lung adenocarcinoma model that GPR35 knockout resulted in significantly smaller tumors compared to wild-type mice, and reduced expression of GPR35 correlated with better overall survival (OS) and progression free survival (PFS). However, to the best of our knowledge, the expression and biological role of GPR35 in PCa have not been explored previously.

This study aims to address this gap by investigating the potential of GPR35 as a prognostic marker in PCa. Using data from The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO), we analyzed the differential expression of GPR35 mRNA in PCa tissues compared to normal prostate tissues. Immunohistochemistry (IHC) was employed to assess GPR35 expression in PCa, adjacent prostate tissues, and benign prostate hyperplasia (BPH). Survival analyses were conducted to examine the association between GPR35 expression and clinical outcomes, including OS, progression free interval (PFI), and biochemical relapse time (BCR). Additionally, bioinformatic analyses were performed to explore the potential mechanistic pathways underlying the role of GPR35 in PCa.

Materials and methods

Clinical sample collection

A total of 25 tissue samples from PCa cases, including 10 paired adjacent (normal prostate) samples and 20 BPH cases, were collected between December 2021 and July 2022. The inclusion criteria were as follows: patients diagnosed with PCa for the first time at our institution, confirmed by pathological biopsy, and with complete serum and imaging data available. The exclusion criteria were as follows: (1) patients with a history of other malignant tumors; (2) patients with a history of prostate surgery; (3) patients who had undergone surgery, radiotherapy, chemotherapy, or androgen deprivation before participation in this study; (4) patients with severe organic diseases (such as heart failure, cirrhosis, end-stage renal disease, etc.) or other chronic conditions that could interfere with the study outcomes. All tissue samples were preserved in formalin for immunohistochemical experiments. The clinical parameters, including age, clinical stage, Gleason score, tumor size, lymph node metastasis, bladder or seminal vesicle infiltration, and extra-prostate metastases, were collected from the samples. The study population consisted solely of Chinese individuals.

Bioinformatics analysis

The gene expression profiles of GPR35 were retrieved from the TCGA database (https://www.cancer.gov/tcga). The RNA-seq expression of GPR35 in the PCa cohort ($n = 499$) and healthy controls ($n = 52$) was analyzed

using the GEPIA online tool (http://gepia.cancer-pku.cn/index.html). The racial distribution of the TCGA cohort included 12 (2.40%) Asian, 57 (11.42%) Black or African American, and 415 (83.17%) White patients. Additionally, the GSE3325 dataset was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/geo/) to validate the expression of GPR35 mRNA. The OS and PFI times were retrieved from the TCGA database, and the BCR data were obtained from the GEO (GSE54460) database to analyze the associations between GPR35 expression at the mRNA level and patients' prognostic outcomes. GSE3325 consists of 13 PCa samples and 6 paired healthy control samples from patients. GSE54460 includes 106 PCa samples, comprising 22 (20.75%) Black and 48 (45.28%) White patients. Meanwhile, screening for possible independent prognostic factors in patients was conducted to explore the role of GPR35 in PCa.

IHC staining and evaluation of staining

To observe the expression of GPR35 at the protein level, we conducted IHC for our study. IHC testing was carried out using an IHC assay kit (Fuzhou Maixin Biotechnology Company Limited, Fuzhou, China) according to the manufacturer's instructions. Paraffin-embedded slides were deparaffinized in xylene and rehydrated in 100%, 95%, 80%, and 60% ethanol, and then washed with Phosphate-Buffered Saline (PBS). Antigen retrieval was performed by microwave pretreatment in citrate antigen retrieval solution for 15 min until the water temperature cooled to 25–35 °C. Endogenous peroxidase activity was then blocked, followed by serum blocking, and the slides were incubated with primary antibodies against GPR35 (1:500; ab150635 from Abcam, Rabbit polyclonal, Cambridge, MA, USA) overnight at 4 °C. After washing with PBS, the slides were incubated with secondary antibody working buffer at 37 °C for 10 min, followed by incubation with streptavidin-peroxidase buffer for 15 min. Finally, visualization was performed using 3,3'-Diaminobenzidine (DAB) staining, followed by counterstaining with hematoxylin at room temperature, dehydration, and mounting. Negative controls were stained in parallel, with PBS used instead of the primary antibody. All samples were processed simultaneously to avoid errors arising from measurements taken at different times.

The semi-quantitative scoring system was used to assess the expression of GPR35 at the protein level based on the intensity score and percentage of positive cells. The percentage of stained cells was scored using a four-tier system (0, no stained cells; 1, 1-25%; 2, 26-50%; 3, 51-75%; 4, 76-100%). The staining intensity of immunohistochemical scores was classified as follows: 0 (no staining), 1 (light yellow), 2 (brown), and 3 (dark brown). The final immunostaining score was determined by multiplying the intensity score by the percentage of positive cells. The low and high levels of GPR35 were classified as follows: a score < 4 was considered negative, and a score ≥ 4 was considered positive. The IHC results were evaluated blindly by two experienced pathologists. In cases where different scores were obtained, the two pathologists repeated the IHC scoring process until consensus was reached.

Biological functions of GPR35 in PCa

We downloaded the TCGA-PRAD mRNA expression profiles from the University of California, Santa Cruz (UCSC) Xena data portal (https://xenabrowser.net/), which grants access to TCGA data. Subsequently, gene expression profiles were converted from fragments per kilobase of transcript per million fragments mapped (FPKM) to transcripts per million (TPM), followed by log2(TPM + 1) transformation and gene filtering.

In addition, we calculated the correlation coefficients between GPR35 and all other mRNAs in the prostate cancer mRNA expression profile using the Pearson correlation test. We submitted the gene list with correlation coefficients having absolute values > 0.3 to the DAVID database (https://david.ncifcrf.gov/) for online analysis. Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were subsequently performed to investigate the functional mechanisms of GPR35 (only pathway results with $P < 0.05$ were retained). Finally, we used the ggplot2 package in R (version 3.6.1) to visualize the p-values of the enrichment results in ranked order. Additionally, we constructed a protein-protein interaction (PPI) network using the STRING (http://string-db.org) database and set the required interaction score threshold at > 0.4 for analysis.

Statistical analysis

Statistical analyses were performed using SPSS 25.0 (IBM SPSS, Chicago, IL, USA) and R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). The Chi-square test was used to detect associations between GPR35 expression and clinicopathological parameters. The Kaplan-Meier method and Cox regression hazard tests were applied for survival analysis, and hazard ratios (HR) with 95% confidence intervals (CIs) were calculated. $P < 0.05$ was considered statistically significant.

Results

GPR35 expression was significantly upregulated in PCa

To explore the potential roles of GPR35 in PCa, we used the TCGA database to analyze GPR35 mRNA expression and validated the results using GEO datasets. Both analyses showed that GPR35 mRNA expression was significantly higher in the PCa group than in healthy controls ($P < 0.001$ and $P < 0.01$, Fig. 1A and B). Furthermore,

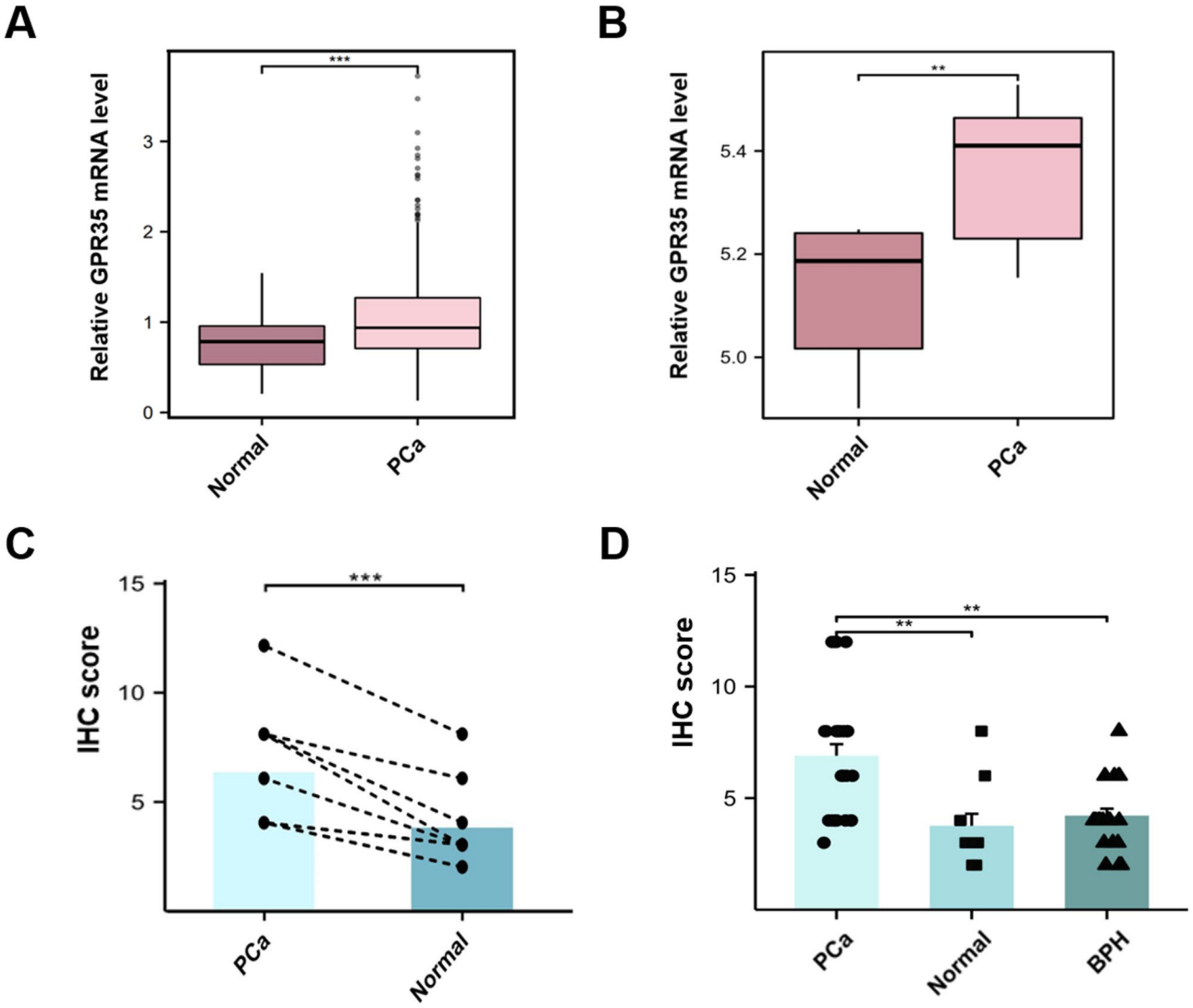

Fig. 1 GPR35 expression was upregulated in PCa tissues. (**A**) Relative GPR35 mRNA expression in PCa patient tissues versus normal samples from the TCGA database. (**B**) Relative GPR35 mRNA expression in PCa patient tissues versus normal samples in GSE3325 from the GEO database. (**C**) GPR35 protein expression in PCa versus paired adjacent normal tissues was detected by IHC. (**D**) Comparison of GPR35 protein expression levels in PCa, adjacent normal tissues, and BPH tissues by IHC. *$P<0.05$, **$P<0.01$, ***$P<0.001$

we confirmed the protein expression of GPR35 in PCa tissues, BPH tissues, and normal tissues using immunohistochemical staining. IHC results indicated that GPR35 protein expression levels were significantly upregulated in PCa tissues compared to BPH tissues and normal prostate tissues ($P<0.01$, Fig. 1C and D). Additionally, GPR35 protein expression was primarily localized to the cell membrane and cytoplasm of cancer cells (Fig. 2).

GPR35 expression is correlated with clinical progression in PCa patients

Clinical data from PCa patients are available in the TCGA database to explore associations between GPR35 mRNA expression and various clinicopathological parameters. As summarized in Table 1; Fig. 3, we found that GPR35mRNA expression in PCa was significantly associated with clinical tumor stage (T stage) ($P<0.001$), lymph node metastasis (N stage) ($P<0.001$), primary therapy outcome ($P=0.009$), residual tumor ($P<0.001$), PSA levels ($P=0.004$) and Gleason score ($P<0.001$). There was no significant association between GPR35 mRNA expression with other clinicopathological features, such as age, race, zone of origin, and metastasis stage ($P>0.05$). Moreover, based on clinical data from a small local sample of patients and immunohistochemical findings, as shown in Table 2; Fig. 4, we found that high GPR35 protein expression was significantly correlated with lymph node metastasis ($P=0.010$). However, GPR35 protein expression was not associated with other clinicopathological features, including T stage, metastasis

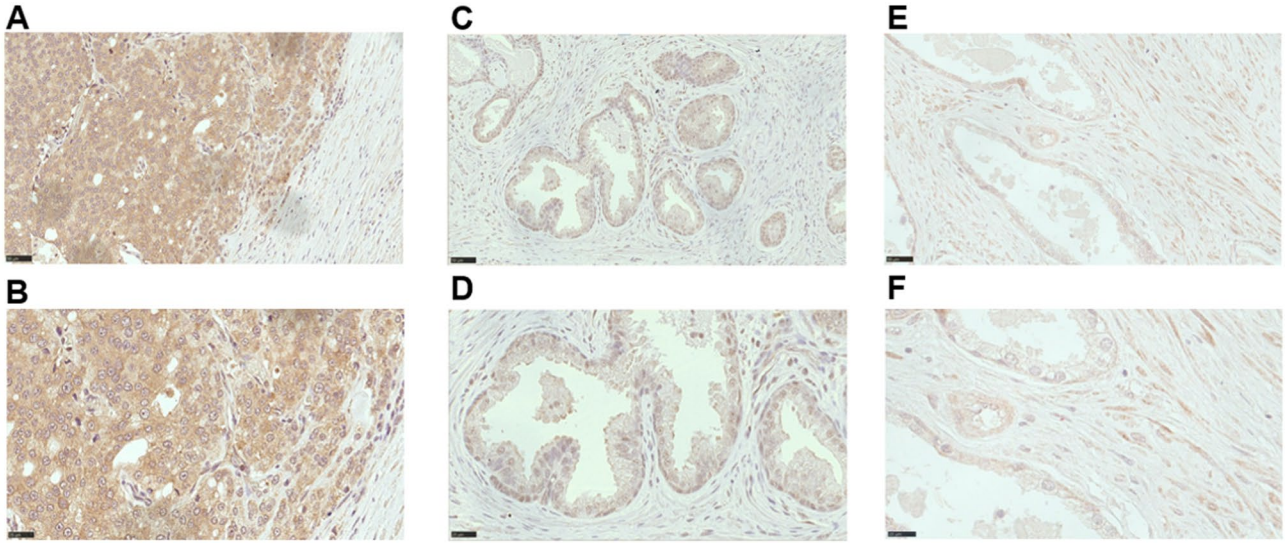

Fig. 2 Immunostaining of GPR35 in PCa, adjacent normal, and BPH tissues. (**A** and **B**) Immunostaining showed strong positive GPR35 expression in PCa. (**C** and **D**) Immunostaining showed moderate positive GPR35 expression in BPH tissues. (**E** and **F**) Representative images of adjacent normal prostate tissue showing low levels of GPR35 expression. Magnification, ×100 and ×200

stage (M stage), clinical stage, tumor size, Gleason score, bladder or seminal vesicle infiltration, and extra-prostate metastases ($P > 0.05$).

Overexpression of GPR35 is associated with poor prognosis of PCa

To investigate whether GPR35 expression levels are associated with OS, PFI, and BCR in prostate cancer patients, we used Kaplan-Meier curves to analyze the correlation between GPR35 expression and the prognosis of PCa patients in the TCGA dataset. As shown in Fig. 5, we found PCa patients with high expression of GPR35 had shorter OS (HR: 3.370, 95% CI: 1.085–10.470, $P = 0.047$), PFI (HR: 3.385, 95% CI: 2.234–5.131, $P < 0.001$), and BCR (HR: 2.229, 95% CI: 1.308–3.801, $P = 0.007$) compared to those with downregulated GPR35 expression. Furthermore, as shown in Table 3, high GPR35 expression was significantly associated with a higher incidence of BCR, confirming the survival analysis result and further supporting its prognostic value in prostate cancer.

GPR35 as an independent prognostic marker in PCa patients

We further employed Cox proportional hazards modeling to assess whether GPR35 was an independent prognostic predictor of survival in PCa patients in the TCGA database. Univariate analyses indicated that T stage ($P < 0.001$), lymph node involvement ($P = 0.046$), serum PSA levels ($P = 0.013$), Gleason score ($P < 0.001$), and GPR35 expression ($P < 0.001$) were unfavorable prognostic factors for PCa patients (Table 4). Multivariate Cox regression models further revealed that high GPR35 expression was a significant independent poor prognostic factor for patients with PCa (HR: 1.915, 95% CI: 1.368–2.682, $P < 0.001$, Table 4).

Biological functions of GPR35 in PCa

Genes with absolute correlation coefficients > 0.3 with GPR35 in PCa were identified through correlation analysis. A total of 1,343 genes showed a positive correlation, while 141 genes were negatively correlated (Fig. 6A). GPR35 and the associated genes were submitted to DAVID for online analysis, and GO enrichment analysis as well as KEGG pathway analysis of GPR35 in PCa were performed. GO enrichment analysis was conducted in three categories: biological process (BP), cellular component (CC), and molecular function (MF). Figure 6B displays the top five results of the GO enrichment analysis. GPR35 was enriched in the following: (i) CCs: cytoplasm, nuclear speck, and centriole; (ii) MFs: protein binding, SNARE binding, and voltage-gated calcium channel activity; and (iii) BPs: DNA repair, T cell receptor signaling pathway, and T cell activation. KEGG was also utilized to identify key pathways associated with GPR35 in PCa. The results suggested that GPR35 was associated with cell metabolism-related pathways (beta-Alanine metabolism, Histidine metabolism, Ether lipid metabolism), cancer-related pathways (PD-L1 expression and PD-1 checkpoint pathway in cancer), immune-related pathways (Primary immunodeficiency, Th1 and Th2 cell differentiation and T cell receptor signaling pathway), cell cycle-related pathways (Oocyte meiosis), and GnRH secretion (Fig. 6C). Additionally, Fig. 6D displays a protein interaction map of the GPR35 protein.

The mutation frequency (Top 10) of high and low expression groups was analyzed using the R package

Table 1 Association of GPR35 mRNA expression with clinicopathologic features of 499 PCa patients

Characteristic	Low expression of GPR35	High expression of GPR35	*P*
n	249	250	
T stage, n (%)			< 0.001
T2	115 (23.4)	74 (15.0)	
T3	128 (26.0)	164 (33.3)	
T4	4 (0.8)	7 (1.4)	
N stage, n (%)			< 0.001
N0	189 (44.4)	158 (37.1)	
N1	18 (4.2)	61 (14.3)	
M stage, n (%)			0.249
M0	223 (48.7)	232 (50.7)	
M1	0 (0)	3 (0.7)	
Primary therapy outcome, n (%)			0.009
PD	12 (3.0)	16 (4.0)	
SD	8 (2.0)	18 (4.5)	
PR	13 (3.2)	21 (5.3)	
CR	179 (44.8)	133 (33.3)	
Race, n (%)			0.576
Asian	5 (1.0)	7 (1.4)	
Black or African American	26 (5.4)	31 (6.4)	
White	214 (44.2)	201 (41.5)	
Age, n (%)			0.054
≥ 60	123 (24.6)	101 (20.2)	
> 60	126 (25.3)	149 (29.9)	
Residual tumor, n (%)			< 0.001
R0	175 (37.4)	140 (29.9)	
R1	55 (11.8)	93 (19.9)	
R2	3 (0.6)	2 (0.4)	
Zone of origin, n (%)			0.697
Central Zone	1 (0.4)	3 (1.1)	
Overlapping / Multiple Zones	49 (17.8)	77 (28)	
Peripheral Zone	47 (17.1)	90 (32.7)	
Transition Zone	4 (1.5)	4 (1.5)	
PSA (ng/ml), n (%)			0.004
< 4	220 (49.8)	195 (44.1)	
≥ 4	6 (1.4)	21 (4.8)	
Gleason score, n (%)			< 0.001
6	25 (5.0)	21 (4.2)	
7	148 (29.7)	99 (19.8)	
8	26 (5.2)	38 (7.6)	
9	49 (9.8)	89 (17.8)	
10	1 (0.2)	3 (0.6)	
Age, mean ± SD	60.14 ± 6.89	61.91 ± 6.65	0.004

maftools, and the tumor mutation burden (TMB) for each prostate cancer sample was calculated (Fig. 7A-B). Correlation analysis between GPR35 expression and TMB was performed using Spearman's correlation, revealing a significant positive correlation (Fig. 7C). The optimal cut-off value for TMB was determined using the R package survminer, dividing the samples into high TMB and low TMB groups. It was found that patients in the low TMB group had significantly better survival rates than those in the high TMB group (Fig. 7D). Similarly, patients with low-risk scores and low TMB also exhibited better prognosis than the other three groups (Fig. 7E).

Discussion

One of the challenges in clinical PCa management is the lack of reliable prognostic biomarkers [17]. Although the PSA test has significantly improved the rate of early diagnosis of PCa [18, 19], it still falls short in meet the clinical need in terms of predicting clinical outcomes [20, 21]. Moreover, an increasing number of researchers have explored prognostic markers for PCa, such as PSA derivatives and prostate health index (PHI) [21–23]. However, these markers are imperfect and yield only modest efficacy. Therefore, there is an urgent need to identify novel biomarkers to assist physicians in evaluating disease prognosis. This study aimed to investigate the expression and clinical significance of GPR35 in PCa. This study is the first to explore the clinical significance of GPR35 in prostate cancer by integrating the TCGA and GSE datasets, along with a small-scale local validation, thereby addressing a gap in this field. Compared to single-cohort studies, the integration of multi-center data effectively mitigates platform biases, thereby enhancing the reliability and broader applicability of the findings. Although no direct studies have yet investigated the role of GPR35 in prostate cancer, we hope that this pioneering work will lay a solid foundation for future research on the role of GPR35 in prostate cancer and other cancer types.

In the present study, we found that both GPR35 mRNA and protein levels were upregulated in PCa tissues. Similarly, earlier studies have reported that GPR35 is overexpressed in various human cancers, such as colon cancer [13, 14, 24, 25], pancreatic cancer [14], breast cancer [15], non-small-cell lung cancer (NSCLC) [26], gastric cancer [27, 28] and endometrial cancer [29], where it promotes tumor growth and development [12]. These studies suggest that GPR35 is overexpressed in a wide range of tumors, but does not appear to have a strong tissue or organ specificity. Therefore, it may serve as a universal oncogene. In addition, we confirmed a trend showing that the expression of GPR35 protein was upregulated in the order of normal tissue, BPH, and PCa, indicating that GPR35 may play an important role in the development of prostate cancer.

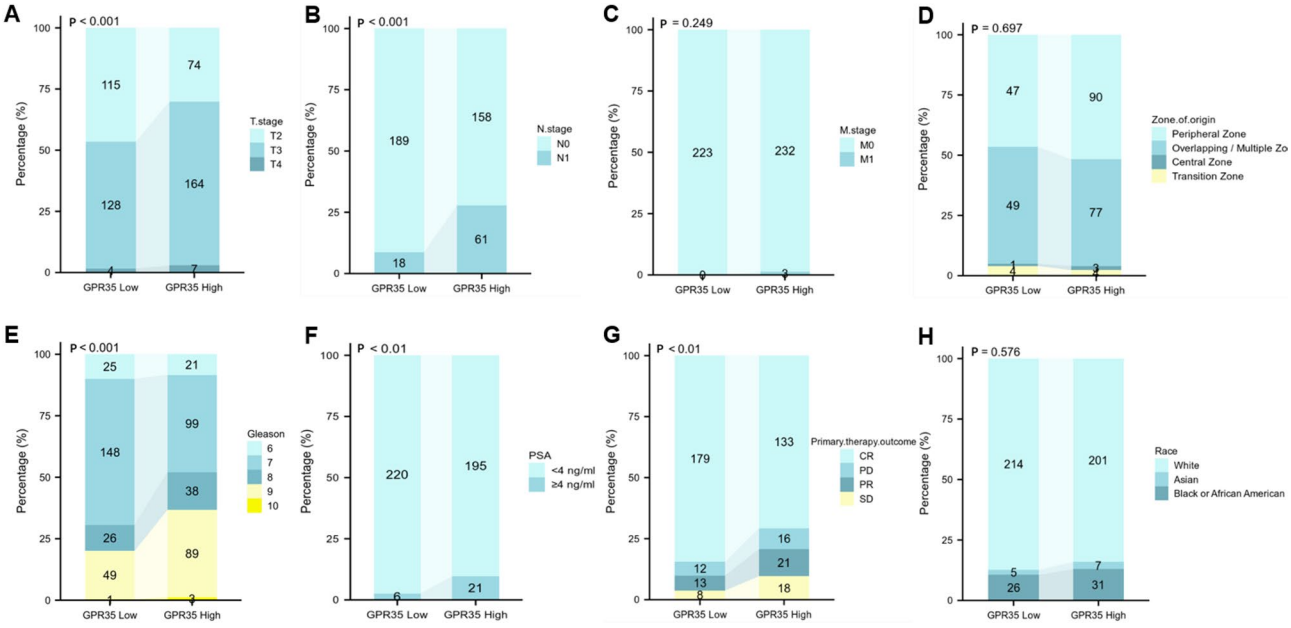

Fig. 3 Association of GPR35 mRNA expression with clinicopathologic features of 499 PCa patients in TCGA. Panels A-H display the distribution of GPR35 expression levels across various clinical variables, including T stage (**A**), N stage (**B**), M stage (**C**), zone of origin (**D**), Gleason score (**E**), PSA levels (**F**), primary therapy outcome (**G**), and race (**H**)

GPR35 is likely involved in the onset and progression of PCa. Our study found that high GPR35 mRNA expression was significantly correlated with clinical T stage, lymph node metastasis, primary therapy outcome, residual tumor, PSA levels, and Gleason score. More importantly, our immunohistochemical experiments revealed that GPR35 expression was associated with lymph node metastasis. However, while other pathological parameters did not show a significant correlation in the IHC experiments, this may be attributed to the small sample size. Nonetheless, existing cross-cancer studies have demonstrated the broader applicability of similar results. High expression of GPR35 has been reported to be associated with gender, regional lymph node involvement, T stage, and tumor histological grade in pancreatic cancer patients [14]. Similar results have also been observed in colorectal cancer, with evidence suggesting correlations between the expression of GPR35 and American Joint Committee on Cancer (AJCC) staging, T-staging, and tumor histological grading [14]. Additionally, GPR35 expression was found to be associated with high Ki-67 expression [15], suggesting a potential correlation with high histological grade and proliferative activity. In gastric cancer, GPR35 expression levels correlate with patient age and tumor tissue type [27]. These findings consistently indicate that GPR35 overexpression is closely related to the progression of various cancers, underscoring its significant value as a potential clinical tumor marker across multiple organs. Recent studies suggest that GPR35, as a potential oncogene, promotes tumor growth and metastasis [30]. The oncogenic effect of GPR35 may be mediated through the following mechanisms: a more recent report demonstrated potential molecular pathways of GPR35 in promoting tumor progression, including the activation of GPR35 increased Na/K-ATPase activity, triggering the kinase Src, Erk, and Akt activation, thereby promoting glycolysis, cell proliferation, and oncogenic signaling [31]. Furthermore, the activation of GPR35 promotes tumor progression primarily by enhancing epithelial cell proliferation and influencing the tumor microenvironment through the coordination of macrophages in spontaneous and colitis-associated colon cancer [32, 33]. Additionally, in lung cancer, GPR35 activation promoted the development of the tumor by increasing the production of IL-5 and IL-13, thereby facilitating the formation of the ILC2-MDSC axis [16]. Additionally, high GPR35 expression in NSCLC is also associated with activation of

Table 2 Clinicopathologic variables and GPR35 protein expression in PCa patients

Characteristic	Low expression of GPR35	High expression of GPR35	*P*
n	9	16	
GPR35, median (IQR)	4(4, 4)	8(7.5, 9)	< 0.001
T stage, n (%)			0.373
T1	1(4.0)	3(12.0)	
T2	2(8.0)	7(28.0)	
T3	6(24.0)	6(24.0)	
N stage, n (%)			0.010
N0	9(36.0)	8(32.0)	
N1	0(0)	8(32.0)	
M stage, n (%)			0.444
M0	9(36.0)	15(60.0)	
M1	0(0)	1(4.0)	
TNM, n (%)			0.432
I-II	2(8.0)	6(24.0)	
III-V	7(28.0)	10(40.0)	
tumor size, n (%)			0.405
< 1.5 cm	8(36.0)	12(48.0)	
≥ 1.5 cm	1(4.0)	4(16.0)	
extra-prostate metastases, n (%)			0.166
Presence	0(0)	3(12.0)	
Absence	9(36.0)	13(52.0)	
bladders or seminal vesicle infiltration, n (%)			0.835
Presence	2(8.0)	3(12.0)	
Absence	7(28.0)	13(52.0)	
vascular invasion, n (%)			0.317
Presence	4(16.0)	4(16.0)	
Absence	5(20.0)	12(48.0)	
Perineural invasion, n (%)			0.915
Presence	6(24.0)	11(44.0)	
Absence	3(12.0)	5(20.0)	
Gleason score, n (%)			0.509
< 8	5(20.0)	11(44.0)	
≥ 8	4(16.0)	5(20.0)	

the β-arrestin-pAkt signaling pathway, which may lead to chemotherapy resistance [26]. This suggests that GPR35 not only predicts the prognosis of diseases other than PCa but may also serve as a potential therapeutic target for PCa patients in clinical applications. Therefore, while the precise biological mechanisms of GPR35 in prostate cancer remain to be fully elucidated, the accumulating evidence supports its role as a promising prognostic marker and potential therapeutic target, highlighting the need for further investigation into its clinical applicability in PCa management.

Our study provides evidence that GPR35 is a reliable biomarker for the prognosis of PCa. We found that high GPR35 expression was correlated with OS, PFI, and BCR in PCa patients by Kaplan-Meier survival analysis, which indicates that patients in the group with overexpression of GPR35 had shorter survival times and worse prognosis. Furthermore, multivariate Cox regression analysis demonstrated that GPR35 is an independent poor prognostic factor for prostate cancer patients (HR: 1.915, 95% CI: 1.368–2.682), with a superior HR compared to PSA, as reported in the study by Hwang et al. (HR: 1.774, 95% CI: 1.673–1.881) [34]. Although PSA is widely used for screening and early diagnosis, its limitations in monitoring disease progression and assessing prognosis are well documented [9, 20, 21]. In contrast, GPR35, as an emerging biomarker, is highly expressed in association with multiple clinicopathological features of prostate cancer, demonstrating greater potential in prognostic evaluation. Furthermore, GPR35 may complement PSA by overcoming its limitations in prognostic evaluation, particularly by improving the accuracy of predicting outcomes for patients with normal PSA levels but underlying tumors. Therefore, GPR35, as an emerging biomarker, offers a more accurate reference for prostate cancer prognosis and holds potential as a valuable tool in clinical practice, aiding the optimization of individualized treatment strategies.

Although our study provides preliminary evidence supporting the potential of GPR35 as a prognostic biomarker for PCa, several limitations should be acknowledged. First, despite our efforts to control for potential heterogeneity by rigorously selecting local cohort participants, the sample size remains relatively small. Moreover, the study primarily relies on samples from a single region in China, which may limit the external validity and generalizability of the findings. Therefore, future studies should aim to expand the sample size and include patients from diverse regions and ethnic backgrounds to further validate the

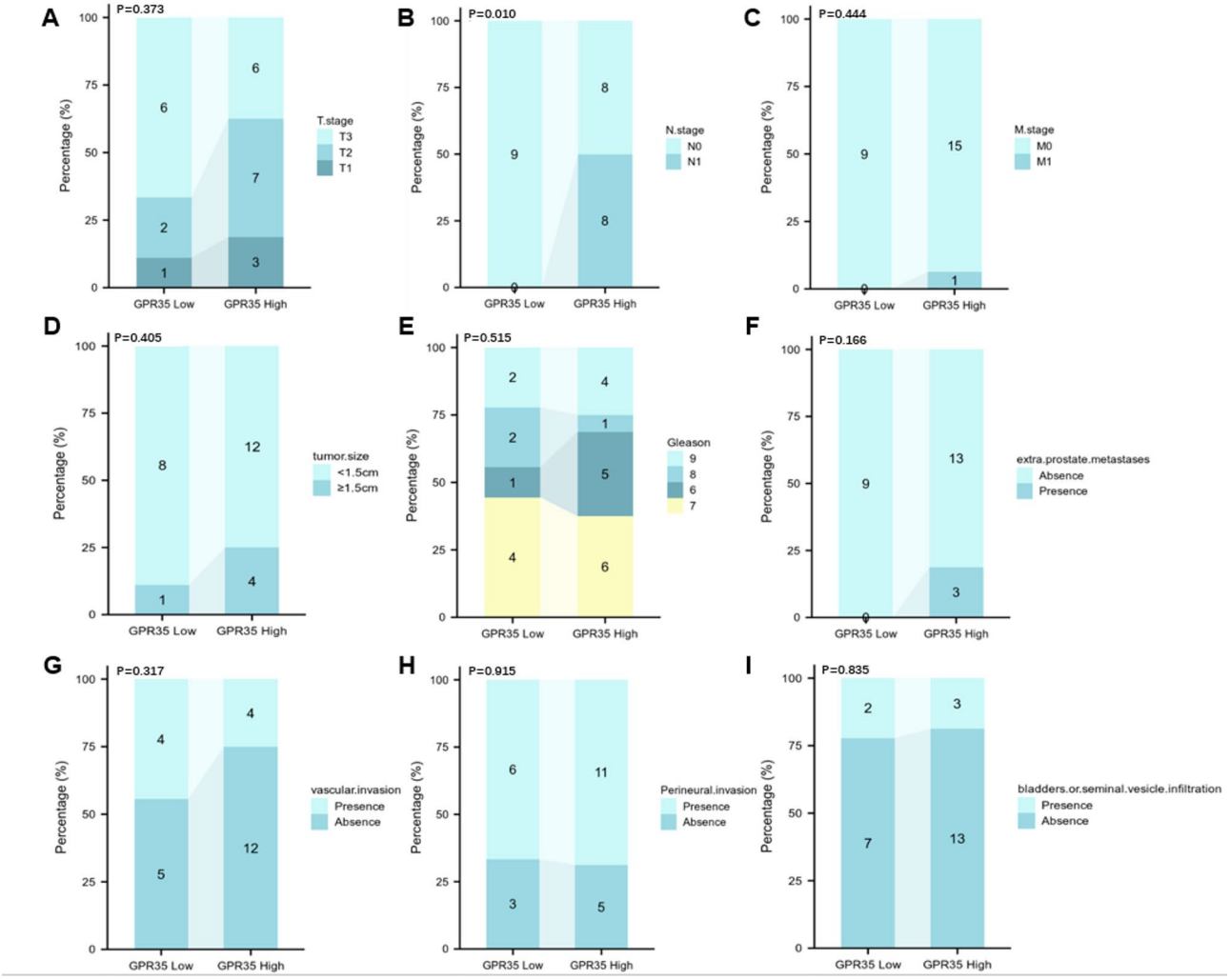

Fig. 4 Immunohistochemical expression of GPR35 and its association with clinicopathologic variables in PCa. Panels A-I display the distribution of GPR35 expression levels across various clinical variables, including T stage (**A**), N stage (**B**), M stage (**C**), tumor size (**D**), Gleason score (**E**), extra-prostate metastases (**F**), vascular invasion (**G**), perineural invasion (**H**), and bladder or seminal vesicle infiltration (**I**)

clinical relevance of GPR35 across different populations. Second, while this study focused on the association between GPR35 expression in PCa tissues and prognosis, the exploration of the specific molecular mechanisms and biological functions of GPR35 in prostate cancer cells remains limited. Although existing data support its potential as a prognostic marker, further research is needed to investigate its roles in cancer initiation, metastasis, and chemoresistance, particularly through in vitro functional validation experiments and animal models. Additionally, although this study relied on follow-up data from public databases for prognostic analysis, the lack of follow-up data in our local cohort limits the long-term follow-up information for the local population. To further validate the predictive capacity of GPR35, future studies should include more extensive follow-up data from local cohorts and analyze the prognostic value of GPR35 under various treatment regimens. Finally, this

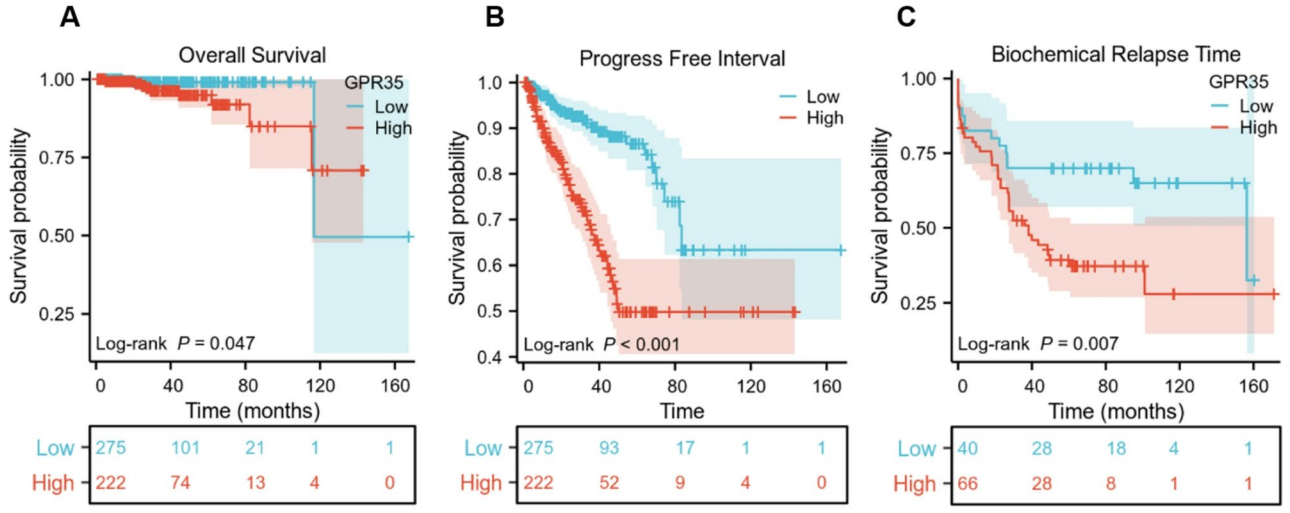

Fig. 5 Kaplan-Meier survival analysis of (**A**) overall survival, (**B**) progress free interval, and (**C**) biochemical relapse time (GSE54460) for GPR35 expression in PCa

Table 3 Association of GPR35 mRNA expression with BCR and other clinical characteristics

Characteristic	Low expression of GPR35(%)	High expression of GPR35(%)	*P*
n	40	66	
BCR, n (%)			0.007
Absence	26 (24.5)	25 (23.6)	
Presence	14 (13.2)	41 (38.7)	
Race, n (%)			0.746
White	22 (31.4)	26 (37.1)	
Black	11 (15.7)	11 (15.7)	
T stage, n (%)			0.494
1	3 (2.9)	11 (10.5)	
2	29 (27.6)	44 (41.9)	
3	7 (6.7)	10 (9.5)	
4	0 (0)	1 (1.0)	
Gleason score, n (%)			0.363
5	1 (0.9)	0 (0)	
6	5 (4.7)	5 (4.7)	
7	31 (29.2)	49 (46.2)	
8	2 (1.9)	8 (7.5)	
9	1 (0.9)	4 (3.8)	
PSA (ng/ml),	7.11 (5.29, 10.63)	7.2 (5.60, 14.65)	0.215
Age, mean ± SD	61.25 ± 6.38	61.03 ± 6.91	0.874

Table 4 Univariate and multivariate analysis of PFI survival

Characteristic	*N*	Univariate analysis		Multivariate analysis	
		HR (95%CI)	P	HR (95%CI)	*P*
T	382		< 0.001		
T1-T2	134	reference		reference	
T3-T4	248	3.829(2.023–7.250)	< 0.001	2.188(1.092–4.382)	0.027
N	382		0.056		
N0	313	reference		reference	
N1	69	1.678(1.010–2.789)	0.046	0.794(0.456–1.356)	0.398
Age	382	1.023(0.989–1.057)	0.186	-	
PSA (ng/ml)	382		0.013		
< 4	357	reference		reference	
≥ 4	25	3.153(1.445–6.879)	0.004	1.681(0.758–3.728)	0.202
Gleason score	382	-	< 0.001	-	
6&7	211	reference		reference	
8&9&10	171	4.532(2.732–7.516)	< 0.001	3.242(1.859–5.653)	< 0.001
GPR35	382	2.037(1.529–2.713)	< 0.001	1.915(1.368–2.682)	< 0.001

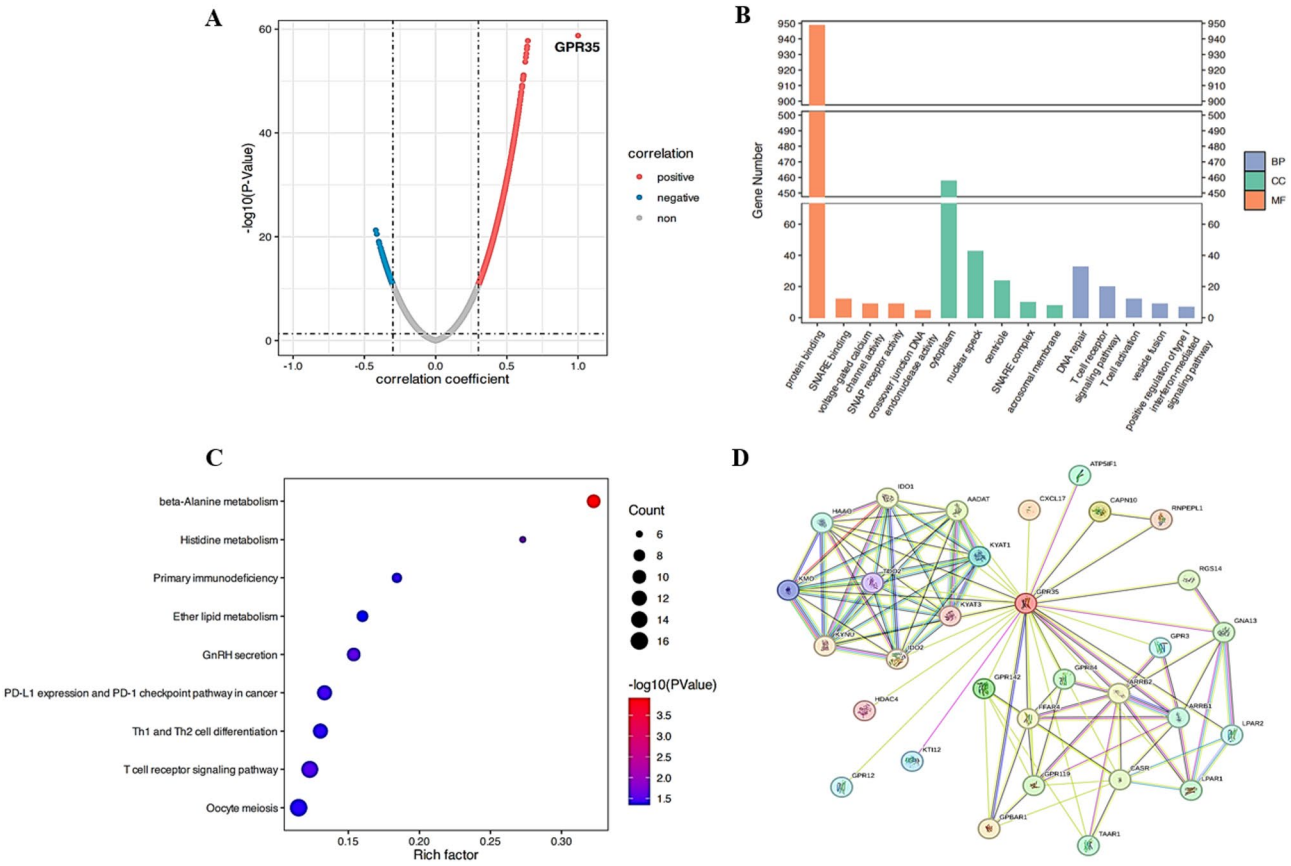

Fig. 6 Analysis of the biological function of GPR35 in PCa. (**A**) A volcano plot of correlation coefficients of GPR35 with all other mRNAs in the prostate cancer mRNA expression profile using the Pearson correlation test. (**B**) Part of the bar chart for enriched GO items, only the 5 top GO terms are shown. The length of the bars is proportional to the number of genes. (**C**) Enrichment plots of KEGG pathways. The size of the nodes is proportional to the number of genes. (**D**) Construction and analysis of protein-protein interaction (PPI) network

study did not fully control for all potential confounding factors that could influence GPR35 expression, such as patient age, comorbidities, and prior treatment history. Future research should account for these confounding variables and further assess the independence and predictive value of GPR35 across various clinical contexts.

Conclusion

For the first time, we provide evidence that high GPR35 expression is significantly correlated with poor prognosis in prostate cancer (PCa) patients, suggesting its potential as a biomarker that may help clinical oncologists deliver more targeted and effective treatment.

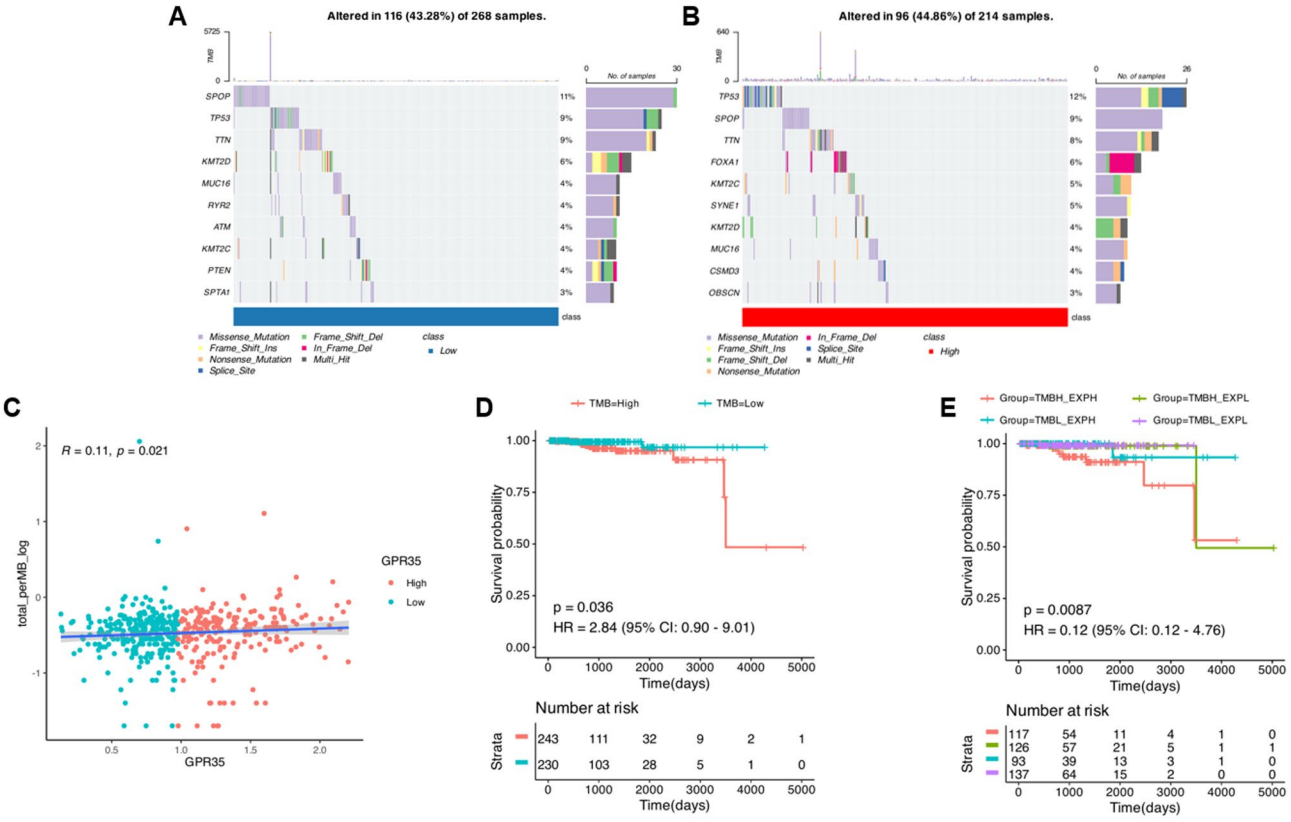

Fig. 7 Correlation of GPR35 expression with tumor mutational landscape and prognostic indicators in prostate cancer. (**A**) Mutation landscape of PCa samples with low GPR35 expression. (**B**) Mutation landscape of PCa samples with high GPR35 expression. (**C**) Correlation between GPR35 expression and TMB. (**D**) Survival probability based on TMB levels in PCa patients. (**E**) Survival probability in different TMB groups based on expression levels of GPR35

Supplementary Information

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Supplementary Material 1

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Author contributions

T.Z., X.L. and K.Z. conceived and designed the experiments, performed the experiments, and authored, revised or reviewed drafts of the article; J.L., A.M., M.H., N.Z., F.Z., and S.H. performed data collection; J.L., W.W., and M.Y. provided analysis and interpretation of data; J.F. and X.M. reviewed the paper. All authors read and approved the final paper.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the General Hospital of Xinjiang Military Region of the Chinese People's Liberation Army and conducted in accordance with the 1996 Declaration of Helsinki. The Ethics Committee of the General Hospital of Xinjiang Military Region of the Chinese People's Liberation Army waived informed consent from participants because this study involved routinely collected medical data that were managed anonymously at all stages, including the data cleaning and statistical analysis stages.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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