SNAP25 Is a Potential Prognostic Biomarker for Prostate Cancer

Longjiang Di

The Second Affiliated Hospital of Harbin Medical University <https://orcid.org/0000-0003-4426-2216>

Maoli Gu

department of urology, the second affiliated hospital of harbin medical university

Yan Wu

department of ultrasound, the second affiliated hospital of harbin medical university

Guoqiang Liu

department of neurosurgery, the second affiliated hospital of harbin medical university

Lishuo Zhang

department of urology, the second affiliated hospital of harbin medical university

Yifei Li

department of urology, the second affiliated hospital of harbin medical university

Wenjing Zhang (✉ wenjingzhang@hrbmu.edu.cn)

Second Affiliated Hospital of Harbin Medical University <https://orcid.org/0000-0001-8471-3080>

Primary research

Keywords: synaptosome associated protein 25, chemokines, migration, prostate cancer

Posted Date: October 12th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-956253/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Cancer Cell International on April 7th, 2022.
See the published version at <https://doi.org/10.1186/s12935-022-02558-2>.

Abstract

Background Prostate cancer is one of the most lethal cancers in male individuals. The Synaptosome associated protein 25 (SNAP25) gene is a key mediator of multiple biological functions in tumours. However, its significant impact on the prognosis in prostate cancer remains to be elucidated.

Methods We performed a comprehensive analysis of the Cancer Genome Atlas dataset (TCGA) to identify the differentially expressed genes between prostate cancer and normal prostate tissue. We subjected the differentially expressed genes to gene ontology analysis and Kyoto Encyclopedia of Genes and Genomes functional analysis, and constructed a protein-protein interaction network. We then screened for pivotal genes to identify the hub genes of prognostic significance by performing Cox regression analysis. We identified SNAP25 as one such gene and analysed the relationship between its expression in prostate cancer to poor prognosis using Studio R.

Results TCGA database demonstrated that SNAP25 was significantly downregulated in prostate cancer, and that its expression was significantly correlated with the Gleason score and pathological TNM stage of patients. The association between SNAP25 expression and tumour-infiltrating immune cells was evaluated using the Tumour Immune Estimation Resource site. Gene set enrichment and gene ontology analyses were used to analyse the function of SNAP25. We found that SNAP25 expression strongly correlated with overall survival in the Gleason score. In addition, SNAP25 was involved in the activation, differentiation, and migration of immune cells, and its expression was positively correlated with immune infiltration, including of B cells, CD8+ T cells, CD4+ T cells, neutrophils, dendritic cells, macrophages, and natural killer cells. SNAP25 expression was also positively correlated with chemokines/chemokine receptors, suggesting that SNAP25 might regulate the migration of immune cells. These molecular experiment results validate the low expression of SNAP25 seen in prostate cancer cells.

Conclusion Our findings indicate a relationship between SNAP25 expression and prostate cancer, demonstrating that SNAP25 is a potential prognostic biomarker due to its vital role in immune infiltration.

Background

Prostate cancer (PCa) is the most common cancer in male individuals worldwide, with peak incidence at 70–79 years of age, and is the second most deadly cancer among male individuals in the United States, after lung cancer [1]. Androgen deprivation therapy remains the most common first-line treatment for patients with advanced PCa, but numerous patients with metastatic PCa develop castration resistance post-therapy, which is inextricably linked to their high mortality rate [2, 3]. The pathogenesis of PCa is complicated and involves a large number of genetic aberrations [4]. In recent years, the tumour microenvironment (TME) has been reported to be associated with the progression of prostate cancer [5–7]. Immunotherapy is a promising strategy that has shown significant anticancer effects in prostate cancer [8, 9], and tumour-infiltrating immune cells have been shown to affect patient prognosis as well as the antitumour effects of immunotherapy [10–13]. However, the molecular immune mechanisms

associated with prostate cancer remain unclear. Therefore, the identification of new therapeutic biomarkers, specifically ones that are closely associated with prostate cancer immune infiltration, is an urgent need. The ability to correlate a quantitative physiological characteristic with the risk or progression of PCa is essential for early diagnosis and improved prognosis.

Synaptosome associated protein 25 (SNAP25) is a soluble, N-ethylmaleimide-sensitive factor attachment protein receptor complex that is essential for the regulation of neurotransmitter release, synaptic messaging, secretory vesicle extravasation, intercellular signalling, and ion channel opening [14-16]. SNAP25 has been seen as potentially important for normal vesicle fusion and lysosomal trafficking [17, 18]. In recent years, a growing number of studies have identified an association between SNAP25 levels and gastric cancer [19], colon cancer [20], hepatocellular carcinoma [21], lung cancer [22] and papillary thyroid cancer [23]. However, the functions and mechanisms associated with SNAP25 activity in the immunologic and pathogenetic progression of prostate cancer remain to be explored.

With the rapid development of high-throughput sequencing technologies, the mechanistic exploration of a large number of diseases has become possible [24]. The Cancer Genome Atlas (TCGA) is a landmark project that provides data on 32 human cancers gathered using genome sequencing, which has facilitated significant advancement in our understanding of the molecular basis of cancer. In this study, we conducted a large-scale bioinformatic investigation to elucidate the functional role of the SNAP25 protein in prostate cancer.

To this end, we obtained gene expression data from the TCGA and validated the survival value of gene expressions in different prostate cancer cohorts. Due to our finding that the effect of SNAP25 protein on survival prognosis became more pronounced as the pathological grading of prostate patients became more advanced, the SNAP25 protein was validated as a prognostic biomarker and selected for further analysis. We then used integrative analysis and multiple visualization methods to explore the mechanisms of SNAP25 in prostate cancer. We investigated the expression levels of the SNAP25 gene and analysed the correlation between the SNAP25 levels and patient clinical staging prognosis. We also used gene ontology (GO) analysis, gene set enrichment analysis (GSEA), and several methods to explore the potential functions of the SNAP25 gene in tumour development and the immune microenvironment. These findings suggested a potential mechanism for SNAP25 action, and provided new insights into the important role of SNAP25 in PCa.

Methods

Data Source and Processing

Clinical and molecular data (including mRNA expression and mutations) for prostate adenocarcinoma comprising 498 tumours and 52 normal tissue in close proximity, as well as patient information, as of May 2020 were downloaded from TCGA (<https://portal.gdc.cancer.gov/>). The database includes mRNA expression profiles for 20,530 differently expressed genes (DEGs). Thereafter, genes that were

differentially expressed between tumour and normal tissue were evaluated using the 'Limma' package of the R software (version 3.48.3). DEGs with a threshold $|\log_2FC| > 2$ and an adjusted p-value < 0.01 were selected for further analysis.

Functional enrichment analysis

To reveal the biological functions of the significant DEGs, Gene Ontology (GO) analysis, including analysis of the molecular functions, cellular components, and biological processes, as well as the Kyoto Encyclopaedia of Genes and Genomes (KEGG) enrichment analysis were conducted using the R package 'clusterProfiler [25]' with adjusted p value < 0.01 and q value < 0.01 . The results of the functional enrichment of hub genes were visualized using the R package 'GOplot [26]'.

Protein-protein interaction analysis

DEGs were screened using the STRING database (<https://string-db.org/>) in the protein-protein interaction (PPI) network. After disregarding the disconnected nodes and screening for relevant DEGs using an interaction score > 0.4 , selected PPI networks were further visualized using the Cytoscape software (version 3.8.2). The DEGs with a degree ≥ 10 were identified as hub DEGs using the cytoHubba plugin.

Heatmap analysis

Heatmap analyses were conducted using the 'heatmap' package.

Prognostic analysis

Univariate and multivariate Cox analyses were performed using hub-DEG expression data and data regarding the clinical factors (age, sex, stage, and grade) of patients with PCa, obtained from TCGA, to determine the prognostic value of hub DEGs, with p-values < 0.05 in both methods considered prognostically relevant. Kaplan-Meier survival curves were plotted between the survival states and the different groups based on the median or optimal cut-off values of the gene expression levels of prognostically relevant DEGs. The primary endpoint was disease-free survival (DFS). Statistical significance was set at $p < 0.05$.

TIMER Database Analysis

The Tumour Immune Estimation Resource (TIMER) [27] is a comprehensive web server for assessing tumour-infiltrating immune cells across different cancers (<https://cistrome.shinyapps.io/timer/>). TIMER includes more than 10,000 samples across the multiple cancer types in TCGA. It applies a partially

deconvoluted linear least square regression method to work out the abundance of immune infiltrates. We assessed the correlation between SNAP25 expression and immune infiltrates, including neutrophils, CD8+ T cells, CD4+ T cells, dendritic cells, B cells, and macrophages, in tumours.

Clinical significance in TCGA

To validate the clinical relevance of prognosis determination via SNAP25, expression profiles were compared between tumours and adjacent normal tissues. Additionally, the relationship between patient information, including Gleason score, TNM stage, and the expression levels of prognostic SNAP25 were analysed. The t-test and one-way analysis of variance (ANOVA) were used to compare two groups and multiple groups, respectively. Statistical significance was set at $p < 0.05$.

GSEA analysis

To investigate the potential signalling pathways between prognostic DEGs with different expression levels, we used the TCGA-based hallmark ('hallmark.all.symbols.gmt') gene set collection to carry out GSEA [28]. Nominal p-values < 0.05 and false discovery rate q-values < 0.25 were considered to be statistically significant.

Functional Experiments

Cell line and culture

PC3 (castration-resistant prostate cancer) and RWPE-1 (normal prostatic epithelial) cells were purchased from Biotechnology Co. (iCell Bioscience Inc., Shanghai, China). Cell lines have recently been identified using the STR method. The PC3 cells were cultured in an RPMI-1640 medium (Sigma, Saint Louis, MO, USA) with 10% FBS at 37 °C in an environment with 5% CO₂. RWPE-1 cells were grown in a Keratinocyte Serum Free Medium (iCell Bioscience Inc., Shanghai, China) at 37 °C in an environment with 5% CO₂.

Western blot analysis

Total protein was extracted using RIPA lysis buffer (Beyotime, Shanghai, China) according to the manufacturer's instructions. Subsequently, 10% sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE) was performed to separate the proteins, and a western blot analysis was conducted to determine the expressions of the proteins of interest, based on the experimental protocols documented in a previous study. Primary antibodies against SNAP25 (1:1000, Wanleibio, Shenyang, China) and β -actin (1:1000, ZSGB-BIO, Beijing, China) were used to probe corresponding proteins. Specific protein bands were detected with an ECL western blotting substrate (Beyotime, Shanghai, China) using

the Fluorescence/Chemiluminescence Imaging System (CLINX Science instruments, Shanghai, China). and analysed using ImageJ software. Each experiment was repeated at least 3 times.

Immunofluorescence

Immunofluorescence staining was performed to detect the expression of SNAP25 in PC3 and RWPE-1 cells. Briefly, cells were fixed with 4% paraformaldehyde for 30 min, permeabilized with 0.5% Triton X-100 for 15 min, and then blocked with goat serum. Subsequently, cells were incubated with an anti-SNAP25 (1:200, Wanleibio, Shenyang, China) antibody at 4 °C overnight, followed by incubation with an FITC-conjugated secondary antibody (ZSGB-BIO, Beijing, China) in the dark for 1 h. Cells were imaged using an immunofluorescence inverted microscope (Leica Dmi8, Wetzlar, Germany). The expression of SNAP25 was quantified using the average fluorescence intensity values generated by the ImageJ software.

Statistical Analysis

Statistical analysis was performed primarily using the ImageJ software (1.38e) and the Statistical Package for the Social Sciences (SPSS 26.0). Statistical significance was set at $p < 0.05$. Results were visualized using GraphPad Prism 9.2.0. All experiments were repeated three times for validation.

Results

Analysis of differentially expressed DEGs in prostate cancer

After careful screening, we selected 498 prostate cancer samples and 52 normal prostate tissue samples, including 20,530 DEGs from TCGA, for further analysis of mRNA expression profiles. Figure 1a shows a volcano plot of 319 DEGs, comprising 82 downregulated genes and 227 upregulated genes ($|\log_2FC| > 2$ and adjusted P value < 0.01). Figure 1b shows the PPI network analysis results, obtained using the STRING tool and the Cytoscape software, including 304 nodes and 457 interactions.

Identification of the biological functions of DEGs

To explore the biological role of differential genes, we performed GO and KEGG enrichment analyses, with the results shown in Figure 2a, 2b. The main biological processes in which the DEGs were involved include cancer-related foreign body metabolism, drug response, oxidation-reduction, and cell adhesion (Figure 2a). The cellular components closely associated with the DEGs were organelle membranes, extracellular matrix, and plasma membranes (Figure 2b). In addition, the DEGs were significantly enriched in molecular functions related to most ion binding and enzyme activities (Figure 2c). Analysis of the KEGG pathway demonstrated that the DEGs were mainly involved in chemotoxicity, drug metabolism, and xenobiotic activities (Figure 2d).

Selection and analysis of hub DEGs.

To explore differentially expressed genes that play an important role in prostate cancer pathogenesis, we performed a detailed analysis of DEGs using the STRING database and visualized them with Cytoscape. Figure 1b represents the PPI network, including 304 nodes and 457 interactions. We then ranked hub DEGs by degree using the Cytoscape plugin cytoHubba. A gene module centred on nine hub DEGs (MYH6, SNAP25, CLU, CCK, GATA3, KRT5, NCAM1, PENK, and RBFOX3) was identified (Figure 3a). As shown in Figure 3b, we performed a functional analysis of the hub DEGs involved in this module using DAVID (<https://david.ncifcrf.gov/>), and visualised the enrichment analysis using R packages to reveal the biological roles of the hub DEGs. Moreover, the heat map showed the expression levels of pivotal DEGs (Figure 3c). It revealed that the pivotal DEGs were different between the prostate cancer samples and non-cancerous samples, and correlated with patient age and the clinical stage of the cancer.

Prognosis analysis of Hub DEGs

To assess the independent prognostic value of hub DEGs, we extracted clinical factors (age, sex, stage, and grade) of prostate cancer patients in the TCGA cohort for univariate and multivariate Cox analyses. SNAP25, MYH6, and CCK were identified as significant factors affecting DFS in the model. Survival curves highlighted that low expression of SNAP25, MYH6, and CCK was significantly associated with poor DFS ($p < 0.05$, Figure 4a). Furthermore, the expression status of SNAP25 was significantly associated with prognosis in patients with pathologically graded PCa ($P < 0.0001$, Figure 4b). This suggests that SNAP25 can be considered a prospective biomarker for PCa and, to our knowledge, has not been previously reported as such. Thus, SNAP25 was selected for further analysis.

Clinical significance of SNAP25 and relationship with tumour-infiltrating immune cells

To gain more insight into the relationship between SNAP25 and the clinical presentation of human prostate cancer, we first analysed its mRNA expression levels in tumour and normal samples. The SNAP25 mRNA was expressed at significantly low levels in PCa cells ($p < 0.01$; Figure 5a). We next explored the relationship between clinical TNM staging of prostate cancer and SNAP25 expression. As shown in Figure 5b, SNAP25 expression decreased as patients progressed through T stage, with low expression of SNAP25 corresponding to the late T stage ($p < 0.01$). Samples with stage N1 had lower SNAP25 expressions than those with stage N0 ($p < 0.01$; Figure 5c). As the examination of M-stage distant metastases in prostate cancer are rarely performed clinically, too few M1-stage samples are available to rigorously assess a correlation with SNAP25. In addition, SNAP25 expression was negatively correlated with the clinicopathological Gleason score ($p < 0.01$; Figure 5d), decreasing as the Gleason score increased from 6 to 10. Based on these results, SNAP25 mRNA expression was found to be clinically significant for PCa.

Based on the TIMER database, SNAP25 was closely associated with tumour-infiltrating immune cells in a variety of cancer types, including colon cancer, hepatocellular liver cancer, lung squamous cell carcinoma, testicular cancer, and prostate cancer (Supplementary Figure S1). Of these, we found that SNAP25 had the most important influence on tumour-infiltrating immune cells in prostate cancer (Figure 5e). SNAP25 expression was negatively correlated with the purity of prostate cancer ($r = -0.497$, $p < 0.01$) and positively correlated with the abundance of several immune cell types, including B cells ($r = 0.251$, $p < 0.01$), CD4+ T cells ($r = 0.404$, $p < 0.01$), CD8+ T cells ($r = 0.273$, $p < 0.01$), neutrophils ($r = 0.322$, $p < 0.01$), macrophages ($r = 0.452$, $p < 0.01$), and dendritic cells ($r = 0.382$, $p < 0.01$). To verify these findings, we evaluated the relationship between SNAP25 expression and tumour-infiltrating immune cells derived from gene expression data using the MCPcounter method. SNAP25 expression showed a strong positive relationship with myeloid dendritic cells, T cells, monocyte lineage, B lineage, cytotoxic lymphocytes, and natural killer cells (Figure 5f). Thus, SNAP25 may play a key role in the immune regulation of prostate tumours.

Linear correlations and PPI networks between SNAP25, chemokines, and chemokine receptors

To further explore the relationship between SNAP25 and immune cell migration in prostate cancer, we incorporated chemokines and chemokine receptors into the analysis [29], as shown in Figure 6a–p. We discovered that SNAP25 expression was closely correlated with that of chemokines and chemokine receptors ($p < 0.01$), including CX3CL1, CX3CR1, CCL4, CCR5, CCL22, CCR4, CCL23, XCL1, XCR1, CXCL9, CXCR3, CXCL1, CXCR2, CXCR6, CCL5, and CCR1. The correlation images of the two different y-axes were obtained using GEPIA (<http://gepia.cancer-pku.cn/>). These chemokines and chemokine receptors appeared to be upregulated with increased SNAP25 expression levels. This implies that high expression of SNAP25 may facilitate the migration of immune cells to prostate tumour tissue.

To better understand the interactions between SNAP25 and chemokines or chemokine receptors, we used the STRING database for PPI network analysis and visualized them using Cytoscape. The PPI network shown in Figure 7 indicated that SNAP25 has known or predicted interactions with the chemokines mentioned above, as well as chemokine receptors through the linkage of the FGF1 and SYT1 proteins. Chemokine, chemokine receptor, and SNAP25 interactions were extracted from the STRING database and then collated for visualisation using the Cytoscape tool. These results indicate that SNAP25 may be involved in the migration of immune cells in prostate tumour tissues by regulating the expression of the FGF1 and SYT1 proteins.

External validation of SNAP25 using molecular experiments

Except for validating the role of SNAP25 in TCGA datasets, the expression level of SNAP25 in prostate cancer cells and normal prostate cells was examined by western blotting. Consistent with results using

TCGA data, SNAP25 expression at the protein level was significantly lower in the tumour group than in the normal group (Figure 8a, 8b). Next, we performed immunofluorescence experiments and observed a significant decrease in the expression level of SNAP25 in cancer cells (Figure 8c, 8d). These results showed that decreased expression of SNAP25 predicted a poor prognosis for PCa.

GSEA of SNAP25

GSEA was performed using samples with high and low SNAP25 expression levels to examine the pathways of enrichment. In the marker genome, downregulation of SNAP25 was clearly involved in cancer pathways, prostate cancer, and the WNT, TGF- β , and MAPK signalling pathways (Figure 9a–e). Downregulation of SNAP25 was also involved in immune effector processes, immune system development, and the innate immune response (Figure 9f–h). Thus, these enriched pathways are not only closely associated with prostate carcinogenesis, but also have a very important regulatory role in tumour immunity.

Discussion

PCa is one of the most lethal genitourinary tumours in male individuals [30]. In this study, bioinformatics analysis of TCGA data was conducted to screen for the distinct genetic characteristics between PCa and normal samples, resulting in the identification of the SNAP25 protein as prognostically significant. SNAP25 was expressed at low levels in prostate cancer and positively correlated with the Gleason score and TNM stage. SNAP25 may also be involved in the biological process by which immune cells enter tumour tissue and improve the TME, thereby influencing the development of prostate cancer and patient prognosis. In addition, we performed molecular experiments to verify the differential expression of SNAP25 in PCa and normal samples. Thus, SNAP25 was found to be a potential immune-related biomarker for PCa. Our findings provide deeper insights into the mechanisms of SNAP25 in prostate cancer.

Using a threshold of $|\log_2 FC| > 2$, we identified differentially expressed genes that were associated with GO terms and the KEGG pathway. Our GO pathway analysis showed that, in terms of molecular function, ion binding, calcium binding, and peroxide activation are closely associated with prostate cancer. For example, multiple ion-binding channels have been reported to mediate intracellular calcium regulation associated with the hallmarks of prostate cancer pathophysiology, including enhanced proliferation, survival, and invasion of cancer cells [31]. In terms of biological processes, differential genes are involved in cell adhesion [32], oxidation-reduction [33] and other processes that are closely related to prostate cancer. Moreover, results of cellular component analysis mainly focused on organelles involved in biosynthesis, cellular exosomes, and extracellular compartments. For example, mitochondrial dysfunction, abnormal organelle membrane potential [34] and exosome action [35] severely affect prostate cancer cells. In addition, KEGG pathway analysis revealed that xenobiotic metabolism, cytochrome P450 metabolism of xenobiotics, and chemical carcinogenesis were significantly associated

with prostate cancer [36]. In particular, a variety of endogenous and exogenous compounds can alter the expression levels of cytochrome P450 in humans, which in turn affects prostate cell metabolism and facilitates prostate cancer development [37]. We further screened the dataset for important hub genes and observed that some of them showed a meaningful chance of survival based on Cox regression analysis. In addition, we found that the SNAP25 gene among them significantly influenced the survival prognosis of gleason pathology grading in prostate patients for the first time. This has not been previously reported in PCa. Therefore, we focused on SNAP25 in PCa for further studies.

In addition to its neurological aspects, SNAP25 research in oncology has received considerable attention in recent years. Recent studies have claimed that neural signalling is of great importance in regulating tumour metabolism and have validated the role of this biological function in regulating SNAP25 expression in gastric cancer metabolism [19]. Therefore, we performed a clinical relevance analysis of the SNAP25 gene in prostate cancer patients using the TCGA database. Results showed that development of the T-stage in prostate cancer patients was negatively correlated with the expression of SNAP25. Patients with stage N1 had lower SNAP25 expression than those with stage N0 disease. In addition, as the Gleason score increased from 6 to 10, the expression of SNAP25 decreased.

In recent years, the tumour microenvironment and immune evasion have received significant attention in the diagnosis and treatment of a variety of malignancies. Although the immune response is heterogeneous, immunotherapy has revolutionized the field of therapeutic oncology. Studies have shown that the tumour microenvironment, particularly the immune microenvironment of tumours, is important for the prognostic outcome of patients with advanced prostate disease [38, 39]. However, few reports have described the function of SNAP25 in the TME. Using the TIMER, we found that SNAP25 affected tumour-infiltrating immune cells in prostate cancer. SNAP25 may promote the infiltration of various immune cells in PCa, including B cells, macrophages, dendritic cells, NK cells, neutrophils, and CD8+ T cells. The MCPcounter method also verified that SNAP25 was positively correlated with the immune infiltration of various types of T cells, B cells, macrophages, NK cells, monocyte lines, neutrophils, fibroblasts, as well as other cells. Activation of NK cells and CD8+ T cells promotes anti-tumour immunity through massive secretion of various cytokines and release of perforin and granzyme [40]. Increasing infiltration of B cells contributes to tumour elimination by engaging in antigen-dependent cell-mediated cytotoxicity [41]. DCs are the most potent antigen-presenting cells, and their infiltration can help present tumour-associated antigenic peptides, or tumour-specific antigens to T cells [42]. Neutrophils can directly kill tumour cells by releasing reactive oxygen species, promote T cell activation, and recruit pro-inflammatory (M1) macrophages, anti-inflammatory (M2) macrophages, and T regulatory cells [43]. Tumour-associated macrophages [44] have been reported to play a dual role in tumour development. M1 macrophages secrete pro-inflammatory cytokines and are involved in antigen presentation and immune monitoring, whereas M2 macrophages express inhibitory cytokines. Furthermore, activated fibroblasts are important in recruiting immune cells and regulating tumour immunity [45]. Thus, SNAP25 may play a key role in the regulation of TME in prostate cancer by participating in cellular and humoral immunity and stimulating anti-tumour function.

It is well known that chemokines control the localization and migration of immune cells. Chemokines are critical for the movement and homeostasis of immune cells [46]. We have analysed the association between SNAP25 and chemokines/chemokine receptors to explore the potential mechanisms associated with SNAP25 in immune cell migration of PCa. The CX3CL1/CX3CR1 interactions is involved in the activation of cytotoxic T lymphocytes and NK cells and is associated with the recruitment of T cells, NK cells and monocytes [47]. The CCL4/CCR5 interaction promotes the recruitment of T cells, DCs, monocytes, and NK cells [48, 49], while CCL22/CCR4 and XCL1/XCR1 interactions play an important role in the recruitment of T cells and NK cells [50, 51]. CCL5/CCR1 is involved in macrophage and NK cell migration as well as T cell-DC interaction [52]. The CCL23/CCR1 interaction affects the migration of monocytes, neutrophils, and T cells, while CXCL9/CXCR3 facilitates recruitment of effector T cells [53]. Finally, CXCL1/CXCR2 and CXCL16/CXCR6 are related to neutrophil recruitment and natural killer T cell migration, respectively [54, 55]. In the present study, SNAP25 was found to positively correlate with the above listed chemokine/chemokine receptors, and SNAP25 also has known or predicted interactions with them through the regulation of SYT1 and FGF1, according to the PPI network. This suggests that SNAP25 may increase immune infiltration in prostate cancer by regulating the migration of immune cells in the tumour microenvironment.

Although the clinical significance and function of SNAP25 in prostate cancer have not been reported previously, the present study demonstrates its potential as a prognostic biomarker. Interestingly, results of GSEA showed that SNAP25 was involved in many biological processes associated with prostate cancer, including the WNT, MAPK, and TGF- β signalling pathways. In addition, SNAP25 was also involved in immune and metabolic processes, such as immune effector processes, immune system development, and innate immune responses. These results suggest that SNAP25 improves the immune response in prostate cancer through multiple pathways.

This study includes several inherent limitations. First, although the study utilised a comprehensive bioinformatics analysis to elucidate potential functions of SNAP25, it did not explore specific signalling pathways associated with SNAP25 in PCa. In addition, in-depth molecular experiments are needed to validate the more profound mechanisms of SNAP25 and its impact on clinical outcomes in prostate cancer. Furthermore, a tight integration and elaboration of the link between SNAP25 and chemokines/chemokine receptors could facilitate a better understanding of the important relationship between SNAP25 and the immune microenvironment in tumours.

Conclusion

SNAP25 is a microenvironment-related and immune-related gene that predicts a poor prognosis in prostate cancer. Bioinformatic analysis showed that SNAP25 was not only closely associated with the clinical manifestations of prostate cancer, but was also involved in cancer-related signalling pathways as well as immune and metabolic processes, which might provide new targets for studying the underlying mechanisms of prostate cancer.

Abbreviations

SNAP25: Synaptosome associated protein 25

TCGA: The cancer genome atlas dataset

PCa: Prostate cancer

TME: The tumour microenvironment

GO: Gene ontology

KEGG: Kyoto Encyclopedia of Genes and Genomes

GSEA: Gene set enrichment analysis

DEGs: Differently expressed genes

TIMER: The tumour immune estimation resource

PPI: Protein–protein interaction

DFS: Disease-free survival;

OS: Overall survival

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available in TCGA at <https://portal.gdc.cancer.gov>, reference number TCGA-PRAD.

Competing interests

The authors state that they do not have any conflicts of interest.

Funding

This work was supported by National Natural Science Foundation of China (Grant Nos. 82001715), Health and Family Planning Commission of Heilongjiang Provincial (Grant No. 2018286), The Fundamental Research Funds for the Provincial Universities (Grant No. 2017LCZX67), Natural Science Foundation of Heilongjiang Province (Grant No. H2017014).

Authors' contributions

Conceptualization, LD; Methodology, LD, WZ; Investigation, LD, MG; writing—original draft, LD; writing—review & editing, LD, GL, LZ; funding acquisition, YL, WZ; resources, MG, YL; supervision, WZ, YL. All authors read and approved the final manuscript.

Acknowledgements

We would like to thank Editage (www.editage.cn) for English language editing.

References

1. Siegel R, Miller K, Jemal AJCacjfc: **Cancer statistics, 2020**. 2020, **70**(1):7-30.
2. Grossman D, Curry S, Owens D, Bibbins-Domingo K, Caughey A, Davidson K, Doubeni C, Ebell M, Epling J, Kemper A *et al*: **Screening for Prostate Cancer: US Preventive Services Task Force Recommendation Statement**. 2018, **319**(18):1901-1913.
3. Moreira D, Howard L, Sourbeer K, Amarasekara H, Chow L, Cockrell D, Pratson C, Hanyok B, Aronson W, Kane C *et al*: **Predicting Time From Metastasis to Overall Survival in Castration-Resistant Prostate Cancer: Results From SEARCH**. 2017, **15**(1):60-66.e62.
4. Gandhi J, Afridi A, Vatsia S, Joshi G, Joshi G, Kaplan S, Smith N, Khan SJPc, diseases p: **The molecular biology of prostate cancer: current understanding and clinical implications**. 2018, **21**(1):22-36.
5. Casey S, Amedei A, Aquilano K, Azmi A, Benencia F, Bhakta D, Bilsland A, Boosani C, Chen S, Ciriolo M *et al*: **Cancer prevention and therapy through the modulation of the tumor microenvironment**. 2015:S199-S223.
6. Sfanos K, Yegnasubramanian S, Nelson W, De Marzo AJNrU: **The inflammatory microenvironment and microbiome in prostate cancer development**. 2018, **15**(1):11-24.
7. Pasero C, Gravis G, Guerin M, Granjeaud S, Thomassin-Piana J, Rocchi P, Paciencia-Gros M, Poizat F, Bentobji M, Azario-Cheillan F *et al*: **Inherent and Tumor-Driven Immune Tolerance in the Prostate**

- Microenvironment Impairs Natural Killer Cell Antitumor Activity.** 2016, **76**(8):2153-2165.
8. Schepisi G, Cursano M, Casadei C, Menna C, Altavilla A, Lolli C, Cerchione C, Paganelli G, Santini D, Tonini G *et al*: **CAR-T cell therapy: a potential new strategy against prostate cancer.** 2019, **7**(1):258.
 9. Madan R, Gulley JJNru: **Prostate cancer: Better VISTAs ahead? Potential and pitfalls of immunotherapy.** 2017, **14**(8):455-456.
 10. Zhao S, Lehrer J, Chang S, Das R, Erho N, Liu Y, Sjöström M, Den R, Freedland S, Klein E *et al*: **The Immune Landscape of Prostate Cancer and Nomination of PD-L2 as a Potential Therapeutic Target.** 2019, **111**(3):301-310.
 11. Taverna G, Giusti G, Seveso M, Hurle R, Colombo P, Stifter S, Grizzi FJdm: **Mast cells as a potential prognostic marker in prostate cancer.** 2013, **35**(6):711-720.
 12. Zhang Q, Helfand B, Carneiro B, Qin W, Yang X, Lee C, Zhang W, Giles F, Cristofanilli M, Kuzel TJEu: **Efficacy Against Human Prostate Cancer by Prostate-specific Membrane Antigen-specific, Transforming Growth Factor- β Insensitive Genetically Targeted CD8 T-cells Derived from Patients with Metastatic Castrate-resistant Disease.** 2018, **73**(5):648-652.
 13. Erlandsson A, Carlsson J, Lundholm M, Fält A, Andersson S, Andrén O, Davidsson SJTP: **M2 macrophages and regulatory T cells in lethal prostate cancer.** 2019, **79**(4):363-369.
 14. Baker R, Hughson FJNrMcb: **Chaperoning SNARE assembly and disassembly.** 2016, **17**(8):465-479.
 15. Wang T, Li L, Hong WJT: **SNARE proteins in membrane trafficking.** 2017, **18**(12):767-775.
 16. Yoon T, Munson MJCbC: **SNARE complex assembly and disassembly.** 2018, **28**(8):R397-R401.
 17. Manca P, Mameli O, Caria M, Torrejón-Escribano B, Blasi JJN: **Distribution of SNAP25, VAMP1 and VAMP2 in mature and developing deep cerebellar nuclei after estrogen administration.** 2014, **266**:102-115.
 18. Mu Y, Yan X, Li D, Zhao D, Wang L, Wang X, Gao D, Yang J, Zhang H, Li Y *et al*: **NUPR1 maintains autolysosomal efflux by activating SNAP25 transcription in cancer cells.** 2018, **14**(4):654-670.
 19. Rabben H, Andersen G, Olsen M, Øverby A, Ianevski A, Kainov D, Wang T, Lundgren S, Grønbech J, Chen D *et al*: **Neural signaling modulates metabolism of gastric cancer.** 2021, **24**(2):102091.
 20. Zou J, Duan D, Yu C, Pan J, Xia J, Yang Z, Cai SJP: **Mining the potential prognostic value of synaptosomal-associated protein 25 (SNAP25) in colon cancer based on stromal-immune score.** 2020, **8**:e10142.
 21. Li J, Zhang C, Yuan X, Ren Z, Yu ZJAJotr: **Correlations between stemness indices for hepatocellular carcinoma, clinical characteristics, and prognosis.** 2020, **12**(9):5496-5510.
 22. Cao Q, Dong Z, Liu S, An G, Yan B, Lei LJCCI: **Construction of a metastasis-associated ceRNA network reveals a prognostic signature in lung cancer.** 2020, **20**:208.
 23. Yu X, Zhong P, Han Y, Huang Q, Wang J, Jia C, Lv ZJJocp: **Key candidate genes associated with BRAF in papillary thyroid carcinoma on microarray analysis.** 2019, **234**(12):23369-23378.
 24. Zhou C, Zhu C, Liu QJTimm: **Toward in silico Identification of Tumor Neoantigens in Immunotherapy.** 2019, **25**(11):980-992.

25. Yu G, Wang L, Han Y, He QJOajob: **clusterProfiler: an R package for comparing biological themes among gene clusters**. 2012, **16**(5):284-287.
26. Walter W, Sánchez-Cabo F, Ricote MJB: **GOplot: an R package for visually combining expression data with functional analysis**. 2015, **31**(17):2912-2914.
27. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XJNar: **TIMER2.0 for analysis of tumor-infiltrating immune cells**. 2020, **48**:W509-W514.
28. Subramanian A, Tamayo P, Mootha V, Mukherjee S, Ebert B, Gillette M, Paulovich A, Pomeroy S, Golub T, Lander E *et al*: **Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles**. 2005, **102**(43):15545-15550.
29. Shi J, Jiang D, Yang S, Zhang X, Wang J, Liu Y, Sun Y, Lu Y, Yang KJFio: **LPAR1, Correlated With Immune Infiltrates, Is a Potential Prognostic Biomarker in Prostate Cancer**. 2020, **10**:846.
30. Sung H, Ferlay J, Siegel R, Laversanne M, Soerjomataram I, Jemal A, Bray FJCacjfc: **Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries**. 2021, **71**(3):209-249.
31. Yang D, Kim JJBr: **Emerging role of transient receptor potential (TRP) channels in cancer progression**. 2020, **53**(3):125-132.
32. Crowley F, Sterpi M, Buckley C, Margetich L, Handa S, Dovey ZJR, urology ri: **A Review of the Pathophysiological Mechanisms Underlying Castration-resistant Prostate Cancer**. 2021, **13**:457-472.
33. Koroglu-Aydin P, Bayrak B, Bugan I, Karabulut-Bulan O, Yanardag RJTm, methods: **Histological and biochemical investigation of the renoprotective effects of metformin in diabetic and prostate cancer model**. 2021:1-12.
34. Kang B, Siegelin M, Plescia J, Raskett C, Garlick D, Dohi T, Lian J, Stein G, Languino L, Altieri DJCcraojotAAfCR: **Preclinical characterization of mitochondria-targeted small molecule hsp90 inhibitors, gamitrinibs, in advanced prostate cancer**. 2010, **16**(19):4779-4788.
35. Akoto T, Saini SJljoms: **Role of Exosomes in Prostate Cancer Metastasis**. 2021, **22**(7).
36. Yan Y, Mao X, Zhang Q, Ye Y, Dai Y, Bao M, Zeng Y, Huang R, Mo ZJCbsAoDm: **Molecular mechanisms, immune cell infiltration, and potential drugs for prostate cancer**. 2021, **31**(1):87-96.
37. Maksymchuk O, Kashuba VJPrP: **Dietary lipids and environmental xenobiotics as risk factors for prostate cancer: The role of cytochrome P450**. 2019, **71**(5):826-832.
38. Mo R, Han Z, Liang Y, Ye J, Wu S, Lin S, Zhang Y, Song S, Jiang F, Zhong W *et al*: **Expression of PD-L1 in tumor-associated nerves correlates with reduced CD8 tumor-associated lymphocytes and poor prognosis in prostate cancer**. 2019, **144**(12):3099-3110.
39. Anker J, Naseem A, Mok H, Schaeffer A, Abdulkadir S, Thumbikat PJNc: **Multi-faceted immunomodulatory and tissue-tropic clinical bacterial isolate potentiates prostate cancer immunotherapy**. 2018, **9**(1):1591.
40. André P, Denis C, Soulas C, Bourbon-Caillet C, Lopez J, Arnoux T, Bléry M, Bonnafeous C, Gauthier L, Morel A *et al*: **Anti-NKG2A mAb Is a Checkpoint Inhibitor that Promotes Anti-tumor Immunity by**

- Unleashing Both T and NK Cells.** 2018, **175**(7):1731-1743.e1713.
41. Cascalho M, Platt JJI: **Harnessing B cells in immunotherapy.** 2016, **8**(3):237-239.
 42. Gardner A, Ruffell BJTii: **Dendritic Cells and Cancer Immunity.** 2016, **37**(12):855-865.
 43. Giese M, Hind L, Huttenlocher AJB: **Neutrophil plasticity in the tumor microenvironment.** 2019, **133**(20):2159-2167.
 44. Liang P, Henning S, Guan J, Grogan T, Elashoff D, Cohen P, Aronson WJPc, diseases p: **Effect of dietary omega-3 fatty acids on castrate-resistant prostate cancer and tumor-associated macrophages.** 2020, **23**(1):127-135.
 45. Kalluri RJNrC: **The biology and function of fibroblasts in cancer.** 2016, **16**(9):582-598.
 46. Balkwill FJNrC: **Cancer and the chemokine network.** 2004, **4**(7):540-550.
 47. Conroy M, Lysaght JJAiem, biology: **CX3CL1 Signaling in the Tumor Microenvironment.** 2020, **1231**:1-12.
 48. Wang Y, Liu T, Yang N, Xu S, Li X, Wang DJOr: **Hypoxia and macrophages promote glioblastoma invasion by the CCL4-CCR5 axis.** 2016, **36**(6):3522-3528.
 49. Liou J, Lee C, Day YJAAtoJotTSOA: **The immune aspect in neuropathic pain: role of chemokines.** 2013, **51**(3):127-132.
 50. Araujo-Pires A, Vieira A, Francisconi C, Biguetti C, Glowacki A, Yoshizawa S, Campanelli A, Trombone A, Sfeir C, Little S *et al*: **IL-4/CCL22/CCR4 axis controls regulatory T-cell migration that suppresses inflammatory bone loss in murine experimental periodontitis.** 2015, **30**(3):412-422.
 51. Lei Y, Takahama YJM, infection: **XCL1 and XCR1 in the immune system.** 2012, **14**(3):262-267.
 52. Liao W, Yen H, Liao C, Tseng T, Lan C, Liu CJAjocr: **DSE regulates the malignant characters of hepatocellular carcinoma cells by modulating CCL5/CCR1 axis.** 2019, **9**(2):347-362.
 53. Xiu W, Luo JJB: **CXCL9 secreted by tumor-associated dendritic cells up-regulates PD-L1 expression in bladder cancer cells by activating the CXCR3 signaling.** 2021, **22**(1):3.
 54. Sawant K, Xu R, Cox R, Hawkins H, Sbrana E, Kolli D, Garofalo R, Rajarathnam KJJoi: **Chemokine CXCL1-Mediated Neutrophil Trafficking in the Lung: Role of CXCR2 Activation.** 2015, **7**(6):647-658.
 55. Huang Y, Zhu X, Du M, Li DJJoi: **Human trophoblasts recruited T lymphocytes and monocytes into decidua by secretion of chemokine CXCL16 and interaction with CXCR6 in the first-trimester pregnancy.** 2008, **180**(4):2367-2375.

Figures

Figure 1

The differentially expressed DEGs between PCa and normal samples. (a) Volcano plot showing differentially expressed DEGs between prostate cancer (PCa) and normal prostate samples. (b) The PPI network of DEGs was constructed using Cytoscape. Upregulated genes are marked in red; downregulated genes are marked in green.

Figure 2

Functional enrichment analysis of differentially expressed genes. (a-c) GO analysis of representative DEGs, including biological process (BP), cellular component (CC), and molecular function (MF). (d) KEGG pathway terms in DEGs. The y-axis shows enriched GO and KEGG terms.

ontology. $p < 0.05$ was considered statistically significant. (c) Heat map of hub DEGs. The colour layout therein from blue to red indicates the expression levels of hub DEGs from down to up.

Figure 4

Survival curves of hub prognosis of DEGs. (a) Prognostic analysis of the training and validation datasets. relationship between hub DEGs expression in TCGA and DFS. (b) Relationship between SNAP25 expression in TCGA and OS in Gleason pathology-graded prostate cancer patients.

Figure 5

Correlation between SNAP25 expression and clinical manifestations, and tumor-infiltrating immune cells of PCa. (a) Expression of SNAP25 between cancer and normal samples. The relationship between SNAP25 expression and T-stage (b), N-stage (c) and pathological Gleason score (d). (e) SNAP25 expression was significantly negatively correlated with tumour purity and positively correlated with the abundance of several immune cell types in PCa. This included CD8+ T cells, CD4+ T cells, macrophages,

B cells, neutrophils and dendritic cells. (f) A heatmap of the correlation between SNAP25 and T cells, B lineage, monocytic lineage, myeloid dendritic cells, cytotoxic lymphocytes, and natural killer cells completed by the MCPcounter R package. Higher SNAP25 expression was associated with more immune infiltration in PCa.

Figure 6

Scatter plots of SNAP25, chemokines and chemokine receptors. (a-p) SNAP25 was positively correlated with CCL4, CCL5, CCL22, CX3CL1, CCR1, CCR5, CX3CR1, CCL23, CXCL1, CXCR3, CXCL9, CXCR6, XCR1, CCR4, XCL1 and CXCR2.

Figure 7

Protein-protein interaction (PPI) network based on SNAP25 and chemokine/chemokine receptor with a minimum required interaction score >0.4. PPI network between SNAP25 and 12 chemokines and 8

chemokine receptors.

Figure 8

External validation of SNAP25 in molecular experiments. (a) Western blot analysis of SNAP25 between PCa and normal clinical samples. β -actin was used as an internal control. (b) Quantification of SNAP25 protein levels. (c) Immunofluorescence analysis of SNAP25 (green) in normal prostate cells and prostate cancer cells. Scale bar, 50 μ m. (d) The relative mean fluorescence in each group was calculated. Data

were presented as the means $\pm$ SD, n = 3. *P < 0.05, **P < 0.01, and ns, P > 0.05, no significant difference.

Figure 9

Analysis of important signalling pathways associated with SNAP25 by GSEA. (a-e) The most important KEGG signaling pathways associated with down-regulated SNAP25. (f-h) The most important GO biological processes associated with down-regulated SNAP25.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigureS01.pdf](#)