

The associations of SNF5 with prognosis and immune responses in bladder cancer

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Research

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Abstract

Background

Bladder cancer (BC) is the second most common malignancy in the urinary system. Improving survival has been hampered by high heterogeneity and drug resistance. SNF5, a subunit of the SWI/SNF chromatin-remodeling complex, is commonly lost or inactivated in multiple malignancies. The role of SNF5 in bladder cancer has not yet been elucidated. This is the first exploration into the associations of SNF5 with prognosis and its functions in BC.

Methods

Using datasets from The Cancer Genome Atlas projects and our institution, we investigated the predictive value of SNF5 in bladder cancer, as well as the relationships with clinical characteristics. The differential genes expression analysis, gene ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, gene set enrichment analyses (GSEA) were performed to investigate the functions of SNF5. The Immune Cell Abundance Identifier (ImmuCellAI) algorithm was used to infer the fractions of tumor-infiltrating lymphocytes (TILs). Next, we conducted gene set variation analysis (GSVA) and Tumor Immune Dysfunction and Exclusion (TIDE) algorithm to assess the effects of SNF5 on antitumor response and sensitivity to immune checkpoint blockade (ICB).

Results

SNF5 had the potential to predict bladder cancer and a negative correlation with survival. SNF5 was involved in immune response. Low expression of SNF5 promoted infiltration of immune cells into tumor, enhanced the abilities to process and present antigens by activating the MHC-T cell receptor activation pathway. Moreover, decreased SNF5 activated CTLA-4 pathway, upregulating CTLA-4 expression, but SNF5 did not modify the expression of PD-1 or PD-L1. A higher TIDE score was generated in the low-expression group, which indicated that patients with low expression might derive greater benefit from ICB.

Conclusions

Our findings indicated that SNF5 was not only a good biomarker for diagnosis and prognosis of BC, but a potential new target in the immunotherapy for BC.

Background

Bladder cancer, the ninth most common carcinoma, is annually responsible for 165,000 deaths and 430,000 new cases worldwide [1]. Radical cystectomy combined with chemotherapy has long been considered the conventional therapeutic strategy for bladder cancer [2]. However, due to the great

heterogeneity and drug resistance of bladder cancer, prognoses widely differ among individuals even if they are confirmed to have disease in the same stage. Despite advances in medical technology, the 5-year survival rate has shown little improvement over the past three decades [3]. Hence, treatment refinement and development are urgently needed.

An increasing cohort of studies has indicated that the tumor microenvironment, especially immune-infiltrating cells in this microenvironment, reflects the tumor-related immune response and exerts beneficial or negative effects on tumor initiation, progression and treatment in various cancers [4–6]. Immunotherapy, which is based on the immune response to tumors, has received increasing attention and has recently been deeply studied as an additional therapeutic strategy. This is well exemplified in the successful development of immune checkpoint inhibitors, such as antibodies against programmed cell death 1 (PD-1) and PD-1 ligand (PD-L1), which block the immunosuppressive interaction between immune cells and tumor cells [7].

SNF5, also known as SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily B, Member 1 (SMARCB1), is one of the members of the SWI/SNF ATP-dependent chromatin-remodeling complex and is known for its frequent inactivation in aggressive tumors [8]. In rhabdoid tumors, its inactivation dramatically shortens the life span of relatively young patients [9]. However, the associations of SNF5 with survival and the immune response against tumor cells in bladder cancer have not been clarified.

The present study, to our knowledge, is the first analysis of the role of SNF5 in bladder cancer. We investigated the relationship between SNF5 and clinical outcomes in The Cancer Genome Atlas datasets and our own data. Differential gene expression analysis, gene ontology (GO) analysis and gene set enrichment analysis (GSEA) were applied to explore the pathways affected by SNF5 expression. Immune Cell Abundance Identifier (ImmuCellAI), a recently developed metagenomic approach, was used to quantify 24 tumor-infiltrating immune cell types in bladder cancer to study the associations of SNF5 with tumor-infiltrating immune cells. The findings in this paper may provide new insight into the effects of SNF5 on bladder cancer, shed light on the possible mechanism linking SNF5 and tumor-immune interactions and offer guidance for regimen selection and refinement.

Materials And Methods

Patients And Study Design

Bladder tumor tissue specimens were obtained from 157 patients with bladder cancer who underwent surgery at the Department of Urology, Southwest Hospital of Army Medical University. Informed consent was obtained from all the patients. This study was approved by the Institutional Review Board (IRB 2012XLC02) of Southwest Hospital. A total of 408 patients from the TCGA were included in this study, and their level-3 RNA-Sequencing (RNA-Seq) data and corresponding clinical characteristics were analyzed.

Immunohistochemistry

All tumor samples were processed, fixed with 10% formalin and embedded in paraffin. Five-micrometer-thick slides were cut, deparaffinized and rehydrated. Antigen retrieval was conducted in citrate buffer (0.01 mol/L, pH 6.0), followed by blocking endogenous peroxidase activity with a 3% hydrogen peroxide solution. Then, the slides were blocked with goat serum and incubated with an anti-SNF5 antibody (dilution 1:200; Bethyl Laboratories) at 4 °C overnight and a horseradish peroxidase (HRP)-conjugated anti-rabbit secondary antibody. Finally, the sections were dehydrated, cleared, and mounted with a coverslip. Two pathologists reviewed all the sections independently. A semiquantitative scoring system was used according to the percentage of positive cells (0, 0–10%; 1, 10–25%; 2, 26–50%, 3, 51–70%; and 4, >70%) and the staining intensity (0, negative; 1, weak; and 2, strong). The final score was calculated by adding the two scores together. Sections with a score less than 4 were labeled low SNF5 expression, whereas those with a score equal to or greater than 4 were considered to have high expression.

Differential Gene Expression Analysis And GO Analysis

We identified differentially expressed genes (DEGs) with the DESeq2 package. Genes with a p value < 0.05 and $\log_2|\text{fold change}| > 1.0$ were considered DEGs. We performed GO enrichment analysis of the DEGs to identify gene function clusters.

GSEA And Gene Set Variation Analysis

GSEA of the DESeq2 differential expression results was conducted to evaluate possible biological mechanisms correlated with the SNF5 mRNA expression levels in tumor samples from the TCGA database. We used the clusterProfiler package and gene sets from the C2 collection and C5 collection in the Molecular Signatures Database (MSigDB) in this analysis. A false discovery rate (FDR) < 25% and $P < 0.05$ were used to define significant enrichment. Gene set variation analysis (GSVA) was implemented using the GSVA package to assess pathway activity.

Immune infiltration analysis and prediction of immune checkpoint blockade therapy response

ImmuCellAI is a newly developed algorithm used to evaluate the concentrations of infiltrating immune cells precisely [10]. The proportions of 24 immune cell types (CD4 + T cells (CD4T), CD8 + T cells (CD8T), CD4 + naive T cells (CD4 naive), CD8 + naive T cells (CD8 naive), central memory T cells (Tcm), effector memory T cells (Tem), Tr1 cells, induced regulatory T cells (iTreg), natural regulatory T cells (nTreg), T helper (Th) 1 cells, Th2 cells, Th17 cells, T follicular helper cells (Tfh), cytotoxic T cells (Tc), mucosal-associated invariant T cells, exhausted T cells (Tex), gamma delta T cells (Tgd), natural killer T (NKT) cells, B cells, macrophages, monocytes, neutrophils, dendritic cells (DC) and NK cells) were assessed in bladder cancer tissue specimens based on the mRNA-Seq data. To analyze the influence of SNF5 on tumor-infiltrating immune cells (TIICs) in bladder cancer, we sorted 408 samples from the TCGA database

in decreasing order based on the SNF5 expression level and extracted the upper-third and lower-third of these samples for subsequent analysis. $P < 0.05$ was considered the criterion to define a type of lymphocytes as affected by the expression of SNF5. Tumor Immune Dysfunction and Exclusion (TIDE) algorithm is a newly developed tool to accurately assess tumor immune evasion, consistently presenting favorable performance in prediction of response to immune checkpoint inhibitors [11, 12]. The patient with high TIDE score had much chance of immune escape. We predicted the potential immune checkpoint blockade (ICB) response with the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm.

Statistical Analyses

Statistical analyses were performed by using R software (version 3.6.3). Kaplan-Meier curves were analyzed by the log-rank method to determine the statistical significance of survival differences. Univariate and multivariate Cox analyses were applied to evaluate the relationships between SNF5 expression and clinical factors and their effects on survival. Differences between two groups were analyzed with a two-tailed Student's t-test for continuous data. Correlations were evaluated by calculating the p value and Spearman's R value. A correlation was considered relevant when the absolute value of R was greater than 0.1. Statistical significance was defined as $P < 0.05$ if not otherwise specified.

Results

Baseline Clinical Characteristics

One hundred and fifty-seven patients with bladder cancer were enrolled in this study. Eighteen patients had pTa, 49 had pT1, 51 had pT2, 21 had pT3, and 18 had pT4. The detailed clinical characteristics are shown in Additional file 1: Table S1. Four hundred and eight patients with bladder cancer in the TCGA dataset were included in this study. The detailed clinical characteristics of these patients (including age, sex, race, AJCC stage and subgroups based on mRNA clusters) are listed in Additional file 2: Table S2.

Associations Between SNF5 Expression And Clinical Characteristics

First, we plotted a receiver operating characteristic (ROC) curve to study the clinical value of SNF5. It showed promising power to predict bladder cancer, as the area under the curve (AUC) was 0.781 (Fig. 1a). To investigate the relationships between SNF5 and clinical factors, Cox analysis was applied (Table 1). Univariate Cox analysis revealed that age, SNF5 level, tumor size, lymph node status, metastasis status and AJCC stage were significantly related to survival. Multivariate analysis indicated that age and SNF5 level were independent prognostic factors for survival in bladder cancer. Next, Kaplan-Meier survival analysis of the TCGA cohort data was plotted to evaluate the correlation between the SNF5 expression level and survival time of patients with bladder cancer. A clear separation between the patients with

higher SNF5 expression and those with lower expression was observed, and low expression of SNF5 conferred a poor prognosis in bladder cancer (Fig. 1b). Similar results were also obtained for the 88 patients with bladder cancer. The patients whose tumor sections showed high expression had a more favorable prognosis than their counterparts (Fig. 1c and Fig. 1d). These findings indicate that low SNF5 expression is associated with a poor prognosis.

Table 1
Univariate and multivariate Cox analyses in TCGA datasets

Variables	Univariate Cox analysis		Multivariate Cox analysis	
	Hazard ratio (95%CI)	P	Hazard ratio (95%CI)	P
Age	1.03 (1.02–1.05)	< 0.001	1.09 (0.75–1.60)	< 0.001
Gender	0.92 (0.73–1.16)	0.471		
Race	0.99 (0.74–1.31)	0.921		
SNF5	0.88 (0.76–1.02)	0.029	0.91 (0.77–1.07)	0.024
Tumor size	1.70 (1.30–2.21)	< 0.001	1.46 (0.65–3.28)	0.355
Lymph node	1.77 (1.42–2.21)	< 0.001	1.25 (0.82–1.93)	0.301
Metastasis	0.85 (1.38–3.92)	< 0.001	1.01 (0.50–2.03)	0.977
AJCC stage	1.64 (1.35–2.01)	< 0.001	1.12 (0.91–1.37)	0.696
<i>CI</i> confidence interval				

SNF5 Is Involved In The Immune Response

To explore the role of SNF5 in bladder cancer, we extracted the upper third and lower third of samples sorted in decreasing order according to the SNF5 expression level and identified the DEGs between the two groups. In total, we obtained 781 significant DEGs in the low-expression group, with 481 upregulated and 301 downregulated genes ($|\log(\text{fold change})| > 1$, $P < 0.05$). The expression profiles of the DEGs in both groups are shown in Fig. 2a. To further investigate the biological functions of the DEGs, we conducted GO analysis after gene annotation. A total of 266 GO terms were identified. Many inflammation-related gene sets, such as leukocyte chemotaxis and cytokine secretion, and immune cell-related gene sets, such as neutrophil-mediated immunity, neutrophil activation, T cell activation and T cell proliferation, were upregulated in the low SNF5 expression group (Fig. 2b). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis implied that the altered genes were mainly involved in cytokine-cytokine receptor interactions, chemokine signaling pathways, IL-17 signaling pathways and T cell receptor signaling pathways (Fig. 2c). Taken together, these results suggest that SNF5 is associated with immune responses.

Relationships Between SNF5 Expression And Tumor-infiltrating Immune Cells

Based on the samples extracted above, we explored the potential connection between SNF5 expression and immune infiltration in bladder cancer. ImmuCellAI was used to precisely estimate the abundances of 24 types of immune cell types. As shown in Figure, SNF5 expression significantly affected the CD4 naive, CD8 naive, Tc, nTreg, Th1, Th2, Tfh, NKT, MAIT, macrophage, NK, neutrophil, Tgd, CD4T, and CD8T fractions. Among the fractions, the Tc, Th1, Th2, Tfh, NKT, MAIT, macrophage, NK, CD4T, Tgd and CD8T fractions were increased in the low-expression group, while the nTreg, CD4 naive, CD8 naive and neutrophil fractions exhibited higher proportions in the high SNF5 expression group (Fig. 2d). The correlations among the tumor-infiltrating immune cell types are shown in Fig. 3a. A heatmap revealed that different subpopulations of tumor-infiltrating immune cells were weakly to strongly correlated. Two points were particularly noteworthy. The Th1, Tex, Tc, Tfh, NK, iTreg, Th2, CD8T, Tfh and CD4T fractions were positively correlated with each other. The CD4 naive, CD8 naive and neutrophil fractions displayed positive relationships with each other but negatively correlated with the other TIIC fractions. As in previous work, we calculated the immune infiltration score (IIS) based on TIICs [13]. The IIS in the low-expression group was remarkably higher than that in the high-expression group (Fig. 3b). The results of the analyses corroborated each other and indicated that patients with bladder cancer with low SNF5 expression have elevated immune cell infiltration.

Low SNF5 expression is correlated with the antitumor response and immune checkpoints

Antigen processing and presentation are fundamental processes in the antitumor response. Here, we first used GSVA to calculate an antigen processing and presentation machinery score (APPMS) in light of a signature defined in previous studies [14, 15]. The APPMS of the low-expression group was significantly higher than that of the high-expression group (Fig. 3b). Next, to guide our exploration of immune pathways crucial for SNF5 function, GSEA was applied using gene sets from the MSigDB v7.0. Consistent with the GSVA findings, the GSEA results revealed that pathways related to antigen processing and presentation, such as the MHC pathway and T cell receptor activation pathway, were enriched in the low-expression group (Fig. 3c and Fig. 3d). Additionally, antitumor response gene sets, such as the cytotoxic T lymphocyte (CTL)-mediated immune response and NO₂-dependent IL-12 pathway in NK cells, were overrepresented in tumor tissue samples with low SNF5 expression (Fig. 4a and Fig. 4b). Considering that the antitumor response of T cells is also influenced by immune checkpoints, we tested whether there was a significant difference in immune checkpoint expression between the two groups. CTLA4 expression was observed to be significantly elevated in the low-expression group, whereas PD-1 expression and PD-L1 presented nonsignificant differences between the groups (Fig. 4c). Additionally, the low-expression group had a significantly lower TIDE score (Fig. 4d). A gene set representing CTLA-4 pathway was enriched in BC patients with low SNF5 expression (Fig. 4e). Collectively, these findings indicated that low SNF5 expression enhanced antigen processing and presentation and the antitumor response. On the other hand, the CTLA4-mediated immune checkpoint mechanism was activated to

suppress the CTL-mediated antitumor response. Hence, compared with the high-expression group, the low-expression group would potentially derive more benefits from anti-CTLA4 immunotherapy.

Discussion

This paper first characterized the role of SNF5 in bladder cancer. We found that SNF5 could serve as a predictive biomarker for bladder cancer and was independent from other clinical characteristics. In the survival analysis, patients with low expression had a worse prognosis than those with high expression in both the TCGA dataset and our cohort, which supports the aggressive driven-tumor activity and early embryonic lethality observed in SNF5-inactivated murine models. To understand how SNF5 functions in bladder cancer, we applied differential gene expression analysis to compare the low- and high-expression groups. GO analysis revealed that the identified DEGs were correlated with immune response features, including T cell proliferation, cytokine secretion, leukocyte chemotaxis and activation of T cells and neutrophils. These results demonstrate that SNF5 is related to the immune response.

Immune cells, which are vital components in the tumor environment, are executors that carry out immune response functions, such as cytokine secretion and interactions with antigens. Increasing evidence has previously established that the infiltration of immune cells into tumors and cross talk between immune cells and tumor cells have tight associations with tumor progression, response to therapy and patient prognosis. Although inspiring results have been observed for PD1/PD-L1 checkpoint immunotherapy in some cancers in recent years, the complete response rate is still only 15–20% [16]. Therefore, a description of the landscape of tumor-infiltrating cells in bladder cancer is required to provide new insight into the tumor environment and could facilitate therapy development, particularly development of individualized treatments.

For assessing TIIC subsets, previous studies have adopted flow cytometry and immunohistochemistry. However, cell injury or loss during sample preparation can present challenges in regard to the credibility and accuracy of results [17]. Utilizing one or several markers to analyze different subpopulations via immunohistochemistry staining can suffer from poor specificity. Additionally, this kind of analysis is likely to be affected by an inherent individual bias or a daily bias that limits consistency and could lead to misleading findings [18]. Developments in bioinformatics make it possible to depict the composition of TIICs based on RNA expression data. The EPIC, TIMER, and MCP-counter tools have been developed and used for evaluating tumor infiltration [19–21]. However, the EPIC, TIMER and MCP-counter tools predict only six to ten T cell subsets. These algorithms neglect the importance of other subpopulations, such as iTregs and Tex, which have been reported to be essential for the immune system and immune response. ImmuCellAI, a newly developed algorithm used in this study, can accurately identify the abundances of 24 T cell subpopulations. Based on ImmuCellAI algorithm, significant differences were detected in CD4 naive, CD8 naive, Tc, nTreg, Th1, Th2, Tfh, NKT, MAIT, nTreg, neutrophil, Tgd, CD4T and CD8T cells between the two groups. These results suggested that these immune cells may influence biological characteristics of tumors. We then calculated the IIS based on TIICs. By comparing low and high SNF5 expression groups, the former showed a significantly higher IIS. Tumor-infiltrating T lymphocytes have

been an indicator of a favorable prognosis in various solid cancers [22]. However, there is also evidence indicating that increased T lymphocyte infiltration has a tight association with a poor prognosis [23]. A previous study has shown that a high CD4T cell density is correlated with adverse outcomes in patients with bladder cancer [24]. Moreover, CD4T cells have been reported to enhance CD8T cell recruitment and infiltration into tumors [25]. These findings are in line with our results.

The efficiency of antigen processing and presentation is fundamental in determining whether the immune response will be effective against tumor cells. We compared the APPMS between the two groups to evaluate this process by applying the GSVA method. A higher APPMS was found with low expression, suggesting that SNF5 is negatively related to antigen processing and presentation. GSEA also confirmed that the MHC pathway, TCR activation pathway and T cell activation pathway were activated in the context of low SNF5 expression. Taken together, the findings for the GSVA, GO analysis and landscape of immune cell infiltration indicate that low SNF5 expression promotes the proliferation and activation of T cells and enhances tumor infiltration and antigen processing and presentation. Next, we explored the expression of immune checkpoint molecules. PD-1 and PD-L1 expression was not significantly different between the groups, but CTLA4 expression was significantly increased in the low SNF5 expression group. The GSEA results also confirmed this. Finally, the prediction of ICB response based on the TIDE algorithm was applied and suggested that the low-expression group might be more sensitive to immune checkpoint therapy. Overall, these results could be interpreted as the mechanism underlying the poor outcomes in patients with low expression, implying that an anti-CTLA4 antibody would show clinical promise in patients harboring low SNF5 expression.

In the present study, we acknowledge some limitations. This exploration was a retrospective analysis mainly based on publicly available data. Second, the samples used for verification were limited. Therefore, further prospective studies with a large number of samples are still required.

Conclusions

In conclusion, SNF5 is an independent prognostic biomarker for BC, and low expression confers adverse outcomes. SNF5 is associated with the immune response to tumors. Low SNF5 expression can enhance tumor infiltration by immune cells, the abilities to process and present antigens and TCR activation. Importantly, low SNF5 expression elevates CTLA4 expression, but does not modify the expression of PD-1 or PD-L1. These findings could lay the foundation for the clinical use of anti-CTLA4 therapy based on the SNF5 expression pattern in bladder cancer.

Abbreviations

APPMS

antigen processing and presentation machinery score;

AUC

area under the curve;

BC
Bladder cancer; DC:dendritic cells;
CTL
cytotoxic T lymphocyte;
DEGs
differentially expressed genes;
FDR
false discovery rate;
GO
gene ontology;
GSVA
gene set variation analysis;
GSEA
gene set enrichment analyses;
HRP
horseradish peroxidase;
ICB
immune checkpoint blockade;
IIS
immune infiltration score;
ImmuCellAI
Immune Cell Abundance Identifier;
KEGG
Kyoto Encyclopedia of Genes and Genomes;
MSigDB
Molecular Signatures Database;
PD-1
programmed cell death 1;
PD-L1
programmed cell death ligand1;
ROC
receiver operating characteristic;
SMARCB1
SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily B, Member 1;
TCGA
The Cancer Genome Atlas projects
TIDE
Tumor Immune Dysfunction and Exclusion;
TIICs
Tumor-infiltrating immune cells;

TILs
tumor-infiltrating lymphocytes;

Declarations

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Authors' contributions

Data analysis and coding were performed by HD. JZ and SL collected samples and conducted immunohistochemistry. LW, JS, YH and XZ prepared and made the figures and tables. JY and ZC reviewed and revised the manuscript. All the authors read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The current study was approved and consented by the Institutional Review Board (IRB 2012XLC02) of Southwest Hospital. Informed consent of all patients was consistent with the Helsinki Declaration.

Consent for publication

Not applicable.

Competing Interests

The authors declare no competing financial interests.

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Figures

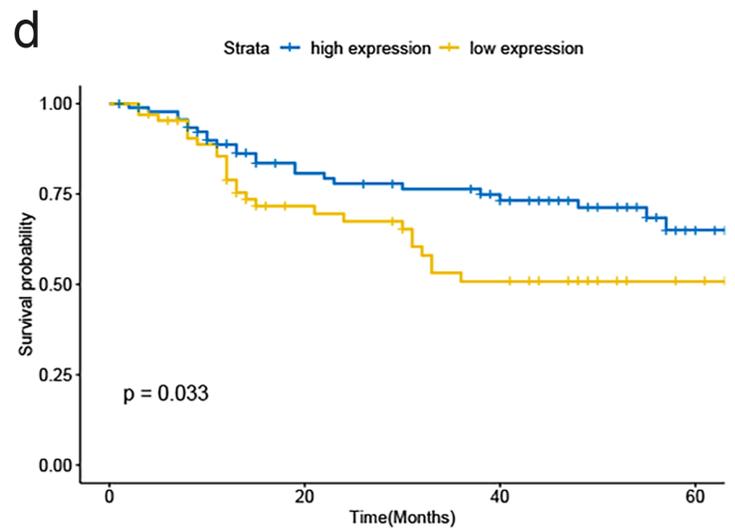
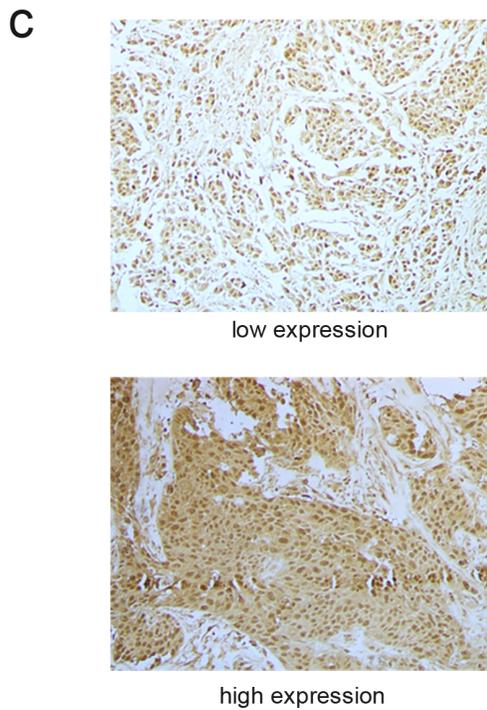
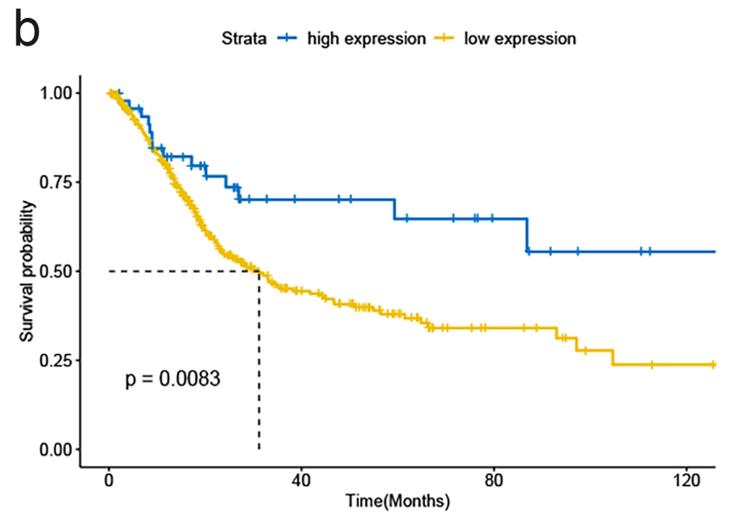
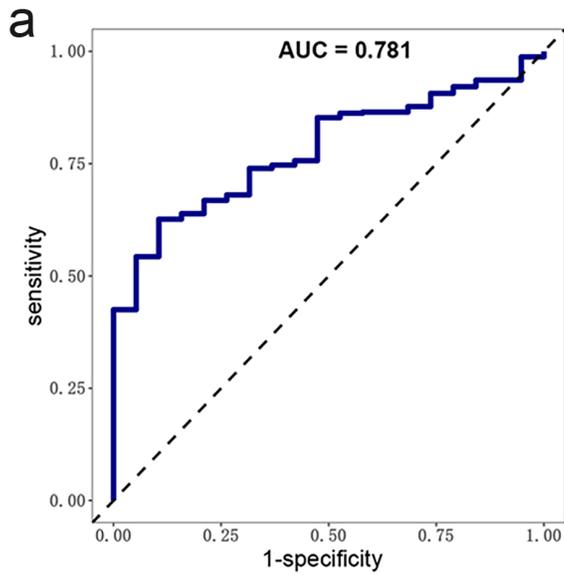


Figure 1

SNF5 was associated with prognosis of BC. a. ROC curves of SNF5 expression to predict BC in TCGA. b. Levels of SNF5 expression and survival based on TCGA. c. Representative immunohistochemical pictures of SNF5 from BC tissues. d. Further Validation of the correlation between SNF5 expression and prognosis of BC patients from our department.

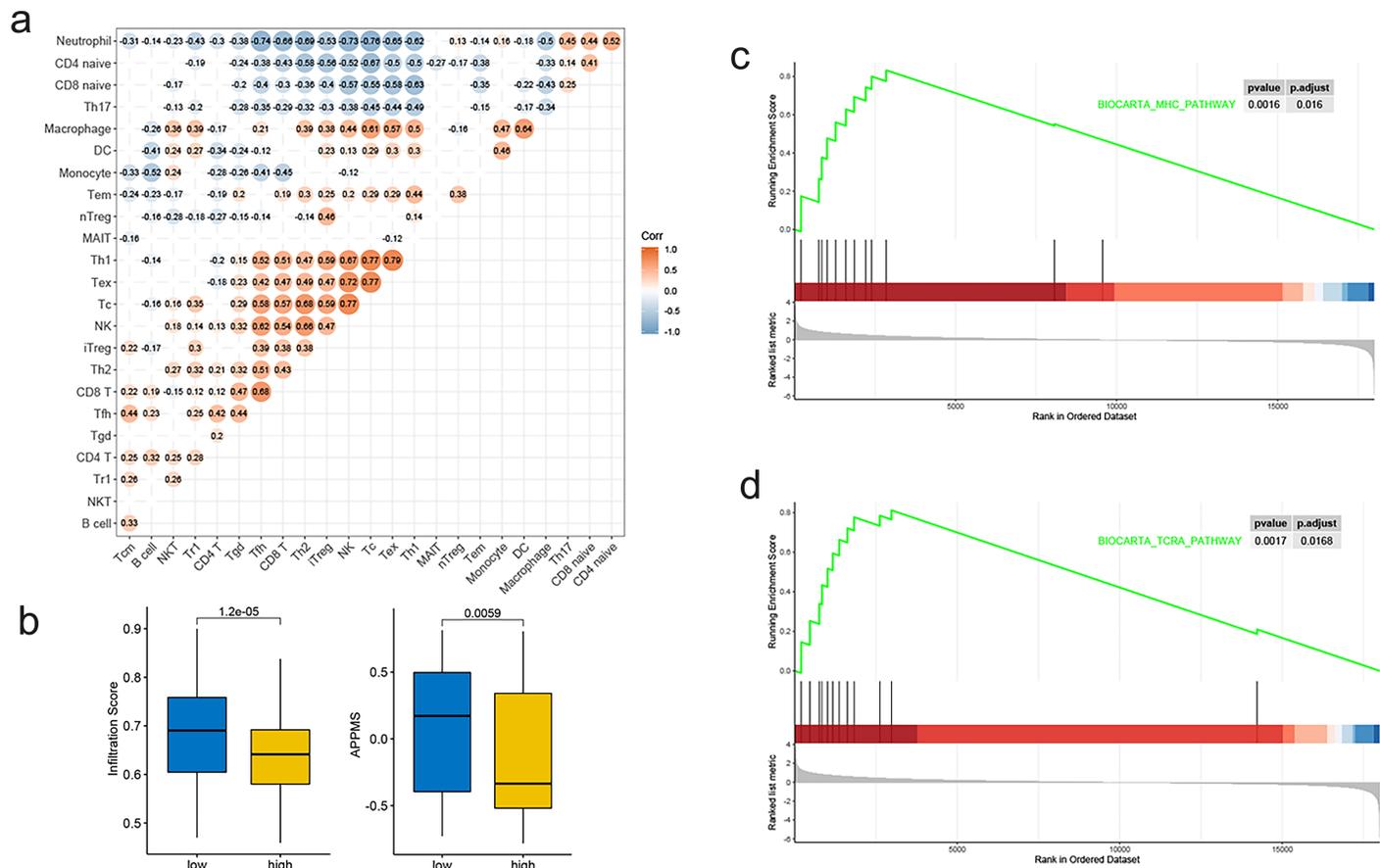


Figure 3

The associations of SNF5 with antigen processing and presentation. a. The correlations of different TIICs. b. The evaluations of infiltration score and APPMS. c. MHC pathway was enriched in low SNF5 expression group. d T-cell receptor activation pathway was enriched in low SNF5 expression group.

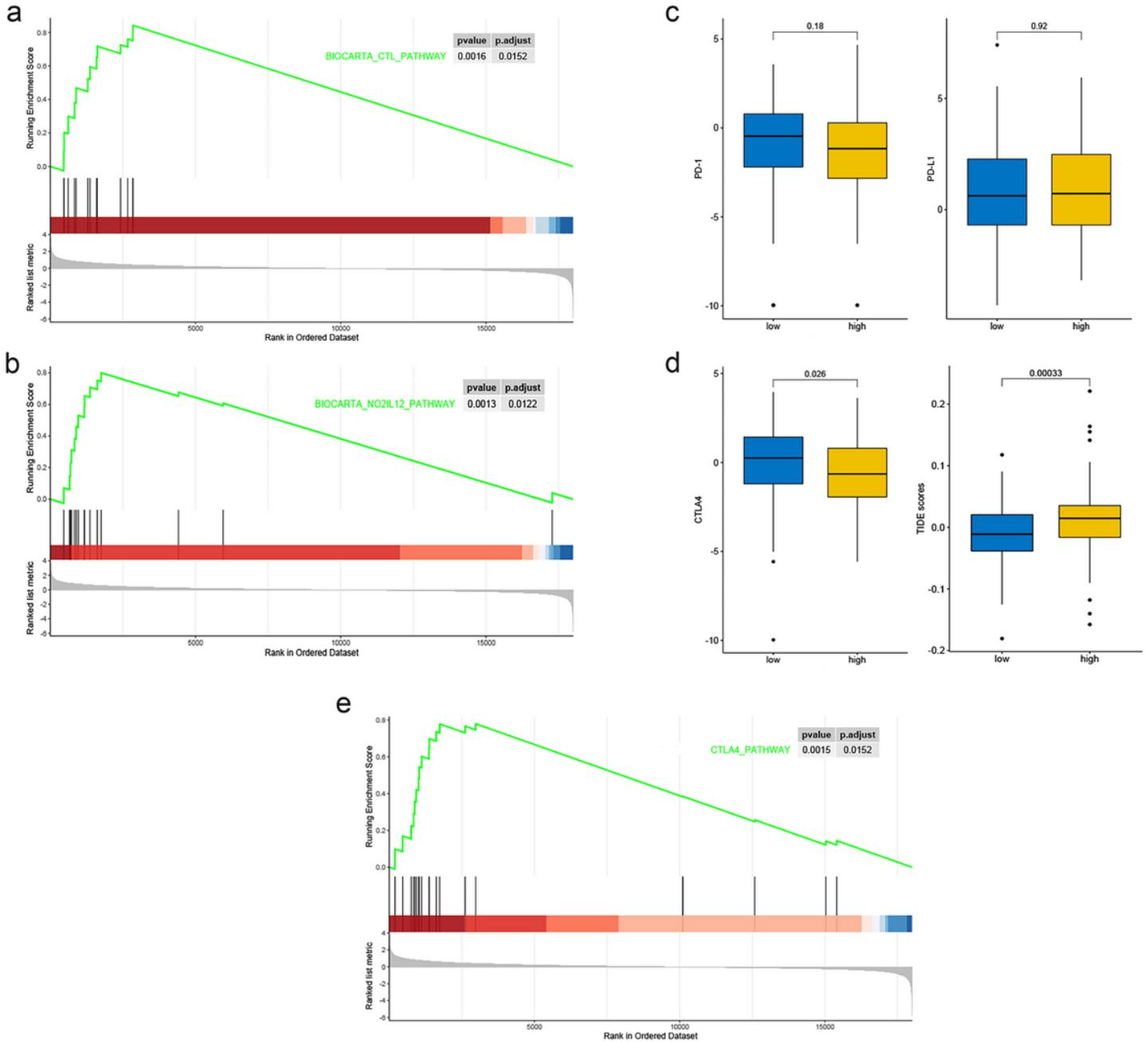


Figure 4

Antitumor responses and immune checkpoints induced suppressive response. a. Cytotoxic T lymphocyte (CTL) pathway was enriched in low SNF5 expression b. NO2-dependent IL12 pathway in NK cells was enriched in patients with low SNF5 expression c. Comparison of expression levels of PD-1 and PD-L1 in the low- and high-expression groups. d. Expression of CTLA-4 in low- and high-expression groups. e. CTLA-4 pathway was enriched in low SNF5 expression group.

Supplementary Files

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

- [Additionalfile2.TableS2.xlsx](#)
- [Additionalfile1.TableS1.xlsx](#)