B7-H6 is a Prognostic Biomarker for Human Renal Clear Cell Carcinoma and Correlates with Immune Cell Infiltration

Ziling Sun
Soochow University Medical College
Hao Qiu
Soochow University Medical College
Shangshang Gao
Soochow University Medical College
Jiamin Wang (wangjiamin@suda.edu.cn)
Soochow University Medical College https://orcid.org/0000-0002-7887-9970

Research Article

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Abstract

The B7 homolog 6 (B7-H6) is a newly discovered co-stimulatory molecule of the B7 family, which found to be widely expressed in various tumors. The purpose of this study is to identify the prognostic significance of B7-H6 and its relation with immune cell infiltration in human clear cell renal cell carcinoma (ccRCC). Using The Cancer Genome Atlas (TCGA) database, Gene Expression Profiling Interactive Analysis (GEPIA), Tumor Immune Estimation Resource (TIMER) database and Kaplan-Meier plotter database, we explored B7-H6 expression and its prognostic significance of ccRCC. Finally, the association between B7-H6 expression and tumor infiltration immune status was estimated via TIMER and CIBERSORT. We found that B7-H6 was remarkably elevated in ccRCC than normal kidney tissues and adjacent tumor-free tissues. Patients with high B7-H6 expression had favorable overall, disease-free survival and progress-free interval. Univariate and multivariate Cox analyses demonstrated that B7-H6 expression was an independent protective factor for overall survival in ccRCC patients.

Moreover, the expression of B7-H6 was correlated with differential immune cell infiltration and various immune biomarkers. This study reveals that increased B7-H6 expression correlates with favorable prognosis in ccRCC. It could be a novel clinical biomarker and provide new insights for future treatments in ccRCC.

Introduction

Kidney renal clear cell carcinoma (ccRCC or KIRC) is the most common subtype of kidney cancer with high morbidity and mortality. Despite the growing understanding of carcinogenesis, improved early diagnosis and ameliorative treatment strategies for patients with ccRCC, the prognosis of patients still has not essentially improved, up to a third of patients will present as or develop metastases. With a high tumor recurrence rate after resection and 5-year survival rate of ccRCC is only about 10% [1–4]. Therefore, there is an urgent need to further explore the molecular mechanisms underlying ccRCC, which may be beneficial to develop new therapeutic strategies to improve long-term survival in patients with ccRCC.

The natural cytotoxicity triggering receptor 3 (NCR3LG1), also known as the B7 homolog 6 (B7-H6), is a newly discovered member of the B7 family [5]. Studies found that human B7-H6 was not constitutively expressed in normal tissues but selectively expressed in a variety of tumors, including lymphomas[6], hepatocellular carcinoma[7], gastric carcinoma[8], brain cancer[9, 10], ovarian cancer[11], breast cancer[12], cervical carcinoma [13]. B7-H6 protein has been reported to be a ligand for the NK-cell receptor NKp30, which is an activating receptor of NK cells. NK cells eliminate B7-H6 expressing tumor cells directly by cytotoxicity or indirectly by cytokines release [5, 14]. Additionally, the expression levels of B7-H6 were significantly correlated with a variety of clinical tumor parameters of patients, such as tumor stage, grade, and overall survival time [15, 16, 13]. Recent studies also revealed that B7-H6 protein was involved in the progression of human cancer through multiple mechanisms [17]. However, the interrelation between B7-H6 expression, tumor immunity, and prognosis of patients with ccRCC remains unclear.

This study was conducted to investigate the prognostic potential of B7-H6 and its association with tumor infiltration status. Using multiple databases, B7-H6 expression was examined, its prognostic significance was also assessed. The relationship between B7-H6 expression and tumor immune cell infiltration was also analyzed. Our study suggests that B7-H6 has the potential to become a novel predictor to evaluate prognosis and immune infiltration for ccRCC patients. These results may provide a basis for exploring B7-H6 as a potential new target for immunotherapy of ccRCC.

Materials And Methods

Data and patients

ccRCC patients datasets (539 ccRCC samples and 100 normal samples), including transcriptome sequencing data and corresponding clinical information, were extracted from The Cancer Genome Atlas (TCGA) KIRC dataset (http://cancergenome.nih.gov/tcga/) and GTEx database (https://www.gtexportal.org/home /) utilizing the R/Bioconductor/TCGA package in May 2021. Meanwhile, the corresponding clinicopathological and survival information were also acquired from the TCGA database and integrated into the RNA-seq data.

Prognosis Analysis in patients with ccRCC

In order to evaluate the significance of B7-H6 expression level in the prognosis of ccRCC, survival package in R computing language (version 3.6.3), The Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html) and KM plotter databases (https://kmplot.com/) were applied separately. The median value of B7-H6 expression was utilized to identify high/low NCR3LG1 expression patients and the *p* value was set as 0.05.

Systematic Analysis of Immune Infiltration

The Tumor Immune Estimation Resource (TIMER) (https://cistrome.shinyapps.io/timer/) is a comprehensive tool providing immune infiltrates analysis across TCGA tumors [18]. Immune cell infiltration and immune biomarker expression correlated with B7-H6 expression were evaluated with Spearman's correlation analysis using the TCGA KIRC dataset. Since ssGSEA can determine the immune cell population in a tumor sample according to gene expression data [19,20], we estimated the Immune-cell infiltration levels in ccRCC tumors using ssGSEA with the gsva package in the R software. Spearman rank correlation analysis was then performed to examine relationships between B7-H6 gene expression and corresponding immune-cell infiltration levels.

To further explore relationships between expression of B7-H6 and possibly gene biomarkers of tumor-infiltrating immune cells. The gene markers included markers of $CD8^+$ T cells, T cells (general), B cells, T-helper 1 (Th1) cells, T-helper 2 (Th2) cells, follicular helper T (Tfh) cells, T-helper 17 (Th17) cells, regulatory T (Treg) cells, Eosinophils, neutrophils, natural killer (NK) cells, dendritic cells (DC), monocyte, Macrophage and exhausted T cells.

Statistical analyses

Most of the analyses were conducted using R software (version 3.6.3). Univariate and multivariate Cox regression analyses were conducted to verify the correlations between NCR3LG1 expression and survival. Some statistical tests were performed with IBM SPSS Statistics 26.0. A standard Student's t-test, paired t-test or Mann-Whitney U-test was used to compare the difference within two groups. ANOVA analysis or Kruuskal-Wallis test was used to compare difference among more than two groups. *p*-value < 0.05 was considered statistically significant. The gene expression correlation was accessed by Spearman's R and statistical significance. The absolute value of R greater than 0.1 was considered to be relevant and *p*-value < 0.01 was considered statistically significant.

Results

B7-H6 is aberrantly expressed in tumors

To explore the difference of B7-H6 expression between tumors and normal tissues, we compared the messenger RNA (mRNA) expression levels of B7-H6 between tumors and normal tissues of various cancer types from the TCGA database and GTEx. The results showed that compared to correspondingly normal tissue, B7-H6 expression was significantly higher in 9 types of tumor tissues (CHOL, COAD, ESCA, KICH, KIRC, KIRP, PAAD, READ and STAD) while significantly lower in 15 types of tumor tissues (ACC, BLCA, CESC, GBM, LAML, LIHC, LUAD, LUSC, OV, PRAD, SKCM, TGCT, THCA, UCEC and UCS) (Fig. 1a).

B7-H6 expression in ccRCC tumor samples (n = 539) and normal samples (n = 100) was also compared and the result demonstrated that B7-H6 expression was markedly increased within ccRCC cancer samples compared to normal kidney samples (Fig. 1b). Furthermore, the expression of B7-H6 in 72 paired ccRCC samples and adjacent normal samples was also analyzed, we found that the B7-H6 expression in ccRCC tissues was significantly upregulated compared with the expression in the adjacent normal tissues (Fig. 1c).

Association Between B7-h6 Expression And Clinicopathological Characteristics In Ccrcc Patients

The B7-H6 expression data and clinicopathological information from 539 patients with ccRCC were obtained from the TCGA database KIRC cohort for analysis. The ccRCC patients in TCGA KIRC cohort were divided into B7-H6 high-expression and B7-H6 low-expression groups with the median value of B7-H6 expression as a cut-off value. The relationship between the clinical parameters and expression level of B7-H6 was shown in Fig. 2. The results showed that the mRNA expression level of B7-H6 gradually decreased with the increase of tumor stage, histologic grade and pathologic stage in ccRCC (Fig. 2a, 2c and 2d). The expression of B7-H6 in patients with distant metastasis was significantly lower than patients without distant metastasis. Still, the expression level was no significant difference between

ccRCC patients with or without lymphatic metastasis (Fig. 2c and 2b). The study also found that the expression of B7-H6 was significantly higher in the survive group than in the deceased group. We then analyzed the correlations between clinical parameters and B7-H6 expression and found that B7-H6 expression was significantly correlated with T (tumor) stage ($p < 0.001$), M (distant metastasis) stage ($p < 0.001$), Pathologic stage ($p < 0.001$), gender ($p = 0.025$) and as well as age ($p = 0.043$). (Table 1).

The up-regulated B7-H6 expression, younger age (< 60) and negative distant metastasis are independent prognostic factors of favorable prognosis.

High B7-h6 Expression Is Associated With Favorable Survival In Ccrcc

Overall survival (OS), disease specific survival (DSS), relapse free survival (RFS) and progression free survival (PFS) are four common prognostic monitoring indexes used to basically summarize the prognosis and survival of cancer patients. To explore the potential prognostic significance of B7-H6 expression in ccRCC, different datasets were used to analyze the patient survival between low and high B7-H6 expression level groups in ccRCC patients. Clinical data for ccRCC were downloaded from TCGA database and analyzed by R software. The result showed that high B7-H6 expression was significantly positively associated with the OS, DSS and progress free interval of patients with ccRCC (Fig. 3a, 3b and 3c). Using the Kaplan-Meier plotter database, we found that the high expression level of B7-H6 was significantly correlated with the favorable prognostic survival rate (HR = 0.35, $p = 3.3e\text{-}10$, Fig. 3d). We also generated survival curves for overall survival and disease-free survival based on GEPIA using the log-rank test and the Mantel-Cox test. High B7-H6 expression was significantly associated with favorable OS and DFS (Fig. 3e, 3f). These results indicated that low expression of B7-H6 may be one of the causes of poor prognosis, and this gene could be a prognostic biomarker for ccRCC patients.

To explore whether low expression of B7-H6 is an independent risk factor for OS in ccRCC patients, univariate and multivariate Cox analyses were performed. The univariate Cox analysis indicated that low B7-H6 expression was associated with a worse overall survival time. Other clinical parameters, such as Age, Histologic grade (G3&G4 vs. G1&G2), Pathologic stage (Stage III & Stage IV vs. Stage I & Stage II), T stage (T3 & T4 vs. T1 & T2), N stage (N1 vs. N0) and M stage (M1 vs. M0) also correlated with the overall survival time (Table 2). For verifying the prognostic value of B7-H6 in ccRCC patients, we performed the multivariate analysis. The results showed that only B7-H6 expression level and M stage (M1 vs. M0) were independently associated with the overall survival time, which means that B7-H6 expression level in evaluating patients' clinical prognosis is superior to T stage, N stage and Pathologic stage. These findings indicated that B7-H6 expression was an independent protective factor for OS in ccRCC patients (HR = 0.477, $p = 0.002$, Table 2).

Correlation Analysis Between B7-h6 Expression And Tumor Infiltrating Immune Cells

Tumor infiltrating immune cells affect the antitumor efficacy and survival of patients in various cancers [21, 22]. ccRCC is one type of immunogenic cancers with abundant infiltrating immune cells [1, 23]. Therefore, we analyzed the correlation of B7-H6 expression with tumor infiltrating immune cells and tumor purity with TIMER database. The results displayed that the B7-H6 expression level had obviously positive correlation with tumor purity(r = 0.184, p = 6.72e-05), infiltrating levels of B cells (r = 0.187, p = 5.44e-05), $CD4^+$ T cells (r = 0.143, p = 2.05e-03), macrophages (r = 0.281, p = 1.38e-09), neutrophils (r = 0.224, p = 1.24e-06), and dendritic cells (r = 0.201, p = 1.49e-05) in ccRCC, but no significant association with $CD8^+$ T cells (Fig. 4a).

To further determine the effect of B7-H6 expression on tumor immune cell infiltration, we performed ssGSEA to assess the association between the immune cell infiltration pattern and B7-H6 level based on transcriptome profiling data for 539 KIRC cohort in TCGA database. Spearman correlation analyses revealed that high B7-H6 expression was mainly associated with low infiltration of some immune cell types (Fig. 4b), especially Treg cells and cytotoxic cells. We also observed positive correlations of B7-H6 expression level with infiltration of several types of immune cells, such as Eosinophils, central Memory T (Tcm) cells, and T helper 17 (Th17) cells (Fig. 4b).

Furthermore, the infiltrating proportions and differences of immune cells between the B7-H6 high and B7-H6 low groups were estimated via the CIBERSORT tool. The results displayed that Eosinophils (p < 0.001), central Memory T (Tcm) cells (p < 0.001), T helper 17 (Th17) cells (p < 0.001), neutrophils (p < 0.001) and Th cells (p < 0.001) shared a higher proportion in B7-H6 high expression group compared with low expression group (Fig. 4c). While the proportion of Treg cells, cytotoxic cells, CD56bright NK cells, Th1 and Th2 cells were significantly lower (p < 0.001) (Fig. 4c). We further investigated if the expression of B7-H6 was associated with immune biomarker sets of different immune cells in ccRCC. As expected, a significant correlation was obtained between the expression of B7-H6 and most of the immune markers of immune cells in ccRCC after tumor purity modulation was performed (Table 3). Results showed that B7-H6 expression was strongly linked to STAT5B (Treg cell), STAT6 (Th2 cell), and STAT3 (Th17 cell) (Fig. 5n and Fig. 5o). B7-H6 was also positively linked to STAT1, TNF-α (Th1 cell), IRF5, INOS (M1 Macrophage), CD163, MS4A4A (M2 Macrophage), most markers of Neutrophils (CD66b, CD15, CD11b), some markers of dendritic cell (HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-DPA1, BDCA1, BDCA4, CD11c and CD144) (Table 3). Moreover, B7-H6 levels showed a negative association with markers of natural killer cell (KIR2DL4, XCL1and CD7) and B cell (CD19 and CD79a) (Fig. 5). Since B7-H6 expression was significantly negatively correlated to infiltration of NK cells, we then evaluated the correlation between B7-H6 and its receptor NKp30 (NCR3) of NK cells. The analyses revealed that B7-H6 expression was inversely correlated with NKp30 expression (r = -0.130, p = 0.002), indicating a potential NK inhibitory role for B7-H6 in ccRCC (Fig. 4). In addition, B7-H6 expression in ccRCC showed a positive correlation with TIM-3 in T cell exhaustion but a negative association with PD-1, LAG3 and GZMB (Table 3 and Fig. 5).

Overall, the results demonstrated that B7-H6 had a complicated relationship with immune cell infiltration in ccRCC.

Discussion

Although significant progress in diagnosis and treatment has been made over the past decades, ccRCCs are still incurable and the 5-year survival remains poor. Therefore, efficient prognostic indicators and novel therapeutic strategies are urgently needed. Immunotherapy including targeting immune checkpoint molecules has been proved to have clinical value and may provide potential survival benefits for patients with ccRCC [1]. Studies focusing on the immunoregulatory molecules that regulate tumorigenesis or tumor immunity may provide potential therapeutic strategies for patients with ccRCC.

As an immune checkpoint molecule of the B7 family, B7-H6 (NCR3LG1) performs complex functions during tumorigenesis and tumor progression. On the one hand, B7-H6 protein induces tumorigenesis by promoting tumor proliferation and inhibiting apoptosis [16, 17, 14]. Conversely, Knockdown of B7-H6 inhibited cancer cell proliferation, migration and invasion in lymphoma, HCC and glioma [24, 6, 7, 25]. On the other hand, as an immune costimulatory molecule, B7-H6 activates the antitumor activity of NK cells and promotes the release of cytokines by binding to NK cell receptor NKp30 [5]. Due to its dual immunomodulatory nature, the clinical significance of abnormal B7-H6 expression in human cancer remains controversial. B7-H6 has been proved to be associated with low differentiation and high degree of malignancy in many tumors and is considered as an independent risk factor for poor prognosis and short survival in multiple tumors including human breast cancer, cervical cancer and esophageal squamous cell carcinoma [26, 13, 27]. However, there are also exceptions. For example, in our previous study of liver cancer, we found that the survival time of the high B7-H6 expression group was significantly longer than that of low B7-H6 expression group. Univariate and multivariate regression analysis showed that B7-H6 was one of the indicators with good prognosis [28]. Li and colleagues found that the high expression of B7-H6 was associated with good overall survival in patients with gastric cancer [29]. Similar to these findings, Thomas's latest study of B7-H6 expression in SCLC revealed that higher B7-H6 protein was associated with longer progression-free survival in SCLC [30].

The function of B7-H6 in tumorigenesis, progression and prognosis of ccRCC remains unclear. In this study, with several public databases we performed comprehensive analyses integrating mRNA expression with clinical outcomes and tumor immune infiltration to further explore the significance of B7-H6 expression in ccRCC. The aberrant expression levels of B7-H6 between tumor and normal tissues have been observed in multiple cancers. We compared B7-H6 expression in ccRCC with normal tissues using data from the TCGA and GETx databases. The data sets showed that B7-H6 expression in tumor tissues was significantly higher than that in normal tissues and paired non-tumor paracancerous tissues on the mRNA level. The expression level of B7-H6 decreased with the increase of tumor stage, histologic grade and pathologic stage. The Kaplan-Meier curve for OS showed that overexpression of B7-H6 predicted a favorable prognosis. Univariate and multivariate Cox analysis indicated that high expression of B7-H6 was a potential independent protective factor for ccRCC. Why the clinical significance of B7-H6

expression is different in cancers? The possible reasons are as follows. First, in different types of tumors, the pro-tumor function and anti-tumor function of B7-H6 are not exactly the same. Secondly, there is still a pivotal reason that besides cellular B7-H6, soluble B7-H6 is another form of B7-H6 generated by shedding from the extracellular domain of the membrane B7-H6 [31, 10, 32–34]. Studies found that the level of soluble B7-H6 in serum or other body fluids of tumor patients was significantly correlated with the severity of the disease including high-risk neuroblastoma (HR-NB) [10], stage IV melanoma [32]. Mantovani and colleagues' latest research about liver cancer confirmed that membrane B7-H6 of cancer cells was significantly reduced accompanied by significantly increased serum soluble B7-H6. However, the level of gene transcription did not change [35]. Soluble B7-H6 (sB7-H6) directly inhibited NK cell-mediated target cell killing or enhanced tumor immune escape by downregulating NKp30 receptor of NK cells [33, 35]. Given all this, higher expression of tumor cell membrane B7-H6 may be correlated with a lower level of soluble B7-H6 which could explain the longer overall survival in patients with higher B7-H6 expression [29, 35] .

Increasing evidences point to the importance of the immune status in the tumor microenvironment which significantly affects tumor progression, therapeutic efficacy and prognosis. [22, 36]. In-depth understanding the immune cell composition and molecular mechanism of tumor microenvironment is essential to develop effective therapeutic strategies for cancers [37]. Since ccRCC is an immunogenic tumor, accumulating evidences indicate that immunotherapy has been clinically validated as an effective treatment option for a variety of tumors including ccRCC [21], suggesting that immune cell infiltration may play pivotal function in the treatment of ccRCC. So we tried to determine the relationship between B7-H6 expression and immune infiltration status in ccRCC. Our study demonstrated a significant and complicated association between the B7-H6 expression and the status of tumor infiltration in tissues of ccRCC. The results revealed positive relationships between B7-H6 expression and infiltration of Eosinophils, Tcm and Th17 cells, but significant negative correlated to infiltration of Treg cell, cytotoxic cell and NK cell. Human eosinophils possess antitumor activity against a variety of cancer cell lines, including colorectal cancer [38, 39] and melanoma [40]. The number of tumor-infiltrating eosinophils in CRC was positively correlated with good prognosis for patients. Tumor-associated eosinophils also play a role in immunotherapy-induced vascular normalization [38]. T cell memory plays critical role in the antitumor response of solid tumors [41]. Tissue resident central memory T cells (Tcm) can be activated by tumor antigen and perform directly tumor cell killing [42]. T helper 17 (Th17) cells acts as a double-edged sword in cancer. Th17 cell was considered to promote neovascularization and tumor cell proliferation, as well as immunosuppressive activities. However, Th17 cell also can activate cytotoxic T cells, NK cells and neutrophils; promote interferon (IFN)-γ production to mediate antitumor immune responses [43, 44]. In this study, the higher B7-H6 expression positively correlated with eosinophils, Tcm and Th17 cells tend to have a better prognosis in patients with ccRCC

We also demonstrated that B7-H6 expression in ccRCC tumor cells was negatively associated with regulatory T cells (Tregs) cell proportion. Tregs are present in a large number of solid tumors and mainly associated with a poor prognosis, as their principal function is to inhibit the antitumor immune response contributing to immunosuppression. Previous studies indicated that Treg cells in tumors were associated

with a higher pathological stage and poor prognosis of ccRCC [45, 46]. Our finding suggested that B7-H6-expressing ccRCCs may exert weaker tumor immune escape mechanisms than B7-H6-negative tumor cells in the tumor microenvironment.

Across solid tumors, infiltration by $CD8^+$ T cells is associated with an improved prognosis, but, paradoxically in ccRCC, such infiltration has been associated with a worse prognosis [37]. As the first line defense of innate immunity, natural killer cells (NK cells) play pivotal role in anti-tumor immune surveillance. In this study, we found the expression of B7-H6 in ccRCC was negatively correlated with tumor infiltrating NK cells, both CD56bright and CD56dim NK cells. Given that B7-H6 proteins trigger NK cell function by identifying and interacting with NK cell activating receptor NKp30 (NCR3) [9, 35], we evaluated the correlation between B7-H6 and its receptor NKp30 of NK cells. The analyses also revealed a negative association between B7-H6 expression and NKp30, which indicating a potential NK inhibitory role for B7-H6 in ccRCC.

We also analyses the relationships between gene markers of these immune cells. B7-H6 expression was almost positively correlated with the biomarkers of Eosinophils, Tcm cells, Th17 cells, Neutrophils, Monocyte, Macrophage and dendritic cells (DC), and negatively associated with most of the biomarkers of NK cell, general T cell and B cell. Our analysis also revealed that B7-H6 expression was negatively associated with T cell exhaustion-associated markers including PD-1, LAG3 and GZMB, but positively correlated with Tim3 (HAVCR2). Interestingly, although we previously found that the expression of B7-H6 was negatively correlated with the proportion of tumor-infiltrating Treg cells in ccRCC, there was a strong significant positive correlation between B7-H6 expression and biomarker gene STAT5B of Treg cells. All these findings further illustrate the complexity of the composition and regulatory mechanism of tumor-infiltrating immune cells in ccRCC. The relationship between B7-H6 and tumor-infiltrating immune cells is far more complex. It is far from enough to focus on a specific immune molecule or some types of immune cells. Comprehensive and dynamic analysis based on big data will be more conductive to explore tumors' immune mechanisms and seeking more effective therapeutic strategies.

In the present study, multiple databases were used to determine the pattern of B7-H6 in ccRCC. We have confirmed that high B7-H6 expression was associated with favorable overall survival, and B7-H6 expression was strongly associated with tumor immune infiltration. There are still certain limitations in our research. First, our results have not been verified in clinical samples and cannot provide accurate clinical data. Second, our research has limited knowledge about the mechanism of B7-H6 in ccRCC. Moreover, the underlying mechanism of the relationship between B7-H6 expression and immune cell infiltration needs to be further clarified.

Conclusion

Acting as a tumor suppressor in the progression of ccRCC, B7-H6 emerges to be a novel prognostic/predictive biomarker for patients with ccRCC. In addition, the expression of B7-H6 is related to the immune infiltrating cells and affects the survival of ccRCC, which is worthy of further study.

Abbreviations

B7-H6, B7 Homolog 6; NCR3LG1, Natural Killer Cell Cytotoxicity Receptor 3 Ligand 1; mRNA, messenger RNA; ccRCC, clear cell renal cell carcinoma; RCC, renal cell carcinoma; KIRC, kidney renal clear cell carcinoma; OS, overall survival; DFS, disease-free survival; GEPIA, Gene Expression Profile Interactive Analysis; HR, hazard ratio; PFA, prognostic factor analysis; TCGA, The Cancer Genome Atlas.

Declarations

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Author contributions

Study design: JMW, SSG; Data acquisition: HQ, ZLS; Data analysis and interpretation: JMW, SSG, HQ; drafting the manuscript: JMW, ZLS. All authors approved the final version of the manuscript. All authors have read and approved the final version of the article to be published. All authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Data availability The datasets were downloaded from The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/), GTEx database (https://www.gtexportal.org/home /).

Code availability Not applicable.

Conflict of interest

All the authors declare that they have no conflict of interest.

Ethics approval The patients' information involved in our research were obtained from The Cancer Genome Atlas (TCGA) KIRC dataset and GTEx database. All the patients and treatments complied with the principles laid down in the Declaration of Helsinki in 1964 and its later amendments or comparable ethical standards. Informed consent Informed consent was confirmed by all the patients participated in the TCGA- KIRC dataset and GTEx database.

Consent to participate Not applicable.

Consent for publication All the authors consented to the publication of this research.

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Tables

Table 1
Summarizes the correlation between expression of B7-H6 and the ccRCC clinical features in TCGA KIRC cohort.

Clinical parameters	Low expression of B7-H6 (n = 269)	High expression of B7-H6 (n = 270)	P
Gender, n (%)			0.025
Female	80 (14.8%)	106 (19.7%)	
Male	189 (35.1%)	164 (30.4%)	
Age, n (%)			0.043
<=60	122 (22.6%)	147 (27.3%)	
> 60	147 (27.3%)	123 (22.8%)	
T stage, n (%)			< 0.001
T1	108 (20%)	170 (31.5%)	
T2	38 (7.1%)	33 (6.1%)	
T3	115 (21.3%)	64 (11.9%)	
T4	8 (1.5%)	3 (0.6%)	
N stage, n (%)			0.079
N0	118 (45.9%)	123 (47.9%)	
N1	12 (4.7%)	4 (1.6%)	
M stage, n (%)			< 0.001
M0	198 (39.1%)	230 (45.5%)	
M1	55 (10.9%)	23 (4.5%)	
Pathologic stage, n (%)			< 0.001
Stage I	105 (19.6%)	167 (31.2%)	
Stage II	29 (5.4%)	30 (5.6%)	
Stage III	75 (14%)	48 (9%)	
Stage IV	58 (10.8%)	24 (4.5%)	

Table 2
Univariable and multivariable analysis of the correlation of B7-H6 expression with OS in ccRCC patients using TCGA database KIRC cohort.

Characteristics	Total(N)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Gender (Male vs. Female)	539	0.930 (0.682–1.268)	0.648		
Age (> 60 vs. <=60)	539	1.765 (1.298–2.398)	**< 0.001**	1.613 (1.052–2.473)	**0.028**
Histologic grade G3&G4vs.G1&G2	531	2.702 (1.918–3.807)	**< 0.001**	1.601 (0.960–2.670)	0.072
Pathologic stage (Stage III&Stage IV vs. Stage I&Stage II)	536	3.946 (2.872–5.423)	**< 0.001**	1.169 (0.462–2.960)	0.742
T stage (T3&T4 vs. T1&T2)	539	3.228 (2.382–4.374)	**< 0.001**	1.479 (0.648–3.376)	0.353
N stage (N1 vs. N0)	257	3.453 (1.832–6.508)	**< 0.001**	1.404 (0.696–2.831)	0.344
M stage (M1 vs. M0)	506	4.389 (3.212–5.999)	**< 0.001**	2.692 (1.593–4.549)	**< 0.001**
B7-H6 (High vs. Low)	539	0.386 (0.279–0.534)	**< 0.001**	0.477 (0.299–0.760)	**0.002**

Table 3 is available in the Supplemental Files section.

Figures

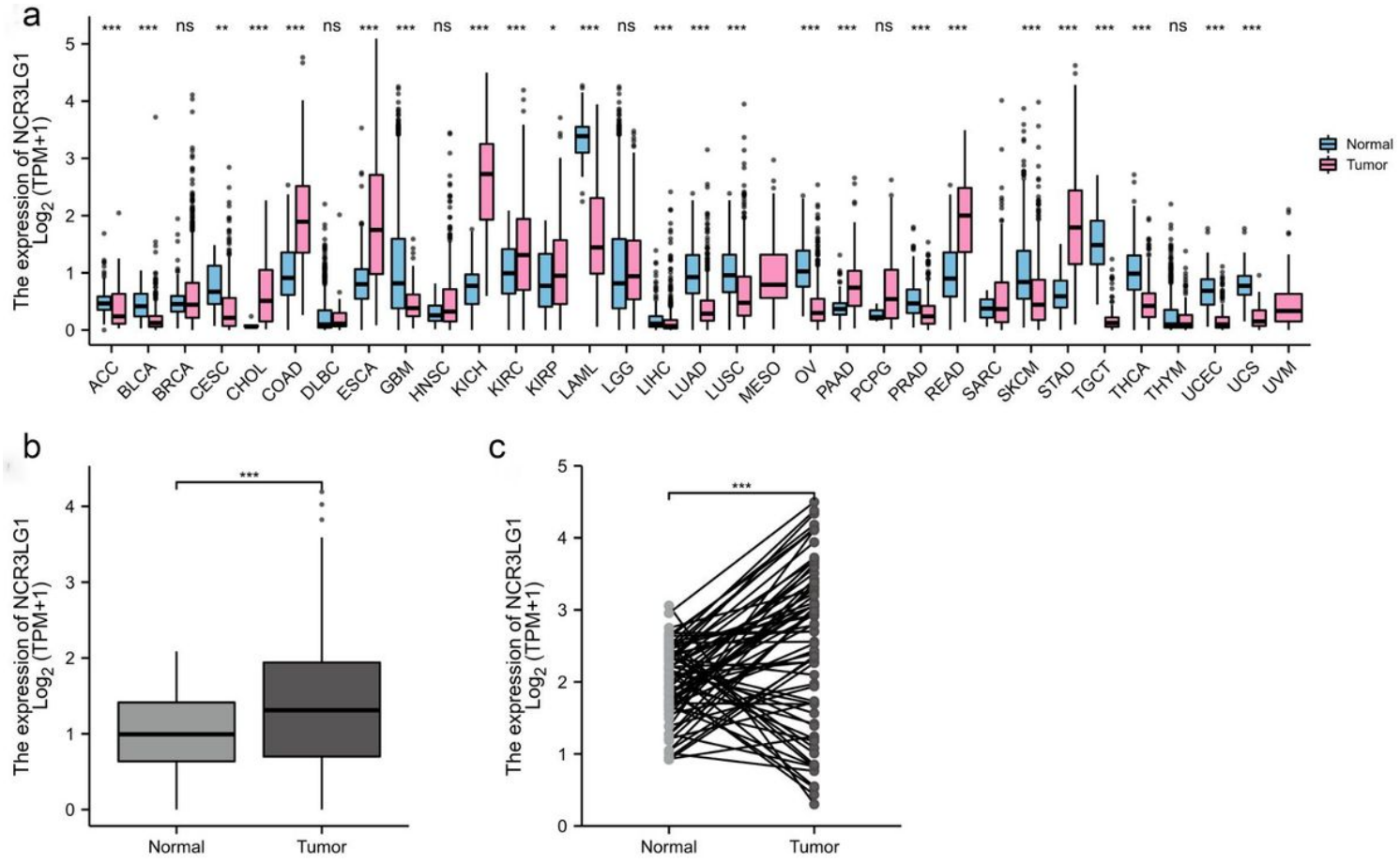

Figure 1

B7-H6 is aberrantly expressed in tumors.

(a) B7-H6 expression in tumor and normal tissues in TCGA and GTEx pan-cancer data.

(b) B7-H6 expression in tumor and normal tissues in ccRCC.

(c) Expression of B7-H6 in 72 paired ccRCC cancer tissues and adjacent normal tissues.

(*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

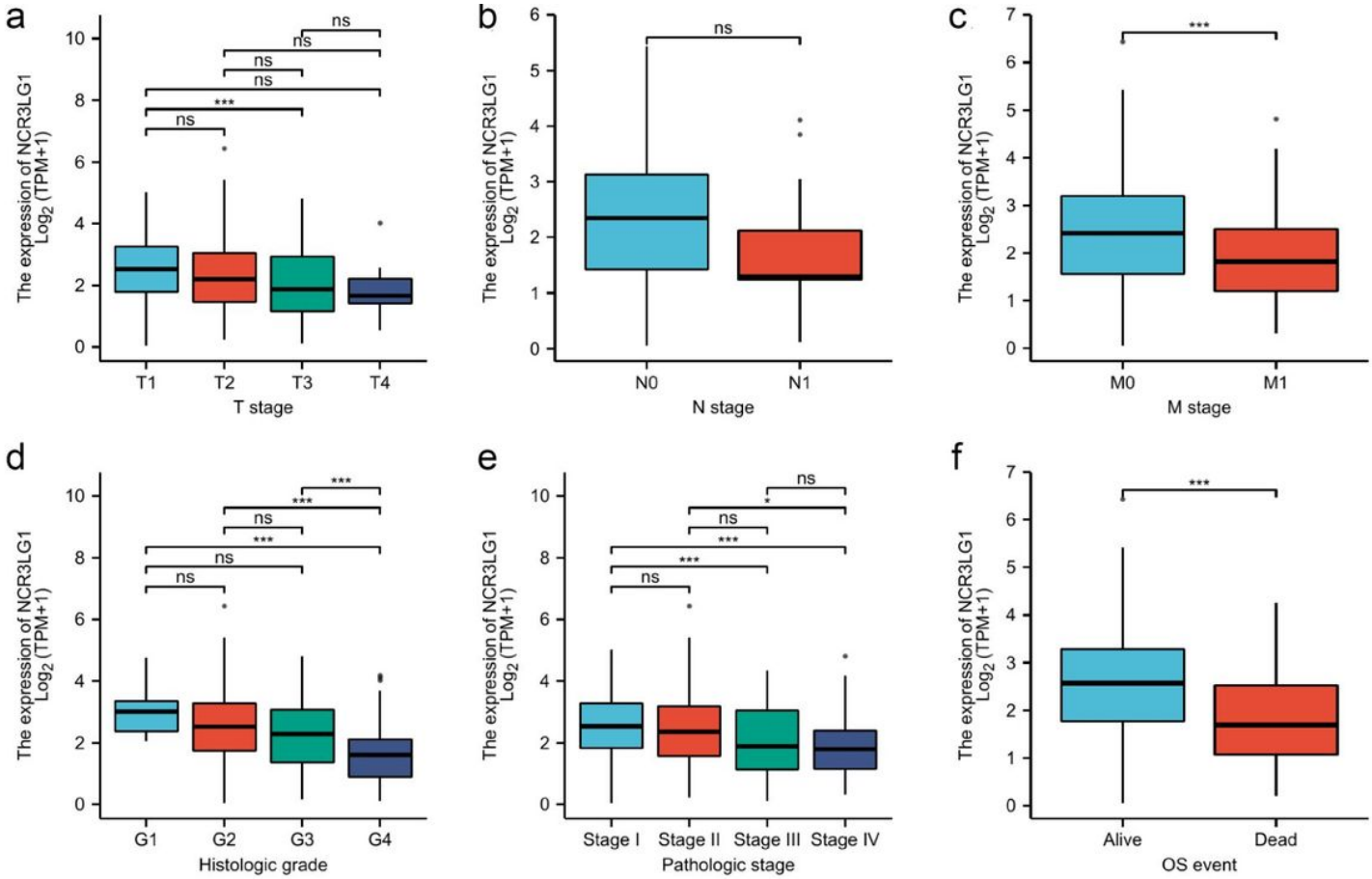

Figure 2

B7-H6 expression and association with clinicopathologic characteristics in ccRCC.

B7-H6 expression in the different (a) T stage, (b) N stage, (c) M stage, (d) histologic stage, (e) pathologic stage, (f) survival state.

(ns, no significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

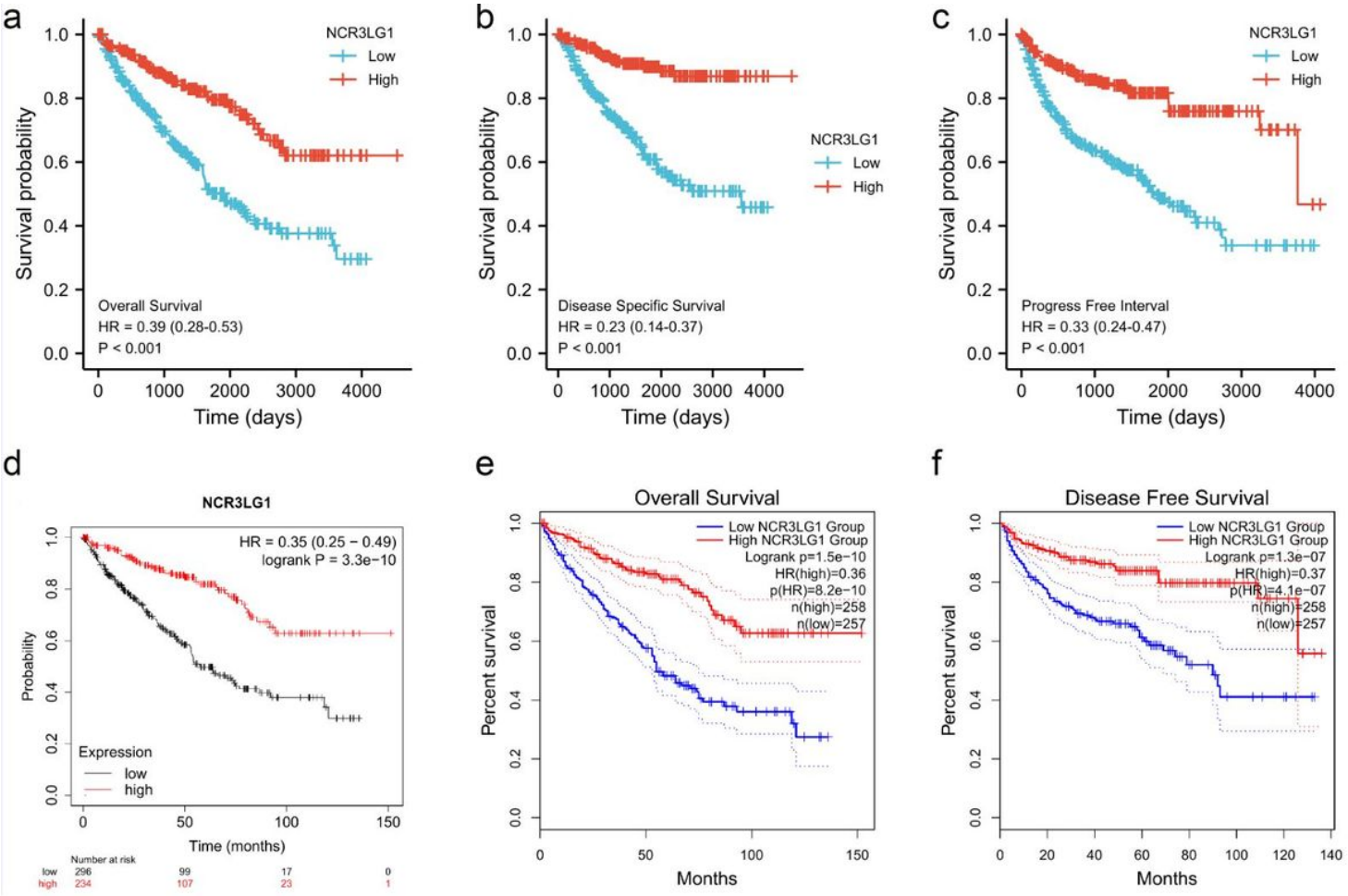

Figure 3

Correlation analysis between B7-H6 expression and prognostic survival in ccRCC patients.

Overall survival, disease specific survival and progress free interval in TCGA database (a, b and c)

OS in Kaplan–Meier Plotter database (d)

OS and DFS in GEPIA database (e, f)

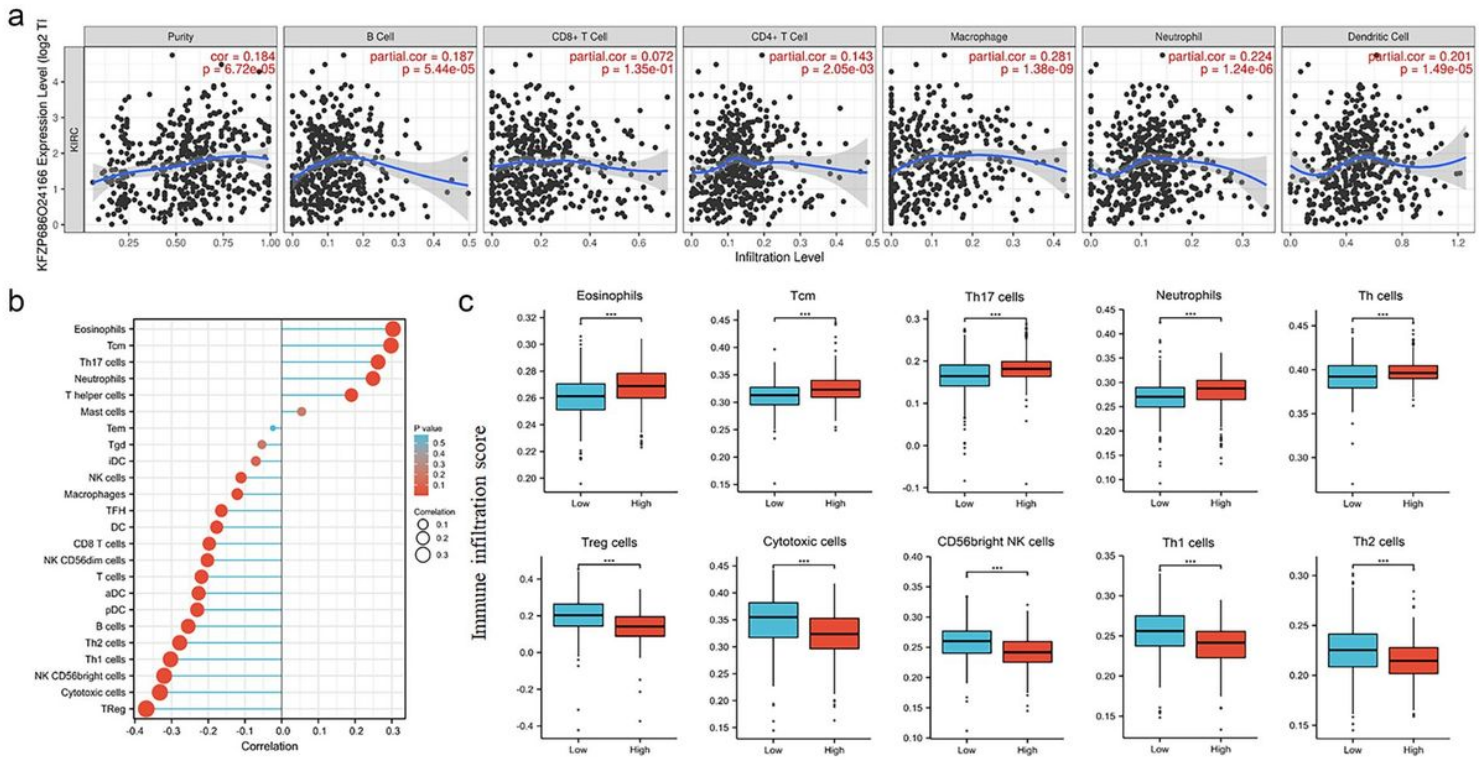

Figure 4

Correlation of B7-H6 expression with tumor immune cell infiltration in ccRCC

(a) Association between B7-H6 expression and tumor immune cell infiltration including purity, B cell, $CD8^+$ T cell, $CD4^+$ T cell, macrophage, neutrophil and dendritic cell in ccRCC.

(b) Correlation of B7-H6 expression with the infiltration levels for various immune cell types. Left, immune cells negatively correlated with B7-H6 expression; right, immune cells positively associated with B7-H6 expression.

(c) Immune cell infiltration level in the high B7-H6 expression group and low B7-H6 expression group in TCGA cohort.

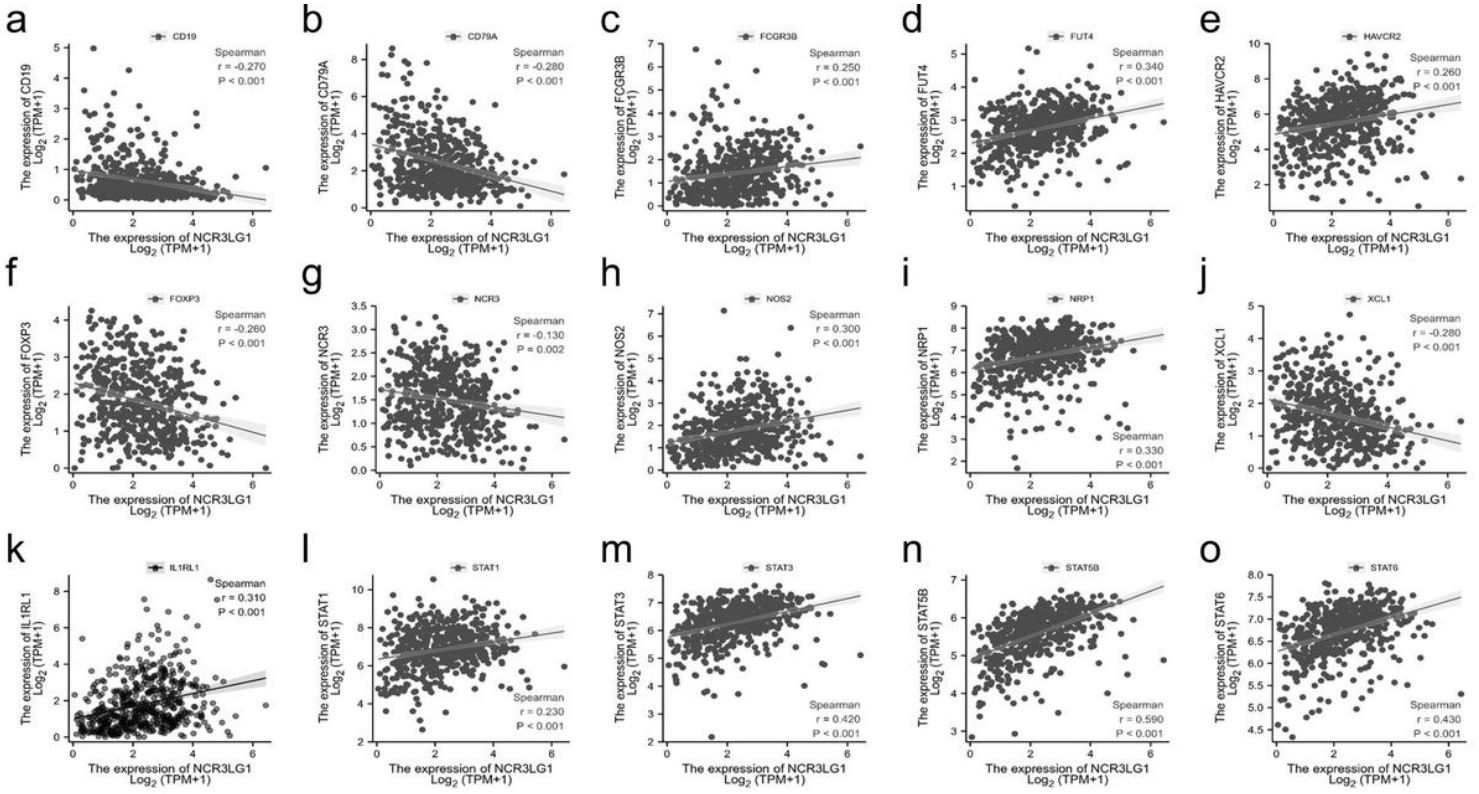

Figure 5

Correlation between B7-H6 expression and immune infiltration associated biomarker genes.

B cells: CD19 (a) and CD79A (b). Monocyte: CD16 (FCGR3B) (c), Neutrophils: CD15 (FUT4) (d), T cell exhaustion: TIM-3 (HAVCR2) (e), Treg: Foxp3 (f), STAT5B (n), NK cell: NKp30 (NCR3) (g), XCL1 (J), M1 Macrophage: NOS2 (h), Dendritic cell: NRP1 (i), Eosinophils: ST2/IL1RL1 (k), Th1: STAT1 (l), Th17: STAT3 (m), Th2 cell: STAT6 (o).

Supplementary Files

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

- Table3.jpg
- Table3a.jpg