Integrated analysis of the underlying immunomodulator and survival signature of STON1 gene in kidney renal clear cell carcinoma

Axiu Zheng

Guangdong Medical University

Jianrong Bai

Guangdong Medical University

Yanping Ha

Guangdong Medical University

Bingshu Wang

Hainan Medical University

Yuan Zou

Guangdong Medical University

Caixia Wu

Guangdong Medical University

Jingci Xing

Guangdong Medical University

Shaojiang Zheng

Hainan Medical University

Zhihua Shen

Guangdong Medical University

Botao Luo

Guangdong Medical University

Wei Jie (✉ wei_jie@hainmc.edu.cn)

Hainan Medical University <https://orcid.org/0000-0002-1869-1458>

Research Article

Keywords: STON1, kidney renal clear cell carcinoma, tumor immune microenvironment, immunomodulators, nomogram, survival, bioinformatics

Posted Date: August 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-847345/v1>

Abstract

Background Stonin 1 (STON1) is an endocytic protein but its role in cancer remains unclear. Here, we investigated the role of STON1 in kidney renal clear cell carcinoma (KIRC). Methods We undertook bioinformatics analyses of a series of public databases to determine the expression and clinical significance of STON1 in KIRC and focused on STON1-related immunomodulator and survival signatures. A nomogram model integrating clinicopathological characteristics and risk scores for KIRC was established and validated. Results Through TGCA and GEO databases, STON1 mRNA was found to be significantly downregulated in KIRC compared with normal controls, and decreased STON1 was related to grade, TNM stage, distant metastasis, and vital status of KIRC. Furthermore, OncoLnc, UALCAN, Kaplan–Meier, and GEPIA2 analyses supported that KIRC patients with high STON1 expression had better overall survival. STON1 was positively associated with mismatch proteins including MLH1, PMS2, MSH2, MSH6 and EpCAM, and was negatively correlated with tumor mutational burden. Interestingly, arm-level deletion of STON1 was clearly related to the abundance of immune cells and the infiltration abundance in the majority of 26 immune cell types that were positively related to STON1 mRNA level in the TIMER database. The TISIDB database revealed 21 immunostimulators and 10 immunoinhibitors that were obviously related to STON1 in KIRC. In univariate and multivariate Cox regression analyses, CTLA4 , KDR , LAG3 , PDCD1 , HHLA2 , TNFRSF25 , and TNFSF14 were screened to establish a risk score model, and the low-risk group had a better prognosis for KIRC. Furthermore, a nomogram integrating clinicopathological characteristics and risk score had better accuracy and practicability in predicating the survival of KIRC patients. Conclusions Decreased STON1 expression in KIRC leads to clinical progression and poor survival. Mechanically, loss of STON1 is associated with the aberrant immune microenvironment in KIRC. Integrated clinicopathological characteristics and risk score derived from STON1 -related immunomodulators can aid the prediction of KIRC survival.

Background

Renal cell carcinoma (RCC) is a type of cancer generally derived from renal tubular epithelial cells, with 73,820 new cases and 14,770 deaths reported in 2019 [1]. Most cases occur in developed countries, and the morbidity rate in men is two-thirds of that in women [2]. There are various subtypes of RCC according to the 2016 World Health Organization (WHO) classification of tumors of the urinary system, such as kidney renal clear cell carcinoma (KIRC), chromophobe renal cell carcinoma, papillary RCC, and collecting duct and renal medullary carcinoma [3], and KIRC accounts for 70% cases of RCC [4].

The current treatment strategy for RCC includes partial nephrectomy, radical nephrectomy, ablative therapies, and targeted therapeutics [5], and its pathogenesis has been widely explored; however, 25% of RCC patients still undergo disease progression or metastasis [6]. Recently, immune checkpoint blockade therapy was developed. Monoclonal antibodies against immune checkpoint blockade molecules, such as CTLA-4 (cytotoxic T lymphocyte antigen 4), PD-1 (programmed cell death 1), and PD-L1 (programmed cell death ligand 1), target the tumor immune microenvironment with an obvious objective response rate for KIRC [7]. Nevertheless, prolonged use of immune checkpoint blockers is often accompanied by toxicity,

including colitis/diarrhea, hepatitis, pneumonitis, myocarditis, myositis, encephalitis, and myasthenia gravis [8]. Thus, there is an urgent need to identify novel biomarkers to guide prognosis prediction and clinical management.

Stonins are an evolutionally conserved family that mediates the recovery and circulation of vesicles at neuromuscular junctions, and consists of Stonin1 (STON1, also known as germ line-specific transcription factor) and Stonin2 (STON2) [9]. The human STON1 protein consists of 735 amino acids and has a predicted molecular mass of 83 kDa[10]. Additionally, the level of *STON1* gene methylation was verified to be a prognostic marker for the progression of obesity and in the development of personalized treatment[11]. High expression of *STON1* as a potential mRNA biomarker in sperm was identified in cigarette smoke-exposed individuals [12]. Furthermore, STON1 has an irreplaceable role in regulating the endocytosis of neuron-glia antigen 2 (NG2). NG2 appears to be oncogene of glioblastoma and melanoma [13], but the potential functions of STON1 in mediating specific molecular mechanisms in various carcinomas remain entirely unexplored.

Over the past few years, various prognostic models have been constructed for the underlying mechanism of KIRC based on metabolism-related genes, long non-coding RNAs, alternative splicing variants, immune-related genes, RNA binding proteins, microRNA related genes, and autophagy-related genes [14–20]. In our current study, the independent prognostic value of *STON1* was validated using online bioinformatics databases. Subsequently, we identified several STON1-related immunomodulators with prognostic indication and used them to build a risk score model. Using this model, risk score was estimated in KIRC patients. Finally, a nomogram integrating clinicopathological parameters and risk score was established to maximize the convenience and efficiency for physicians in predicting the overall survival (OS) time of patients with KIRC.

Methods

Expression of *STON1* in KIRC

The Tumor Immune Estimation Resource (TIMER) database TIMER2.0 (<http://timer.comp-genomics.org/>) was used to evaluate the expression of *STON1* in pan-cancer data from The Cancer Genome Atlas (TCGA), including KIRC[21]. Furthermore, independent KIRC cohorts were downloaded from the Gene Expression Omnibus (GEO) database with the “GEO2R” online tool [22] (<https://www.ncbi.nlm.nih.gov/geo/>), including GSE16441 (platform: GPL6480), GSE16449 (platform: GPL6480), and GSE71963 (platform: GPL6480). STON1 protein expression was assessed using the UALCAN database [23] (<http://ualcan.path.uab.edu/index.html>).

Analysis of the relationships between STON1, clinical phenotype, and prognosis in KIRC

RNA sequencing and related clinicopathological data were downloaded from TCGA using UCSC Xena (<https://xena.ucsc.edu/>), an online explorer that allows users to explore functional genomic data sets for correlations between genomic and phenotypic variables. A total of 491 patients were enrolled in the

cohort after exclusion criteria were applied. The exclusion criteria were as follows: 1) patients without follow-up records; 2) patients without a diagnosis of KIRC; 3) primary tumor could not be evaluated; and 4) stage and grade were not reported. Finally, 491 patients were divided into high and low STON1 expression groups according to the median expression value of STON1. The OncoLnc database (<http://www.oncolnc.org/>) is a platform for immediate survival analysis with TCGA data[24]. The UALCAN database (<http://ualcan.path.uab.edu/>) can provide graphs and plots depicting expression profiles and patient survival information for protein-coding, miRNA-coding, and lincRNA-coding genes [23]. Kaplan–Meier Plotter (<https://kmplot.com/analysis/>) is an accessible online website with the purpose of identifying survival biomarkers[25]. We also analyzed the prognostic value of STON1 expression in KIRC patients using the Gene Expression Profiling Interactive Analysis (GEPIA) browser (<http://gepia2.cancer-pku.cn/#survival>) [26].

Correlation of tumor mutational burden, microsatellite instability, and STON1

The raw data of mRNA profiles in KIRC were downloaded from TCGA dataset. “R.utils”, “rjson”, and “edgeR” R packages were used to process the raw expression matrix. A marginal scatter plot was acquired by the “ggpubr” R package for the KIRC mRNA profile. The relationship between STON1 and tumor mutational burden (TMB) was displayed by the Assistant for Clinical Bioinformatics, an open integrated database (<https://www.aclbi.com/>).

Analysis of *STON1* mRNA levels, immune cells, and immunomodulators in KIRC

The TIMER database was employed to display the correlation between gene copy numbers of *STON1* and immune cells[27]. The Tumor and Immune System Interaction Database (TISIDB) (<http://cis.hku.hk/TISIDB/>) was used to evaluate the relationship between *STON1* and 28 immune cell types, as well as 69 types of immunomodulators[28]. Heatmaps and bubble graphs produced by the “ggplot2” R package intuitively presented *P*-values and Rho values for immune cells and *STON1* expression, while a violin diagram showed the relevance of STON1 and immune subtype in KIRC.

GO, KEGG analysis, and Integrated Network in KIRC

A total of 31 immunoregulators with statistical significance were uploaded into cBioPortal (<http://www.cbioportal.org/>) to identify genes that are co-expressed with immunomodulators[29]. Using the screened 57 co-expressed genes and 31 immunoregulators, gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analysis were performed on the DAVID online tool [30] (<https://david.ncifcrf.gov/>). A *P*-value of less than 0.05 was considered statistically significant. Results are presented as a bubble plot generated using the R package “ggplot2”. A protein interaction network was constructed by Cytoscape software, an open source for visualizing complex networks [31].

Univariate and multivariate Cox regression, Kaplan–Meier survival analysis, and establishment of a STON1-related immunoregulator prognostic signature in KIRC

The processed RNA-sequencing data of KIRC, which contained 504 samples, was randomly divided into a training set ($n=328$) and an internal validation set ($n=176$) at a proportion of 65% via the “caret” R-package. Using the “survminer” and “survival” R packages, univariate Cox analysis was applied to evaluate the correlation between immunomodulators and OS in the training set. Next, we entered 15 immunomodulators with statistical significance into a stepwise multivariate Cox regression model with the purpose of identifying independent prognostic biomarkers.

Risk score was calculated using the following formula:

$$\text{Risk score} = h_0(t) \times \exp \left(\sum \text{coefficient of gene (i)} \times \text{expression of gene (i)} \right)$$

Subsequently, the patients in the training set were stratified into a high-risk group and a low-risk group according to the median risk score. The Kaplan–Meier survival curve was applied to compare the survival outcomes among the two groups, employing R packages of survival, as well as the log rank test. Furthermore, we used a time-dependent ROC curve to evaluate the model accuracy with the “timeROC” R package. Using the “ggrisk” package of R software, the risk graph presented a landscape of the STON1-related immunoregulator prognostic signature.

Validation of STON1-related immunoregulator prognostic signature

To validate the stability of our signature, the risk score was calculated using the coefficient described above. Next, the validation set was also divided into high-risk and low-risk groups based on the median risk score. The Kaplan–Meier curve and log rank test presented the survival difference between the two groups, as well as a risk graph. A time-dependent receiver operating characteristic (ROC) curve further validated the accuracy of the STON1-related immunoregulator prognostic signature.

Construction and estimation of a nomogram based on risk score and clinicopathological parameters

Univariate Cox regression analysis was performed on the training set to screen variables with statistical significance using the “survival” and “survminer” packages of R software. Then, stepwise multivariate Cox regression analysis was executed with the screened variables that were derived from the univariate Cox regression analysis. The results were displayed in a forest plot using the “forest plot” of R package. An integrated nomogram was established with the training set based on stepwise multivariate Cox regression analysis to predict the survival probability at 1, 3, and 5 years, employing the “rms” package of R software. Subsequently, time-dependent ROC curves and calibration curves were used to measure the accuracy and stability of the nomogram in the training set and validation set, as well as the c-index. Although the ROC curve and calibration curve could thoroughly predict the accuracy and stability of the nomogram model, it could not reveal the benefit rate of patients in the model. Decision Curve Analysis (DCA) is a statistical method for evaluating the degree of benefit that KIRC patients can obtain. It is therefore an indispensable validation tool in addition to time-dependent ROC curves and calibration curves [32]. Using the “ggDCA” package in R software, DCA curves were plotted to validate our nomogram better than other clinical parameters for clinical benefit in the training and validation sets.

Statistical analysis

All mRNA expression was normalized by log2 transition. A columnar scatter plot was created by GraphPad Prism (version 8, GraphPad Software, CA, USA). Chi-squared test was performed using IBM SPSS Statistics (version 25) to analyze the association between *STON1* mRNA level and clinicopathological parameters. The correlation analysis between *STON1*, mismatch system, and TMB was performed using Pearson's test in RStudio software (version 4.0.3). For the Kaplan–Meier curve, log-rank test and Cox proportional hazard regression model analysis were performed using RStudio software (version 4.0.3). $P < 0.05$ was considered statistically significant.

Results

STON1 expression was significantly reduced in KIRC

We first identified the transcription level of *STON1* in different TCGA tumors with the TIMER2.0 database. A total of 61 human cancer types were explored. We found that most cancers, including KIRC, kidney renal papillary cell carcinoma (KIRP), kidney chromophobe (KICH), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC), showed decreased *STON1* mRNA, while cholangiocarcinoma (CHOL), glioblastoma multiforme (GBM), and head and neck squamous cell carcinoma (HNSN) displayed upregulated *STON1* mRNA levels (Figure 1a). Moreover, results from three GEO datasets—GSE16441, GSE16449, and GSE71963—also revealed aberrantly downregulated *STON1* mRNA in KIRC ($P < 0.01$, Figure 1b, 1c, 1d). All three series were normalized before the analysis (Figure S1). Finally, the protein level of STON1 in KIRC was also found to be reduced using the CPTAC database ($P < 0.0001$) (Figure 1e).

Correlation between *STON1* expression and clinicopathological characteristics of KIRC patients

The KIRC GDC Expression Matrix was downloaded from the UCSC Xena, which is one of the most comprehensive clinical oncology databases [33]. A total of 491 KIRC patients were included in the cohort after excluding non-KIRC diagnoses and missing clinical information, and were classified into a high expression group ($n = 246$) and a low expression group ($n = 245$) according to the median value of *STON1*. Notably, *STON1* mRNA levels were related to grade ($P = 0.022$), TNM stage ($P = 0.039$), distant metastasis ($P = 0.019$), and vital status ($P = 5.46 \times 10^{-6}$). However, no correlation was found between *STON1* mRNA levels and age ($P = 0.136$), sex ($P = 0.949$), invasion depth ($P = 0.074$), and lymph node metastasis ($P = 0.385$) (Table 1).

STON1 was a protective prognostic factor in KIRC

To further analyze the prognostic value of *STON1*, we performed a survival analysis using four oncological databases. The results indicated that *STON1* expression levels were strongly associated with OS in KIRC. Patients with high expression of *STON1* had a better outcome compared with those with low levels of *STON1* in the OncoLnc database (log rank $P = 9.78 \times 10^{-8}$) (Figure 2a). Similar results were acquired from other oncology databases, including the UALCAN database, the Kaplan–Meier database, and GEPIA2 with TCGA data (Figure 2b, 2c, 2d).

Correlations of *STON1* levels with tumor mutation burden and mismatch repair genes in KIRC

Given the sensitive connection between TMB, microsatellite instability (MSI), and immunotherapeutic response, we focused on their relationship with *STON1* expression levels. Marginal scatter plots revealed that alterations in *STON1* expression were always accompanied by changes in mismatch repair proteins (MLH1, PMS2, MSH2, MSH6, and EpCAM). Moreover, the four mismatch proteins seem to be positively associated with *STON1* (Figure 3a, $P < 2.2 \times 10^{-16}$). Additionally, *STON1* mRNA level was negatively associated with the TMB score ($P = 0.012$) (Figure 3b).

Association between *STON1* expression and immune cells in KIRC

STON1 gene copy numbers were accompanied by changes in immune cell infiltration levels, including that of B cells, CD8⁺ T cells, CD4⁺ T cells, macrophages, neutrophils, and dendritic cells (Figure 4a). Subsequently, we examined the relationship between *STON1* expression levels and the infiltration levels of 26 immune-related cell types. Our data illustrated that the abundance of different subtypes of immune cells was significantly positively (effector memory CD8 T cells, effector memory CD4 T cells, type 1 T helper cells, type 2 T helper cells, regulatory T cells, memory B cells, natural killer cells, immature dendritic cells, eosinophils, mast cells, and neutrophils) or negatively (activated CD8 T cells, CD56 bright natural killer cells, CD56 dim natural killer cells, myeloid-derived suppressor cells, activated dendritic cells, and plasmacytoid dendritic cells) related to *STON1* expression (Figure 4b, 4c). These results demonstrated that *STON1* was positively linked with most immune cells. Furthermore, *STON1* mRNA expression was obviously highest in the C3 subtype (inflammatory) (Figure 4d), which was reported to have the best prognosis in KIRC [34]. This result indicated that *STON1* may have a protective role in KIRC by exhibiting upregulated expression in the C3 immune subtype.

***STON1* may mediate immune response through immunomodulators**

Thirty-one immunomodulators were obviously related to *STON1* in KIRC, including 21 immunostimulators (C10orf54, CD27, CD80, CXCL12, CXCR4, ENTPD1, HHLA2, IL6, IL6R, LTA, NT5E, PVR, RAET1E, TMIGD2, TNFRSF14, TNFRSF17, TNFRSF18, TNFRSF25, TNFSF14, TNFSF15, and TNFSF9) (Figure 5a) and 10 immunoinhibitors (ADORA2A, CD274, CTLA4, IDO1, KDR, LAG3, PDCD1, PDCD1LG2, PVRL2, and TGFBR1) (Figure 5b). We subsequently explored the possible signaling pathways through which *STON1* may modulate the immune response in KIRC using GO and KEGG enrichment analyses. Thirty-one immunomodulators and 57 closely related genes were uploaded onto the DAVID. Biological process analysis revealed that these genes were enriched in the tumor necrosis factor (TNF)-mediated signaling

pathway, T cell co-stimulation, and immune response. Molecular functional analysis revealed that they were enriched in TNF receptor binding, TNF-activated receptor activity, and cytokine activity. Cellular component analysis indicated that STON1 was mainly enriched in the plasma membrane (Figure 6a). KEGG enrichment analysis further confirmed that these genes are tightly related to cytokine–cytokine receptor interactions (Figure 6b). The associations between 31 immunomodulators and 57 closely related genes are presented in a protein-to-protein interaction network (Figure 6c)

Prognostic value of *STON1*-related immunoregulators in KIRC

In total, 504 patients were randomly divided into a training set ($n = 328$) and a validation set ($n = 176$) at a ratio of 65%–35%, which was downloaded from GDC. To explore the prognostic value of *STON1*-related immunomodulators, univariate Cox analysis was first performed on the training set, which identified 15 of the abovementioned immunoregulators with statistical significance (Table 2). We then input these 15 immunomodulators into a stepwise multivariate Cox regression model using the training set. *CTLA4* ($P = 0.0177$), *KDR* ($P = 0.0135$), *PDCD1* ($P = 0.024$), and *HHLA2* ($P < 0.001$) were independent prognostic factors, as shown in Figure 7a. Here, risk scores were obtained using the following formula:

$$\text{Risk score} = \text{EXP}_{(\text{CTLA4})} \times 0.25599 + \text{EXP}_{(\text{KDR})} \times (-0.18132) + \text{EXP}_{(\text{LAG3})} \times 0.18505 + \text{EXP}_{(\text{PDCD1})} \times (-0.25896) + \text{EXP}_{(\text{HHLA2})} \times (-0.16182) + \text{EXP}_{(\text{TNFRSF25})} \times 0.13274 + \text{EXP}_{(\text{TNFSF14})} \times 0.13404.$$

The Kaplan–Meier survival curve indicated that the high-risk score group had worse OS than the low-risk score group in KIRC (Figure 7b). Through time-dependent ROC curves, the Cox model displayed better predictive ability. The AUC of 1-, 3- and 5-year OS was 0.732, 0.733, 0.825, respectively (Figure 7d). The risk factor analysis graph presents the complete landscape of risk scores, patient status, and gene expression profiles (Figure 8a). We used the validation set to verify the predictive value of the seven-gene prognostic signature. Consistent with these results, the Kaplan–Meier curves of the validation set showed that the low-risk group had a better outcome than the high-risk group ($P = 7 \times 10^{-8}$) (Figure 7c). The AUC of 1-, 3-, 5-year OS of our model was 0.795, 0.724, 0.784, respectively (Figure 7e). The risk factor analysis chart is also shown in Figure 8b.

Construction of a prognostic nomogram according to risk scores and clinical parameters

Univariate Cox proportional hazards regression analysis demonstrated that risk score (HR = 1.289, 95% CI = 1.217–1.365, $P = 5.81 \times 10^{-18}$), stage (HR = 1.819, 95% CI = 1.532–2.160, $P = 8.62 \times 10^{-12}$), M (HR = 3.858, 95% CI = 3.573–5.784, $P = 6.47 \times 10^{-11}$), T (HR = 1.892, 95% CI = 1.529–2.342, $P = 4.74 \times 10^{-9}$), and age (HR = 1.039, 95% CI = 1.021–1.056, $P = 9.52 \times 10^{-6}$) were distinctly associated with OS in the training set (Figure 9a). Meanwhile, multivariate Cox proportional hazards regression analysis was implemented to assist with the identification of independent prognostic factors. The independent prognostic factors were risk score (HR = 1.197, 95% CI = 1.127–1.271, $P = 6.71 \times 10^{-9}$), age (HR = 1.038, 95% CI = 1.020–1.057, $P = 3.52 \times 10^{-5}$), and stage (HR = 1.699, 95% CI = 1.420–2.033, $P = 6.76 \times 10^{-9}$) (Figure 9b). Therefore, a comprehensive nomogram prognostic model was constructed according to all independent prognostic factors screened

by multivariate Cox proportional hazards regression analysis of the training set to predict the probability of 1-, 3-, and 5-year OS (Figure 9c). In the training set, AUC of 1-, 3-, and 5-year OS were 0.889, 0.804, 0.790, respectively (Figure 9d). In the validation set, AUC of 1-, 3-, and 5-year OS were 0.882, 0.847, 0.809, respectively (Figure 9e). The AUC of the training set and validation set illustrated our model with good predictive ability for OS. Furthermore, the calibration curves, which reflected the consistency between the predicted risk and the actual risk, showed that the nomogram-predicted probability was well-matched with the ideal line for 1-, 3-, 5-year OS in the training set and validation set (Figure 10a). The same methods were applied to the validation set to further validate the authenticity of the above results (Figure 10b). The concordance probability (C-index) can be used to measure and compare the discriminative power of a risk prediction model. The C-indexes of our nomogram model were 0.783 and 0.795 in the training set and validation set, respectively. Moreover, we also discovered that our nomogram prognostic model showed a better net benefit for predicting OS compared with age, stage, and risk score in the training set and validation set (Figure 10c, 10d).

Discussion

Although early-stage KIRC patients can be cured by partial or complete nephrectomy with a better outcome, approximately 90% of KIRC patients still suffer from recurrence and metastasis[1, 5]. Recently, immune checkpoint blockers, such as nivolumab plus ipilimumab combination immunotherapy, have shown promise in KIRC patients with rapid progression in clinical individualized treatment based on targets of the tumor microenvironment[35], but a considerable number of patients displayed a low or absent immune response[36]. Therefore, further identification of novel immunotherapeutic agents and biomarkers to guide clinical therapy and predict survival of KIRC patients remains challenging. In our current study, we focused on *STON1*, a protein-coding gene involved in vesicle transport. We demonstrated that *STON1* was decreased in most human cancer types including KIRC, and was strongly associated with grade, stage, distant metastasis, and vital status in KIRC. Furthermore, *STON1* overexpression bodes well for prognosis. The above results indicated that *STON1* may as a tumor suppressor and inhibit tumorigenesis and progression. *STON1* was identified as target gene at the rs13405728 locus in polycystic ovary syndrome [37]. However, to date, no reports have published the underlying functions of *STON1* in cancer. A previous study demonstrated that alterations in focal adhesion dynamics, cellular motility, and signaling were induced by the absence of *STON1*. The above processes are intimately correlated with tumor migration. Moreover, as a specific adaptor for NG2 endocytosis, *STON1* deficiency resulted in NG2 accumulation on the cell surface, thus promoting tumor deterioration [13]. Our results indicate that *STON1* may inhibit tumor growth and progression in KIRC.

Mismatch repair genes are related to the human mismatch repair response, which is responsible for the repair of base mismatches that occur during DNA replication[38]. Tumor mutational burden is a novel indicator of mutation quantity[39]. A previous review demonstrated that tumors with mismatch repair possess the capacity to increase the mutational burden, resulting in increased immune checkpoint protein expression including PD-1 and PD-L1. Furthermore, the immunogenic neoepitopes generated by the imbalance of the mismatch system significantly improved the immune response rate [40]. Li *et al.*

revealed that several microsatellite site alterations indicate poor survival of KIRC patients [41], while Chalmers *et al.* stated that the median mutation per MB was 2.7 in renal clear cell carcinoma, and microsatellite instability could increase TMB to some extent [42]. Interestingly, as our study revealed, the mRNA level of *STON1* was positively associated with mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, MSH6, and EpCAM). Therefore, we speculated that TMB aggravation is partly due to increased immunogenic neoepitopes created by the mismatch repair system in the absence of *STON1* in KIRC. Our subsequent results corresponded with previous studies and reinforced our assumption. As the *STON1* expression decreased, the TMB score increased. Hence, *STON1* may be a candidate prognostic biomarker for evaluating the efficacy of immunotherapy responses and further experiments should be conducted to confirm this theory.

KIRC is one of the most immune-infiltrated cancers among all human solid cancer types [43]. The emergence of immune checkpoint targets provides a new direction for tumor immunotherapy. In recent years, CTLA4 inhibitors, PD-1 inhibitors, PD-L1 inhibitors, and PD-L1 inhibitors plus tyrosine kinase inhibitor (TKI) in progressed KIRC patients were approved by the FDA and achieved great curative effect[44]. We conducted a comprehensive bioinformatics analysis to reveal the details of the *STON1*-associated immune response in KIRC. The results of the TIMER database analysis indicated that arm-level deletion of *STON1* was strongly associated with the infiltration level of six immune cell types: B cells, CD4⁺ T cells, CD8⁺ T cells, macrophages, neutrophils, and dendritic cells. Furthermore, our study revealed that the high abundance of most immune cells was positively associated with *STON1* mRNA expression in KIRC, including regulatory T cells (Tregs), type 1 T helper cells (Th1), type 2 T helper cells (Th2), effector memory CD4 T cells (Tem_CD4), effector memory CD8 T cells (Tem_CD8), natural killer cells (NK), neutrophil cells, memory B cells (Mem_B), mast cells, and immature dendritic cells (iDC). Tumor-infiltrating Tem_CD4 was associated with downregulation of CD127, a classic marker of exhausted T-cells [45]. Interferon- γ producing Th1 cells secrete TNF, resulting in tumor destruction. Th1 and Th2 cells can not only recruit NK cells and macrophages near to the tumor site to exert antitumor effects, but also act as enhancers to promote tumor-specific cytotoxic T-lymphocyte (CTL) responses[46]. It is reported that high mast cell density is associated with a longer OS and progression-free survival in metastatic renal cell carcinoma [47]. However, myeloid-derived suppressor cells contribute to immune suppression in the tumor microenvironment[48]. The above results indicated that *STON1* may indirectly inhibit tumor progression by regulating most immune cells in the immune microenvironment.

Our violin plot showed that the mRNA level of *STON1* in KIRC was highest in the C3 subtype but the lowest in C5 subtype. C1–C6 immune subtypes were previously identified from five representative signatures across multiple tumors. Furthermore, the C3 subtype seems to possess a better outcome than the other immune types [34]. We speculated that higher *STON1* expression was remarkably associated with longer OS in C3-dominated tumors. Considering the crucial role of immunomodulators in KIRC, the results obtained from the TISDB database identified 21 *STON1*-associated immune-stimulators and 10 *STON1*-associated immunoinhibitors. Subsequently, GO terms analysis indicated that TNF receptor

binding was highly enriched. Taken together, these results further validated that *STON1* is strongly associated with the activation of the tumor immune microenvironment.

Immune prognostic signatures have been reported previously. Xu *et al.* identified seven immune-related genes and constructed a gene signature, of which the AUC value was 0.751. The signature showed moderate prognostic accuracy in predicting survival time [49]. Consistently, we conducted stepwise multivariate Cox regression with *STON1*-related immunomodulators screened by univariate Cox regression analysis in the training test. A total of seven *STON1*-related immune genes were screened, and *CTLA4*, *KDR*, *PDCD1*, and *HHLA2* displayed a dependent prognostic value in KIRC. All of them had been proven to be tightly associated with human cancer biology. *CTLA4* inhibits T cell activation to some extent [50]; thus, anti-*CTLA4* antibody immunotherapy can enhance the anti-tumor immune effects of T cells [51]. Wu *et al.* reported that autophagy induces *KDR* phosphorylation, which leads to angiogenic mimicry that promotes tumor growth [52]. An anti-PD1 (*PDCD1*) agent series was approved by the FDA and used in various carcinomas with better immune response[53]. Xu *et al.* revealed that *HHLA2* was downregulated and had protective effects in epithelial ovarian cancer[54]. On the basis of this seven-gene immunoregulator signature, KIRC patients were divided into low-risk and high-risk groups according to the median value of the risk score derived from the signature. The Kaplan–Meier survival curve of the training set and validation set revealed that the high-risk score group had poor OS in KIRC. In our survival predictive system, the AUC values for 1-, 3-, and 5-year OS for both the training set and validation set were greater than 0.70, indicating the relative stability of our signature. As for clinicopathological characteristics, we integrated a nomogram consisting of age, stage, and risk score according to the stepwise regression model in the training set. With our nomogram, clinicians could acquire great convenience in predicting 1-, 3-, and 5-year survival. To determine the validity and stability of our nomogram, ROC curve, c-index, calibration curve, and decision curve analyses demonstrated the stability and practicability of our nomogram from different aspects. The 1-, 3-, and 5-year AUC values were 0.889, 0.804, 0.790, respectively, in the training set, and 0.882, 0.847, 0.809, respectively, in the validation set, while the c-indexes were 0.783 and 0.795 in the training set and validation set, respectively. Our model was superior to other single clinical factors in clinical decision-making.

Conclusions

Collectively, lower *STON1* expression in KIRC indicates tumor progression and worse survival outcome. *STON1* may act as a stimulator to induce the activation of the anti-tumor immune system. Our current study provides new insights into the function of *STON1* in the tumor microenvironment. Importantly, our integrated nomogram derived from the risk score of *STON1*-associated immunoregulators and clinical parameters could be used to predict survival in KIRC patients. A limitation of our study is that it lacked external validation because of missing survival data, and cytological functional studies are required to further verify the specific role and molecular mechanism of *STON1* in KIRC.

Declarations

Ethics approval and consent to participate

Not applicable. The data of KIRC patients were obtained from public databases.

Consent for publication

Not applicable.

Availability of data and materials

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

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by grants from the YangFan Plan of Guangdong Province (4YF16007G), and the CAMS Innovation Fund for Medical Sciences (2019-I2M-5-023). The funders had no role in the design of the study, collection, analysis, and interpretation of data, or writing the manuscript.

Authors' contributions

Conceived and coordinated the study: WJ, ZHS and BTL; searched literature and performed statistical analyses: AXZ, JRB, YPH, BSW, YZ, CXW, JCX, and SJZ; drafted the manuscript: AXZ and WJ. Read and approved the final manuscript: all authors.

Acknowledgments

We thank H. Nikki March, PhD, from Liwen Bianji (Edanz) (www.liwenbianji.cn/), for editing the English text of a draft of this manuscript.

Author details

¹Department of Pathology, School of Basic Medicine Sciences; Pathology Diagnosis and Research Center of Affiliated Hospital, Guangdong Medical University, Zhanjiang, 524023, PR China; ²Key Laboratory of Emergency and Trauma of Ministry of Education & Emergency and Trauma College, Hainan Medical University, Haikou 571199, PR China; ³Tumor Institute of the First Affiliated Hospital, Hainan Medical University, Haikou 571199, PR China

Abbreviations

AUC, The area under the curve; C-index, The concordance probability; DCA, Decision Curve Analysis; KIRC, Kidney renal clear cell carcinoma; GEO, Gene Expression Omnibus; GEPIA, Gene Expression Profiling Interactive Analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MMR, Mismatch repair; OS, Overall survival; RCC, Renal cell carcinoma; ROC, Receiver operating characteristic curve; STON1, Stonin 1; TCGA, The Cancer Genome Atlas; TIMER, The Tumor Immune Estimation Resource; TISIDB, Tumor and Immune System Interaction Database; TMB, Tumor mutational burden

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin.* 2019;69(1):7–34.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2018;68(6):394–424.
3. Moch H, Cubilla AL, Humphrey PA, Reuter VE, Ulbright TM. The 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs-Part A: Renal, Penile, and Testicular Tumours. *Eur Urol.* 2016;70(1):93–105.
4. Prasad SR, Humphrey PA, Catena JR, Narra VR, Srigley JR, Cortez AD, Dalrymple NC, Chintapalli KN. Common and uncommon histologic subtypes of renal cell carcinoma: imaging spectrum with pathologic correlation. *Radiographics.* 2006;26(6):1795–806. discussion 1806 – 1710.
5. Hsieh JJ, Purdue MP, Signoretti S, Swanton C, Albiges L, Schmidinger M, Heng DY, Larkin J, Ficarra V. Renal cell carcinoma. *Nat Rev Dis Primers.* 2017;3:17009.
6. Jonasch E, Gao J, Rathmell WK. Renal cell carcinoma. *BMJ.* 2014;349:g4797.
7. Barata PC, Rini BI. Treatment of renal cell carcinoma: Current status and future directions. *CA Cancer J Clin.* 2017;67(6):507–24.
8. Wang DY, Salem JE, Cohen JV, Chandra S, Menzer C, Ye F, Zhao S, Das S, Beckermann KE, Ha L, et al. Fatal Toxic Effects Associated With Immune Checkpoint Inhibitors: A Systematic Review and Meta-analysis. *JAMA Oncol.* 2018;4(12):1721–8.
9. Maritzen T, Podufall J, Haucke V. Stonins—specialized adaptors for synaptic vesicle recycling and beyond? *Traffic* 2010, 11(1):8–15.
10. Martina JA, Bonangelino CJ, Aguilar RC, Bonifacino JS. Stonin 2: an adaptor-like protein that interacts with components of the endocytic machinery. *J Cell Biol.* 2001;153(5):1111–20.
11. Almen MS, Jacobsson JA, Moschonis G, Benedict C, Chrousos GP, Fredriksson R, Schioth HB. Genome wide analysis reveals association of a FTO gene variant with epigenetic changes. *Genomics.* 2012;99(3):132–7.
12. Linschooten JO, Van Schooten FJ, Baumgartner A, Cemeli E, Van Delft J, Anderson D, Godschalk RW. Use of spermatozoal mRNA profiles to study gene-environment interactions in human germ cells. *Mutat Res.* 2009;667(1–2):70–6.

13. Feutlinske F, Browarski M, Ku MC, Trnka P, Waiczies S, Niendorf T, Stallcup WB, Glass R, Krause E, Maritzen T. Stonin1 mediates endocytosis of the proteoglycan NG2 and regulates focal adhesion dynamics and cell motility. *Nat Commun.* 2015;6:8535.
14. Liu M, Pan Q, Xiao R, Yu Y, Lu W, Wang L. A cluster of metabolism-related genes predict prognosis and progression of clear cell renal cell carcinoma. *Sci Rep.* 2020;10(1):12949.
15. Sun Z, Jing C, Xiao C, Li T. Long Non-Coding RNA Profile Study Identifies an Immune-Related lncRNA Prognostic Signature for Kidney Renal Clear Cell Carcinoma. *Front Oncol.* 2020;10:1430.
16. Chen T, Zheng W, Chen J, Lin S, Zou Z, Li X, Tan Z. Systematic analysis of survival-associated alternative splicing signatures in clear cell renal cell carcinoma. *J Cell Biochem.* 2020;121(10):4074–84.
17. Zou Y, Hu C. A 14 immune-related gene signature predicts clinical outcomes of kidney renal clear cell carcinoma. *PeerJ.* 2020;8:e10183.
18. Xiang Y, Zhou S, Hao J, Zhong C, Ma Q, Sun Z, Wei C. Development and validation of a prognostic model for kidney renal clear cell carcinoma based on RNA binding protein expression. *Aging.* 2020;12(24):25356–72.
19. Wang Y, Yao Y, Zhao J, Cai C, Hu J, Zhao Y: Development of an Autophagy-Related Gene Prognostic Model and Nomogram for Estimating Renal Clear Cell Carcinoma Survival. *J Oncol* 2021, 2021:8810849.
20. Huang M, Zhang T, Yao ZY, Xing C, Wu Q, Liu YW, Xing XL. MicroRNA related prognosis biomarkers from high throughput sequencing data of kidney renal clear cell carcinoma. *BMC Med Genomics.* 2021;14(1):72.
21. Li T, Fu J, Zeng Z, Cohen D, Li J, Chen Q, Li B, Liu XS. TIMER2.0 for analysis of tumor-infiltrating immune cells. *Nucleic Acids Res.* 2020;48(W1):W509–14.
22. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, et al. NCBI GEO: archive for functional genomics data sets–update. *Nucleic Acids Res.* 2013;41(Database issue):D991–5.
23. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, Varambally S. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia.* 2017;19(8):649–58.
24. Anaya J. OncoLnc: linking TCGA survival data to mRNAs, miRNAs, and lncRNAs. *PeerJ Comput Sci.* 2016;4:e1780v1.
25. Nagy Á, Munkácsy G, Györfy B. Pancancer survival analysis of cancer hallmark genes. *Sci Rep.* 2021;11(1):6047.
26. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. *Nucleic Acids Res.* 2017;45(W1):W98-w102.
27. Li T, Fan J, Wang B, Traugh N, Chen Q, Liu JS, Li B, Liu XS. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells. *Cancer Res.* 2017;77(21):e108–10.

28. Ru B, Wong CN, Tong Y, Zhong JY, Zhong SSW, Wu WC, Chu KC, Wong CY, Lau CY, Chen I, et al. TISIDB: an integrated repository portal for tumor-immune system interactions. *Bioinformatics*. 2019;35(20):4200–2.
29. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*. 2012;2(5):401–4.
30. Huang da W, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*. 2009;4(1):44–57.
31. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B, Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res*. 2003;13(11):2498–504.
32. Van Calster B, Wynants L, Verbeek JFM, Verbakel JY, Christodoulou E, Vickers AJ, Roobol MJ, Steyerberg EW. Reporting and Interpreting Decision Curve Analysis: A Guide for Investigators. *Eur Urol*. 2018;74(6):796–804.
33. Goldman MJ, Craft B, Hastie M, Repečka K, McDade F, Kamath A, Banerjee A, Luo Y, Rogers D, Brooks AN, et al. Visualizing and interpreting cancer genomics data via the Xena platform. *Nat Biotechnol*. 2020;38(6):675–8.
34. Thorsson V, Gibbs DL, Brown SD, Wolf D, Bortone DS, Ou Yang TH, Porta-Pardo E, Gao GF, Plaisier CL, Eddy JA, et al. The Immune Landscape of Cancer. *Immunity*. 2018;48(4):812–30.e814.
35. Motzer RJ, Tannir NM, McDermott DF, Aren Frontera O, Melichar B, Choueiri TK, Plimack ER, Barthelemy P, Porta C, George S, et al. Nivolumab plus Ipilimumab versus Sunitinib in Advanced Renal-Cell Carcinoma. *N Engl J Med*. 2018;378(14):1277–90.
36. Signoretti S, Flaifel A, Chen YB, Reuter VE. Renal Cell Carcinoma in the Era of Precision Medicine: From Molecular Pathology to Tissue-Based Biomarkers. *J Clin Oncol*. 2018;36(36):JCO2018792259.
37. Cao CH, Wei Y, Liu R, Lin XR, Luo JQ, Zhang QJ, Lin SR, Geng L, Ye SK, Shi Y, et al. Three-Dimensional Genome Interactions Identify Potential Adipocyte Metabolism-Associated Gene STON1 and Immune-Correlated Gene FSHR at the rs13405728 Locus in Polycystic Ovary Syndrome. *Front Endocrinol (Lausanne)*. 2021;12:686054.
38. Luchini C, Bibeau F, Ligtenberg MJL, Singh N, Nottegar A, Bosse T, Miller R, Riaz N, Douillard JY, Andre F, et al. ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: a systematic review-based approach. *Ann Oncol*. 2019;30(8):1232–43.
39. Jardim DL, Goodman A, de Melo Gagliato D, Kurzrock R. The Challenges of Tumor Mutational Burden as an Immunotherapy Biomarker. *Cancer cell*. 2021;39(2):154–73.
40. Dudley JC, Lin MT, Le DT, Eshleman JR. Microsatellite Instability as a Biomarker for PD-1 Blockade. *Clin Cancer Res*. 2016;22(4):813–20.
41. Li X, Tan X, Yu Y, Chen H, Chang W, Hou J, Xu D, Ma L, Cao G. D9S168 microsatellite alteration predicts a poor prognosis in patients with clear cell renal cell carcinoma and correlates with the

- down-regulation of protein tyrosine phosphatase receptor delta. *Cancer*. 2011;117(18):4201–11.
42. Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, Schrock A, Campbell B, Shlien A, Chmielecki J, et al. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med*. 2017;9(1):34.
 43. Vuong L, Kotecha RR, Voss MH, Hakimi AA. Tumor Microenvironment Dynamics in Clear-Cell Renal Cell Carcinoma. *Cancer Discov*. 2019;9(10):1349–57.
 44. Hirsch L, Flippot R, Escudier B, Albiges L. Immunomodulatory Roles of VEGF Pathway Inhibitors in Renal Cell Carcinoma. *Drugs*. 2020;80(12):1169–81.
 45. Dubinski D, Wölfer J, Hasselblatt M, Schneider-Hohendorf T, Bogdahn U, Stummer W, Wiendl H, Grauer OM. CD4 + T effector memory cell dysfunction is associated with the accumulation of granulocytic myeloid-derived suppressor cells in glioblastoma patients. *Neuro Oncol*. 2016;18(6):807–18.
 46. Kennedy R, Celis E. Multiple roles for CD4 + T cells in anti-tumor immune responses. *Immunol Rev*. 2008;222:129–44.
 47. Yao J, Xi W, Chen X, Xiong Y, Zhu Y, Wang H, Hu X, Guo J. Mast cell density in metastatic renal cell carcinoma: Association with prognosis and tumour-infiltrating lymphocytes. *Scand J Immunol*. 2021;93(4):e13006.
 48. Nagaraj S, Gabrilovich DI. Myeloid-derived suppressor cells in human cancer. *Cancer J*. 2010;16(4):348–53.
 49. Shen C, Liu J, Wang J, Zhong X, Dong D, Yang X, Wang Y. Development and validation of a prognostic immune-associated gene signature in clear cell renal cell carcinoma. *Int Immunopharmacol*. 2020;81:106274.
 50. Krummel MF, Allison JP. CD28 and CTLA-4 have opposing effects on the response of T cells to stimulation. *J Exp Med*. 1995;182(2):459–65.
 51. Egen JG, Ouyang W, Wu LC. Human Anti-tumor Immunity: Insights from Immunotherapy Clinical Trials. *Immunity*. 2020;52(1):36–54.
 52. Wu HB, Yang S, Weng HY, Chen Q, Zhao XL, Fu WJ, Niu Q, Ping YF, Wang JM, Zhang X, et al. Autophagy-induced KDR/VEGFR-2 activation promotes the formation of vasculogenic mimicry by glioma stem cells. *Autophagy*. 2017;13(9):1528–42.
 53. Sharpe AH, Pauken KE. The diverse functions of the PD1 inhibitory pathway. *Nat Rev Immunol*. 2018;18(3):153–67.
 54. Xu G, Shi Y, Ling X, Wang D, Liu Y, Lu H, Peng Y, Zhang B. HHLA2 predicts better survival and exhibits inhibited proliferation in epithelial ovarian cancer. *Cancer Cell Int*. 2021;21(1):252.

Tables

Table 1. Association between *STON1* expression and clinicopathological parameters of KIRC patients

Classification	<i>n</i>	Ston1 expression		χ^2	<i>P</i> value
		Low	High		
Age					
<60	228	106	122	2.22	0.136
≥60	263	140	123		
Gender					
Female	165	83	82	0.004	0.949
Male	326	163	163		
Grade					
1	10	4	6	9.641	0.022*
2	214	92	122		
3	195	106	89		
4	72	44	28		
Stage					
I	239	106	133	8.362	0.039*
II	52	30	22		
III	120	61	59		
IV	80	49	31		
Invasion Depth					
T1	245	110	135	6.945	0.074
T2	63	36	27		
T3	172	92	80		
T4	11	8	3		
Lymph node metastasis					
Nx	245	117	128	1.911	0.385
N0	232	120	112		
N1	14	9	5		
Distant metastasis					
M0	414	198	216		

M1	77	48	29	5.469	0.019*
Status					
Alive	325	139	186		
Dead	166	107	59	20.674	5.44e-06****

$P < 0.05$ was considered statistically significant.

Table 2. Univariate regression analysis of immunoregulators

Gene	HR	Z_scores	P_value
KDR	0.757	-5.366	8.07 ^{e-08}
TNFSF14	1.293	4.315	1.60 ^{e-05}
HLA2	0.882	-4.197	2.71 ^{e-05}
TNFRSF18	1.348	3.736	1.88 ^{e-04}
IL6	1.150	3.517	4.38 ^{e-04}
CTLA4	1.250	3.384	7.16 ^{e-04}
TNFRSF25	1.281	3.272	1.07 ^{e-03}
RAET1E	0.749	-3.152	1.62 ^{e-03}
LAG3	1.172	2.997	2.73 ^{e-03}
TNFSF15	0.823	-2.384	1.71 ^{e-02}
PDCD1	1.136	2.373	1.77 ^{e-02}
TGFBR1	0.730	-2.328	1.99 ^{e-02}
ENTPD1	0.764	-2.196	2.81 ^{e-02}
CD80	1.172	2.045	4.08 ^{e-02}
NT5E	0.848	-1.998	4.57 ^{e-02}
LTA	1.147	1.903	5.71 ^{e-02}
CXCL12	0.874	-1.855	6.37 ^{e-02}
IL6R	0.861	-1.796	7.25 ^{e-02}
CD27	1.088	1.480	1.39 ^{e-01}
C10orf54	0.863	-1.293	1.96 ^{e-01}
CD274	0.888	-1.275	2.02 ^{e-01}
TNFRSF14	1.152	1.237	2.16 ^{e-01}
TNFRSF17	1.063	1.126	2.60 ^{e-01}
PVRL2	1.210	1.014	3.10 ^{e-01}

PDCD1LG2	0.922	-0.953	3.41 ^{e-01}
CXCR4	1.097	0.885	3.76 ^{e-01}
TMIGD2	1.070	0.746	4.56 ^{e-01}
IDO1	0.959	-0.673	5.01 ^{e-01}
ADORA2A	0.972	-0.319	7.50 ^{e-01}
PVR	1.032	0.211	8.33 ^{e-01}
TNFSF9	1.010	0.168	8.66 ^{e-01}

Figures

Figure 1

STON1 expression is downregulated in KIRC. (A) STON1 mRNA expression in tumor and normal tissues of different human cancer types in TCGA database. (B–D) STON1 mRNA level in KIRC tumors and adjacent normal tissue in GSE16441, GSE16449, and GSE71963 from GEO database. (E) Protein expression of STON1 in CPTAC samples. * $P < 0.1$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Figure 2

High expression of STON1 indicated better outcome in KIRC patients. Kaplan–Meier curves of overall survival according to high and low expression of STON1 in TCGA data in KIRC patients by exploring the OncoLnc database (A), UALCAN database (B), Kaplan–Meier database (C), and GEPIA2 database (D).

Figure 3

Correlation between STON1 and the mismatch system and tumor mutational burden (TMB). (A) STON1 mRNA level was positively associated with MLH1, PMS2, MSH2, MSH6, and EpCAM, as presented in marginal scatter plots. (B) Relationship between STON1 mRNA level and TMB in marginal scatter plots. The expression values of all genes are presented as logarithmic values.

Figure 4

Association between STON1 and immune cells. (A) The infiltration level of six immune cell subtypes related to copy number of STON1. Correlations between 28 immune cell types and STON1 expression presented in a heat map (B) and bubble plot (C). (D) Violin plot of the abundance of immune subtypes according to STON1 mRNA expression. * $P < 0.1$, *** $P < 0.001$.

Figure 5

Association between STON1 mRNA level and immunostimulators (A) and immunoinhibitors (B).

Figure 6

Comprehensive analysis of STON1-associated immunoregulators. (A, B) Gene ontology and KEGG enrichment analyses in STON1-associated immunoregulators with statistical significance and 57 related genes in KIRC. (C) Protein–protein interaction network according to immunoregulators associated with STON1 and 57 correlated genes.

Figure 7

Establishment and validation of a STON1-related immunoregulator signature. (A) Multiple stepwise regression analysis of STON1-associated immunoregulators screened by univariate regression analysis in the training set was presented in a forest plot. (B-C) Kaplan–Meier survival curves of the risk scores in the training and validation sets. (D-E) Time-dependent receiver operating characteristic curves at 1, 3, and 5 years in the training and validation sets.

Figure 8

Landscape of risk score, survival time, and gene expression profile of KIRC patients in the training and validation sets.

Figure 9

Cox regression analyses of risk score and clinicopathological parameters in KIRC and nomogram construction. (A) Univariate Cox regression analyses and (B) multivariate Cox regression analyses displayed in forest plots. (C) Construction of a nomogram for predicting 1-, 3-, and 5-year overall survival rates in KIRC patients in the training set. (D, E) Time-dependent receiver operating characteristic curves of the nomogram at 1, 3, and 5 years in the training and validation sets.

Figure 10

Verification of the accuracy and practicability of a prognostic nomogram in the training and validation sets. (A, B) Calibration curve of 1-, 3-, and 5-year overall survival in KIRC patients. (C, D) Clinical decision curve of net benefit for clinicians making treatment decisions.

Supplementary Files

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

- [PersonalCover.docx](#)
- [figureS1.tif](#)