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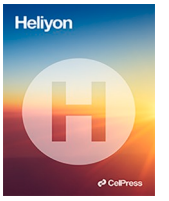

Research article

The predictive value of prognosis and therapeutic response for STAT family in pancreatic cancer

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Background: Signal transducers and activators of transcription (STAT) proteins, well-known cytoplasmic transcription factors, were found to be abnormally expressed in various cancers and play essential parts in the initiation, progression and therapy resistance of cancer. Nevertheless, the functions of different STATs in pancreatic cancer (PC) and their relationship to the prognosis and immune infiltration as well as drug efficacy in PC patients have not been systematically elucidated.

Methods: Expression, prognosis, genetic alterations and pathway enrichment analyses of the STAT family were investigated via Oncomine, GEPIA, Kaplan Meier-plotter, cBioPortal, Metascape and GSEA. Analysis of tumor immune microenvironment was conducted by ESTIMATE and TIMER. "pRRophetic" packages were used for analysis of chemotherapeutic response. Finally, the diagnostic and prognostic value of key STATs were further validated through public datasets and immunohistochemistry.

Results: In this study, only STAT1 mRNA level was significantly increased in tumor tissues and highly expressed in PC cell lines via multiple datasets. PC patients with higher STAT1/4/6 expression had a worse overall survival (OS) and progression-free survival (PFS), while higher STAT5B expression was correlated with better prognosis in the TCGA cohort. The STATs-associated genes were enriched in pathways about the remodeling of tumor immune microenvironment. The STATs levels were significantly correlated with immune infiltration, except STAT6. The STAT1 was identified as a potential biomarker and its diagnostic and prognostic value were further validated at mRNA and protein levels. GSEA showed that STAT1 may be involved in the progression and immune regulations of PC. Moreover, STAT1 expression was significantly related to the level of immune checkpoint, and predicted immunotherapy and chemotherapy responses.

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Conclusion: STAT family members were comprehensively analyzed and STAT1 was identified as an effective biomarker for predicting the survival and therapeutic response, which could be beneficial to develop better treatment strategies.

1. Introduction

Pancreatic cancer (PC) remains highly malignant and has a very miserable prognosis with a 5-year survival rate of about 11% [1] partly due to its poor early detection rate. By 2030, it is expected to rank second as the primary causes of deaths by cancer in the United States [2–4]. Though advances in chemotherapy, target therapy [5], and immunotherapy [6] during the past decades, limited therapeutic effects were observed in PC. Therefore, novel biomarkers for early identification, prognosis and management of PC are urgently needed.

Signal transducers and activators of transcription (STAT) family consist of seven members discovered including STAT1, 2, 3, 4, 5A, 5B, and 6 [7]. Extensive studies have found that STATs play an essential part in immune defense, surveillance, and homeostasis [8,9], so dysregulation of these pathways caused by aberrant STATs expression and activities could lead to various diseases, including cancers [10,11]. During the last two decades, STATs were found to be abnormally expressed in various human cancers and played an important part in the development, growth, invasiveness, immune responses and therapy resistance. Lu et al. [12] showed that inhibiting STAT3 reduced the secretion of immune suppressive cytokines in tumor cells, increased T cell stimulation, and augmented anti-PD-1 treatment in PC. Furthermore, the study found that STAT1 enhances the response to gemcitabine by inhibiting FOXM1 in PC cells [13], whereas suppression of STAT5B impairs the chemoresistance of the tumor cells [14]. STATs may also play dual roles in the development of PC. However, the roles of different STATs in PC patients and their association with the survival and immune infiltration as well as drug efficacy have not been systematically elucidated.

In this study, various cohorts were applied to comprehensively investigate the expression, prognostic value, immune infiltration, functional analysis and drug sensitivity of the STATs in PC. The potential target of STAT family members (STATs) was identified. Then, external datasets GEO, ICGC, and CTPAC were used to validate the value of the STAT1 expression via transcriptional and protein levels. Furthermore, immunohistochemistry (IHC) of PC tissue microarray (TMA) was applied to confirm STAT1 level in PC and paracancerous samples, and validate its prognostic value. Our findings highlight the potential mechanisms and value of the STATs in the prognostic and therapeutic prediction of PC.

2. Methods

2.1. Data acquisition and preprocessing

The transcriptome profiles and survival information of PC were available in the Cancer Genome Atlas (TCGA, https://cancergenome.nih.gov/), The Cancer Genome Collaboratory (ICGC, https://dcc.icgc.org/), Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/). The proteome data were downloaded from CTPAC (https://cptac-data-portal.georgetown.edu/datasets). TCGA-PAAD cohort includes 178 PC samples and 168 cases with complete chemotherapy and survival information more than 30 days were used to estimate the predictive value of STAT1 for the therapeutic response. 269, 125 and 127 PC samples from the ICGC dataset PACA-AU_array, gene expression microarray dataset GSE71729 and proteome dataset PDC000270 with complete survival information were enrolled to validate the prognostic performance of STAT1 level.

2.2. Bioinformatic analysis of STAT family expression

Oncomine [15] was used to compare the different mRNA levels of STAT family members between paracancerous samples in different cancer. GEPIA [16], a comprehensive online tool, was applied to analyze expression of targeted genes between PC and normal tissues, and correlation with tumor stage. EMBL-EBI, containing the RNA-seq data of 1019 human cancer cell lines from the Cancer Cell Line Encyclopedia, was applied to analysis the STAT family levels in PC cell lines (https://www.ebi.ac.uk/gxa/experiments/E-MTAB-2770/Results) [17].

2.3. Survival analysis

Survival analysis of the STAT family was conducted by in Kaplan-Meier (KM) Plotter [18]. To further validate the prognostic value of STAT1 level, samples were divided according to the best cutoff value through the "surv_cutpoint" function of the "survminer" package. Then, the survival distribution was compared using the log-rank test through the "survdiff" function of the "survival" package in R.

2.4. Analysis of genetic alteration in STAT family

The cBioPortal [19] was applied to assess and display genome profiles of STAT family members and their correlation with overall survival (OS) and progression-free survival (PFS) as followed by the online instructions of cBioPortal.

A

Analysis Type by Cancer	Cancer vs. Normal STAT1		Cancer vs. Normal STAT2		Cancer vs. Normal STAT3		Cancer vs. Normal STAT4		Cancer vs. Normal STAT5A		Cancer vs. Normal STAT5B		Cancer vs. Normal STAT6	
Bladder Cancer	4					1		2			1	1	1	
Brain and CNS Cancer	11				3			9	2		4		1	
Breast Cancer	26	1	2		1	2	1	1	1	20		14		
Cervical Cancer	4		1											
Colorectal Cancer	4			2		1		5				1		1
Esophageal Cancer	4							2	2		1			
Gastric Cancer	3				3				2		1			
Head and Neck Cancer	14		3	1	3		1			1		1		
Kideney Cancer	7		1	2	2	1	2				1	2	4	
Leukemia	3	5	1			5		7	1	3		1		3
Liver Cancer	6					1								
Lung Cancer	12	2		2		3		8		5	1	3		4
Lymphoma	23	2	2		2	6	5	12	1	4		6	2	1
Melanoma	2		1		2							1	2	
Myeloma	2				2		2			2		1		
Other Cancer	12	1	6		3			9		1	1	4	3	
Ovarian Cancer	4			1				1		1		4		2
Pancreatic Cancer	7		1									2		
Prostate Cancer					2	2		1			3	2	2	
Sarcoma	5	1		1	1	4	1	2		5		5	1	1

B

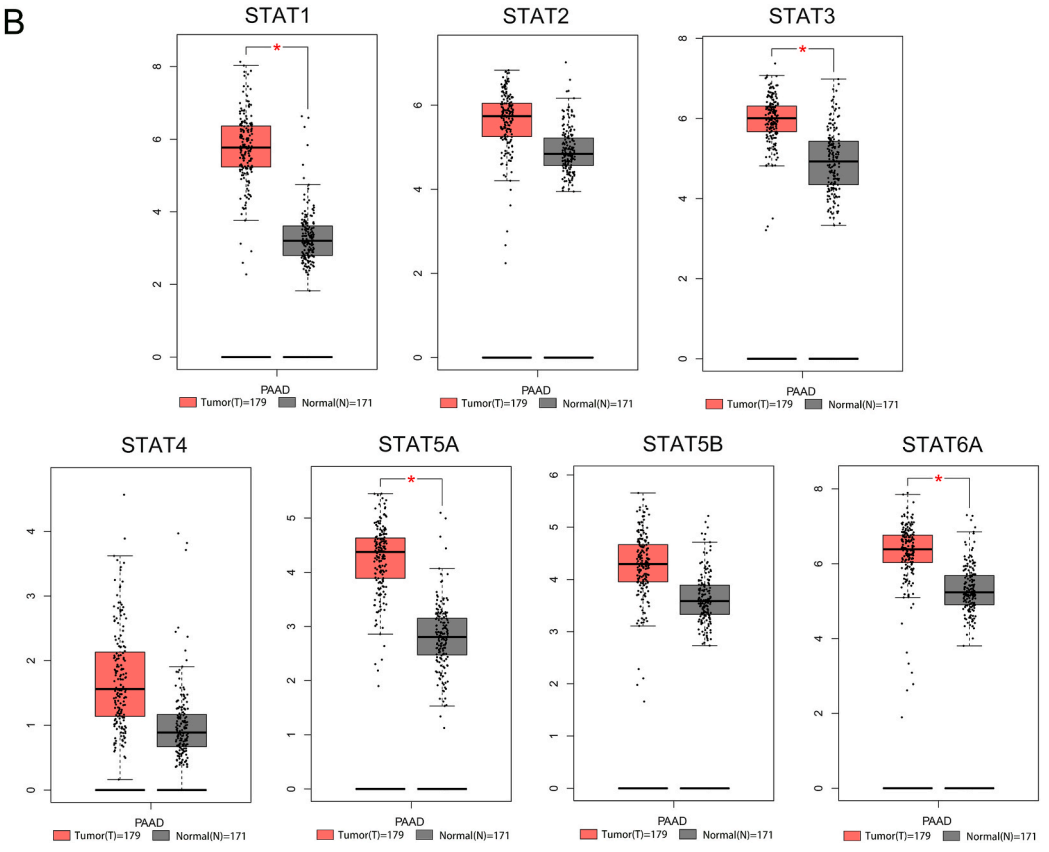

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Fig. 1. The differential expression of STAT family at transcriptional level in Oncomine (A) and GEPIA (B). A: The number in the graph represent the number of datasets with significantly differential STAT expression: upregulated (red), downregulated (blue), which was filtered by the threshold (2-fold change and p value = 0.01). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

2.5. Functional enrichment analyses of STAT family

GEPIA was utilized to obtain 20 highly similar genes of each STATs in PC. Then, GO and KEGG analyses of STATs and their similar genes were performed via Metascape [20].

To further comprehensively analyze the role of STAT1 in PC, GSEA software was performed to evaluate significantly differential pathways between low and high STAT1 expression groups in TCGA cohort. The selected gene set was h.all.v.7.0.symbols.

2.6. Analysis of tumor immune microenvironment

ESTIMATE algorithm [21] was applied to discover relationship between each STATs level and the infiltrations of immune and stroma cells and the TIMER [22] was used to analyze the association between immune cell infiltrates and the expression of STAT family members by R in the TCGA-PAAD cohort. Besides, comparisons of the levels of immune inhibitory checkpoints (CTLA4, CD274 (PD-L1), HAVCR2 (TIM3), IDO1, BTLA, PDCD1LG2, PDCD1 and LAG3) between two groups with different STAT1 expression were conducted in TCGA-PAAD cohort.

2.7. Prediction of therapeutic response

"pRRophetic" [23] package was used to predict the half-maximal inhibitory concentration (IC50) of three commonly used chemotherapy agents in each TCGA-PAAD patients. The immunophenoscore for each TCGA-PAAD patient, obtained from the web-accessible relational database TCIA (The Cancer Immunome Atlas) using machine learning, was applied to predict the responsiveness to anti-CTLA4 and anti-PD-1 antibodies. Then, the differences in clinical responses to chemotherapy and immunotherapy drugs in the STAT1 low- and high-expression group in TCGA cohort were estimated.

2.8. Tissue microarray (TMA) and immunohistochemistry (IHC)

The TMA was constructed, and immunohistochemical staining using anti-STAT1 (Cell Signaling Technology) and analyses were performed as other publications [24].

The scoring criteria were as follows: 0 (negative), 1 (weak), 2 (moderate), 3 (strong). The percentage of the stained tumor cells or normal pancreatic cells was categorized into 5 classes: 0 (negative), 1 (1–25%), 2 (26–50%), 3 (51–75%), and 4 (76–100%). Then, these 2 scores were multiplied to form a staining index. The staining index $\geq$6 was defined as a high expression.

2.9. Statistical analysis

All statistical analyses were conducted by R software (version 4.0.3). Continuous variables were compared using the Student's t-test or Mann–Whitney *U* test, as appropriate.

Table 1
The significant changes of STAT expression in transcription level between different types of pancreatic cancer and normal pancreatic tissues (Oncomine Database).

Gene	Type of Pancreatic Cancer Versus Normal Pancreatic tissue	Fold change	*t*-test	*P*-value	dataset
STAT1	Pancreatic Carcinoma	2.098	6.227	1.10E-05	Segara Pancrea
	Pancreatic Adenocarci-noma	12.225	6.437	1.54E-04	Logsdon Pancreas
	Pancreatitis	3.816	2.842	0.011	Logsdon Pancreas
	Pancreatic Ductal Adeno-carcinoma Epithelia	2.083	2.865	0.006	Grutzmann Pan-creas
	Pancreatic Adenocarci-noma	2.189	3.775	0.002	Iacobuzio-Donahue Pancreas 2
	Pancreatic Carcinoma	3.13	5.527	4.68E-06	Pei Pancreas
	Pancreatic Ductal Adeno-carcinoma	3.069	5.365	6.78E-07	Badea Pancreas
STAT2	Pancreatic Adenocarci-noma	2.901	3.194	0.004	Iacobuzio-Donahue Pancreas 2
STAT3	NA	NA	NA	NA	NA
STAT4	NA	NA	NA	NA	NA
STAT5A	NA	NA	NA	NA	NA
STAT5B	Pancreatitis	−2.022	−3.654	0.003	Logsdon Pancreas
	Pancreatic Adenocarci-noma	−2.827	−1.941	0.04	Logsdon Pancreas
STAT6	NA	NA	NA	NA	NA

3. Results

3.1. Transcriptional levels of STAT family in PC tissues and cell lines

STAT1 mRNA levels were significantly upregulated in PC samples of seven datasets via Oncomine (Fig. 1A). In the Segara dataset [25], STAT1 expression was increased in PC tissues with a foldchange (FC) of 2.098. In the Logsdon dataset [26], the levels of STAT1 in the PC samples were significantly higher than those in the normal samples with a FC of 12.225 and 3.816, respectively. Likewise, the mRNA expression of STAT1 was elevated in PC tissues in the Grutzmann dataset [27] with a FC of 2.083, the pei dataset with a FC of

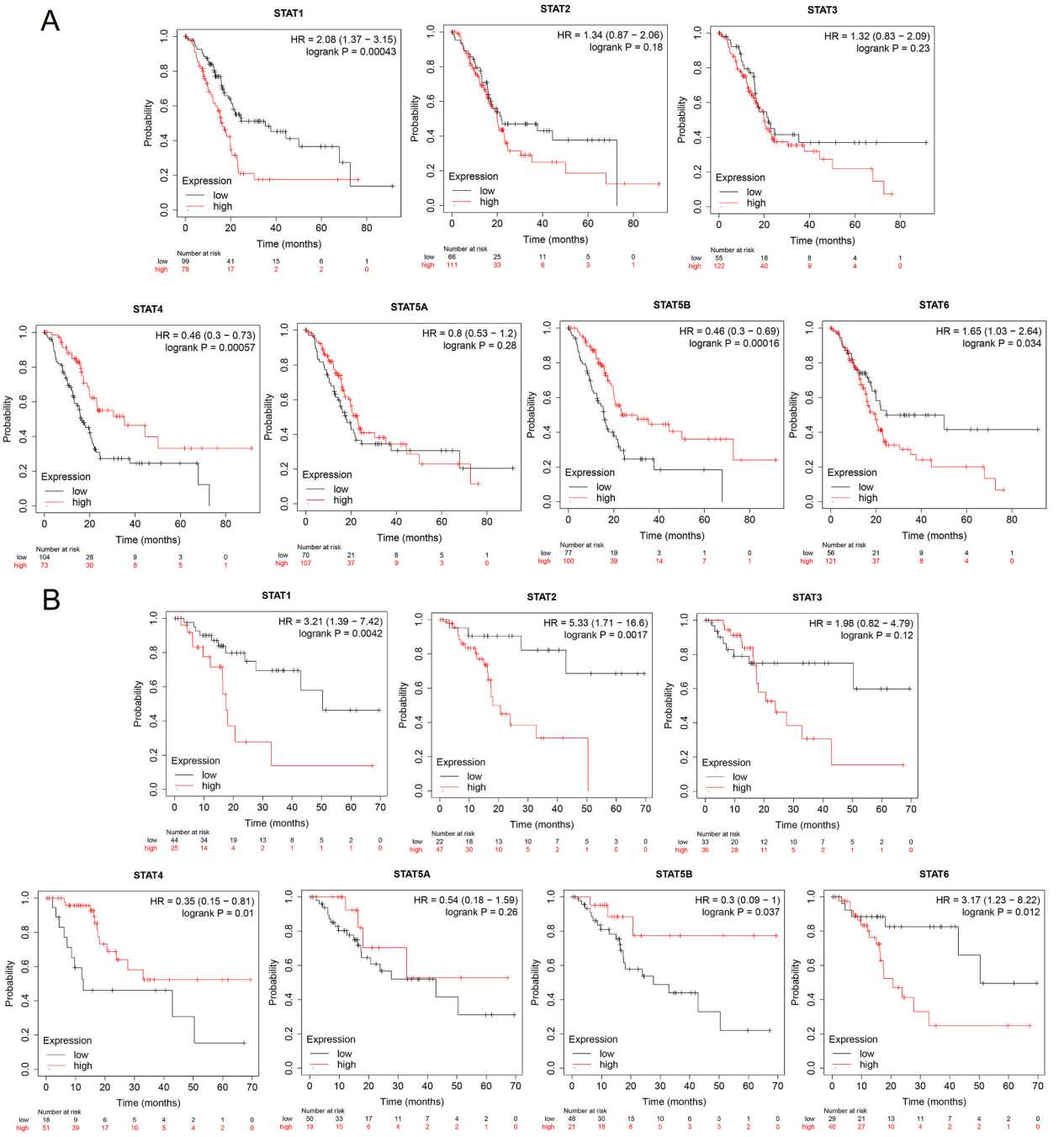

Fig. 2. The prognostic value of STAT family in pancreatic cancer in the TCGA cohort via Kaplan-Meier (KM) Plotter. (A) Overall survival curve of seven STAT members. (B) Relapse-free survival curve of seven STAT members.

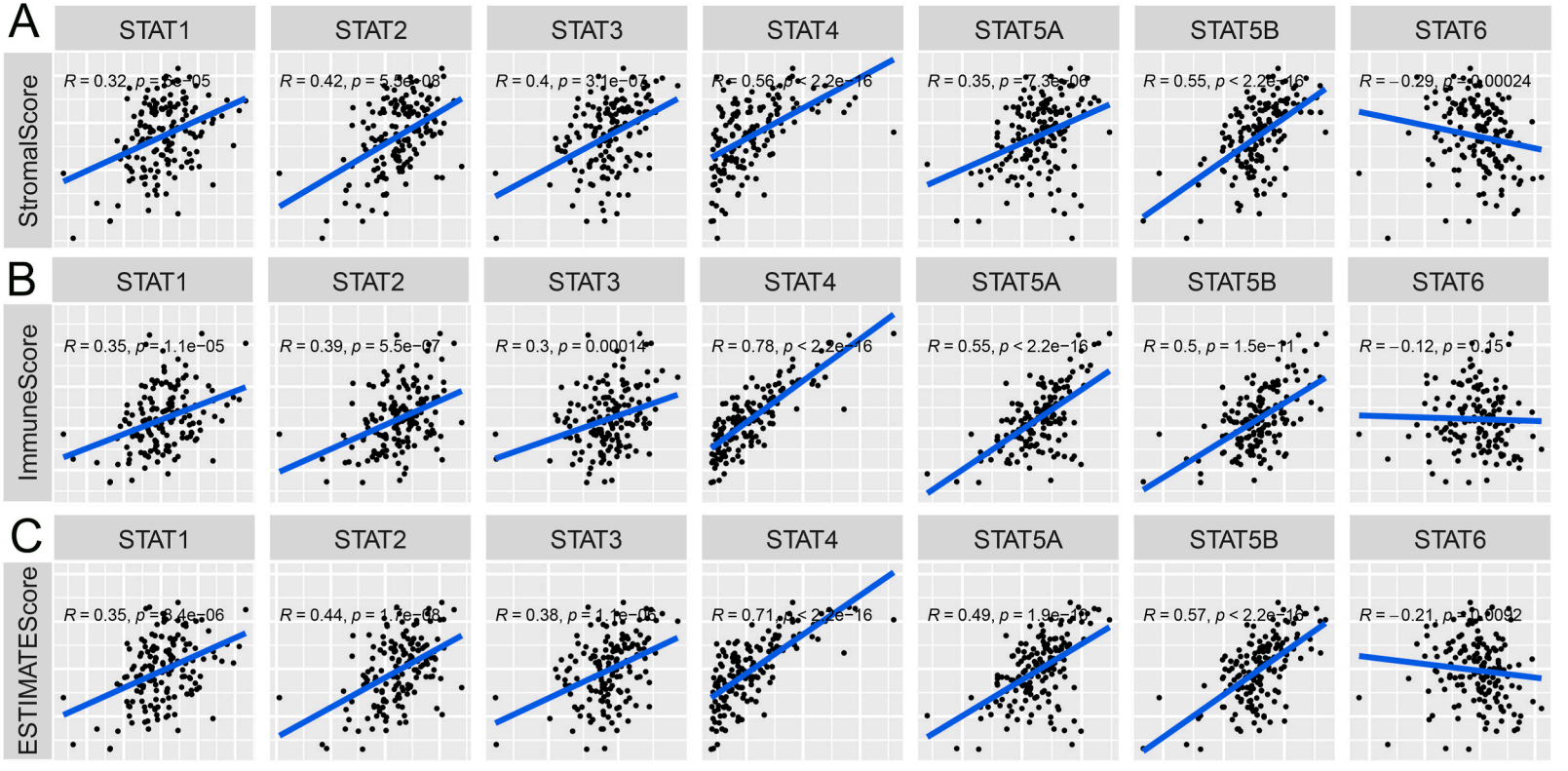

Fig. 3. The association between the expression of STAT family and immuno-stromal scores assessed in the TCGA cohort via ESTIMATE. (A) Correlations between the STAT family expression and the stromal scores. (B) Correlations between the STAT family expression and the immune scores. (C) Correlations between the STAT family expression and the estimate scores.

3.13, the Lacobuzio-Donahue dataset [28] with a FC of 2.189 and the Segara dataset [29] with a FC of 3.069. STAT2 was found to be higher expressed in pancreatic adenocarcinoma (FC = 2.901) in the Lacobuzio-Donahue dataset. However, STAT5B mRNA was significantly lower in PC tissues in the Logsdon dataset (Table 1). There was no significant difference regarding STAT3/4/5A/6 levels between PC and normal tissues (Fig. 1A). Meanwhile, the STAT1 levels were also significantly increased in the most types of cancers, except prostate cancer. STAT4 was found to be lower expressed in many datasets of leukemia and lymphoma. Likewise, the mRNA expression levels of STAT5A and STAT5B were downregulated in most datasets of breast cancer (Fig. 1A).

Then, GEPIA was used to further assess the STATs levels via the TCGA and GTEx cohort. STAT1/3/5A/6 was significantly elevated in PC tissues (Fig. 1B). Besides, the association between STATs and tumor stage in PC was also analyzed. All STATs were not related to the stage (Supplementary Fig. 1, $p > 0.05$), partly because of the limited number of patients with late stages. Moreover, the STATs levels were assessed by EMBL-EBI. STAT1, STAT3 and STAT6 were high-expressed in most cell lines of PC (Supplementary Fig. 2).

3.2. The prognostic values of STAT family in PC

To explore the prognostic values of STATs, the correlation between transcription levels of STATs and survival was evaluated in TCGA cohort via Kaplan-Meier (KM) Plotter. As shown in, PC patients with higher STAT1/4/6 expression had a significantly worse OS, while those with higher STAT5B had better OS (Fig. 2A). Consistently, statistically significant worse RFS was identified in PC patients with STAT1/4/6 higher mRNA expression as well as STAT2. In contrast, PC patients with higher STAT5B were significantly related to better RFS (Fig. 2B).

3.3. Genetic alterations of STAT family in patients with PC

Genetic alterations of 168 PC patients were evaluated using cBioPortal. The percentage of genetic alterations for STATs in PC varied from 5% to 11% (Supplementary Figs. 3A–B, STAT1, 8%; STAT2, 7%; STAT3, 7%; STAT4, 5%; STAT5A, 8%; STAT5B, 11%; STAT6, 10%). Kaplan–Meier survival analysis disclosed that no significant association between the genetic alterations in STATs and OS was observed (Supplementary Fig. 3C, $p > 0.05$) while genetic alterations in STATs were correlated with better RFS (Supplementary Fig. 3D, $p = 0.0199$).

3.4. Co-expression and functional enrichment analysis of STAT family in PC

A statistically significant positive association between the transcriptional patterns of STATs ($R > 0.3$) was observed, except STAT1 and STAT4; STAT1 and STAT5B; STAT4 and STAT5B; STAT5B and STAT6 (Supplementary Fig. 4A). Besides, the top 20 highly similar genes of each STATs in PC were gained by GEPIA (Supplementary Table 1). For instance, the top 20 highly similar genes of STAT1 were LAP3, GBP1, GBP1P1, IFIH1, UBE2L6, GBP4, EPSTI1, PARP14, TAP1, IFIT3, OAS3, CD274, GBP5, B2M, CXCL11, CXCL10, IFIT2, OAS2, TNFSF13B and CMPK2. Then, the biological function of seven STATs and their related genes were evaluated via GO and KEGG analysis after removing duplicates. As shown in Supplementary Fig. 4B, the regulation of cytokine production and signaling pathway mediated by cytokines, positive regulation of immune response, and T cell activation were significantly related to STATs and their similar genes via GO. Additionally, the top 10 KEGG with $p < 0.05$ are displayed. Among them, JAK-STAT signaling, chemokine signaling pathway, RIG—I-like receptor signaling pathway, cytokine-cytokine receptor interaction, primary immunodeficiency and T cell receptor signaling pathway were significantly enriched (Supplementary Fig. 4C). Hence, STATs may play significant roles in the remodeling of the tumor immune microenvironment.

3.5. Patterns of tumor-infiltrating immune cells related to STAT family expression levels

Significant positive correlations were observed between the STAT1/2/3/4/5A/5B expression and the immune-, stromal- and estimate scores via the ESTIMATE algorithm (Fig. 3), further supporting that the STAT family might regulate inflammatory response.

Then, the association between the transcription level of STATs and tumor-infiltrating immune cells (TIICs) was further assessed via TIMER online analysis tool. As shown in Supplementary Fig. 5A, STAT1expression was positively related to the infiltrations of $CD8^+$T cell, Macrophage, Neutrophil and Dendritic cell. STAT2 expression positively correlated with the infiltration of other five types of immune cells except B cell (Supplementary Fig. 5B). STAT3 expression was positively correlated with the infiltration of immune cells except for CD^+4 T cell (Supplementary Fig. 5C), while significant positive correlations were observed in STAT4/5A/5B expression levels with all six types of immune cells infiltration (Supplementary Figs. 5D–F). STAT6 expression was only positively related to the B cell infiltration (Supplementary Fig. 5G). These findings strongly confirm that the STAT family members have significant effects on immune infiltration in PC.

3.6. Validation of STAT1 expression and its prognostic value in resected PDAC

Based on the above analysis, STAT1 was upregulated in PC tissues and the patients with higher STAT1 expression had worse prognosis. The prognostic value of STAT1 expression was further validated in multiple PDAC cohorts. High STAT1 expression group had significantly worse OS in the ICGC and GSE71729 (Fig. 4A–B, $p = 0.023$ and $p < 0.001$, respectively). Meanwhile, a comparison analysis of STAT1 protein abundance between the normal and PDAC tissues also revealed that STAT1 protein level was significantly increased in tumor tissues (Fig. 4C, $p < 0.001$). Likewise, the patient with higher STAT1 protein abundance had a shorter OS than

patients with lower STAT1 expression in the CTPAC cohort (Fig. 4D, p = 0.03). Furthermore, STAT1 staining was administered for PDAC TMA specimens. As two loci were lost in both tumor and paracancerous normal tissues during TMA staining, 29 tumor tissues and 17 paracancerous normal tissues were finally analyzed. STAT1 expression was mainly located on the cytoplasm and nucleus of the tumor cells (Fig. 4E). Consistently, the staining index of STAT1 was markedly higher in cancer samples than those in paracancerous normal samples (Fig. 4F, p < 0.001). In addition, the OS of patients receiving postoperative adjuvant chemotherapy (ACT) with low STAT1 level was significantly longer than those with high level (Fig. 4G, p = 0.004). Therefore, STAT1 expression might be a potential indicator to predict the prognosis of PC patients.

3.7. Gene set enrichment analysis of STAT1 in PC

To more definitely recognize the role of STAT1 in PC, GSEA was conducted between two groups based on the median of STAT1 level in TCGA datasets. GESA revealed gene sets associated to interferon-gamma response, IL-2-STAT5 signaling, inflammatory response, IL-6-JAK-STAT3 signaling, mitotic spindle, KRAS signaling up, TGF-β signaling and TNFA signaling via NFKB were differentially enriched in the STAT1 high expression group (Fig. 5), suggesting that STAT1 may play an essential role in PC development and immune regulation.

3.8. The association between STAT1 expression and hot immune checkpoints and therapeutic responses

CTLA4, CD274 (PD-L1), HAVCR2 (TIM3), IDO1, BTLA, PDCD1LG2, PDCD1 and LAG3 were significantly increased in STAT1 high level group (Fig. 6A), suggesting the presence of potential immunosuppressive mechanism in patients with high STAT1 level. The levels of certain immune checkpoints could be used as promising biomarkers for predicting the response to immune checkpoint blockade. Thus, the clinical responses to CTLA-4 and PD-1 blockade were further estimated. The immunophenoscore (IPS) was applied to assess the potential response to immune checkpoint inhibitors. The IPS-PD-1 and IPS-PD-1+CTLA-4 were significantly higher in STAT1 high expression group, indicating that STAT1 expression might have a role to predict sensitivity to PD-1 and PD-1+CTLA-4 blockade (Fig. 6B). Besides, accumulating evidence indicated that STAT1 signaling of cancer cells was involved in chemoresistance. Thus, the responses to chemotherapy in STAT1 low and high expression groups were also investigated via the pRRophetic algorithm. Gemcitabine and Cisplatin except for Paclitaxel have lower IC50 in STAT1 high expression group, suggesting that the patients in STAT1 high expression group were more sensitive to these two drugs (Fig. 7A, p = 0.027 for Gemcitabine, p < 0.001 for Cisplatin). Then, the predictive value of STAT1 expression in chemotherapeutic response was further investigated. As shown in Fig. 7B–C, the prognosis of patients without chemotherapy was significantly poorer than those with chemotherapy in the high expression group, which was not

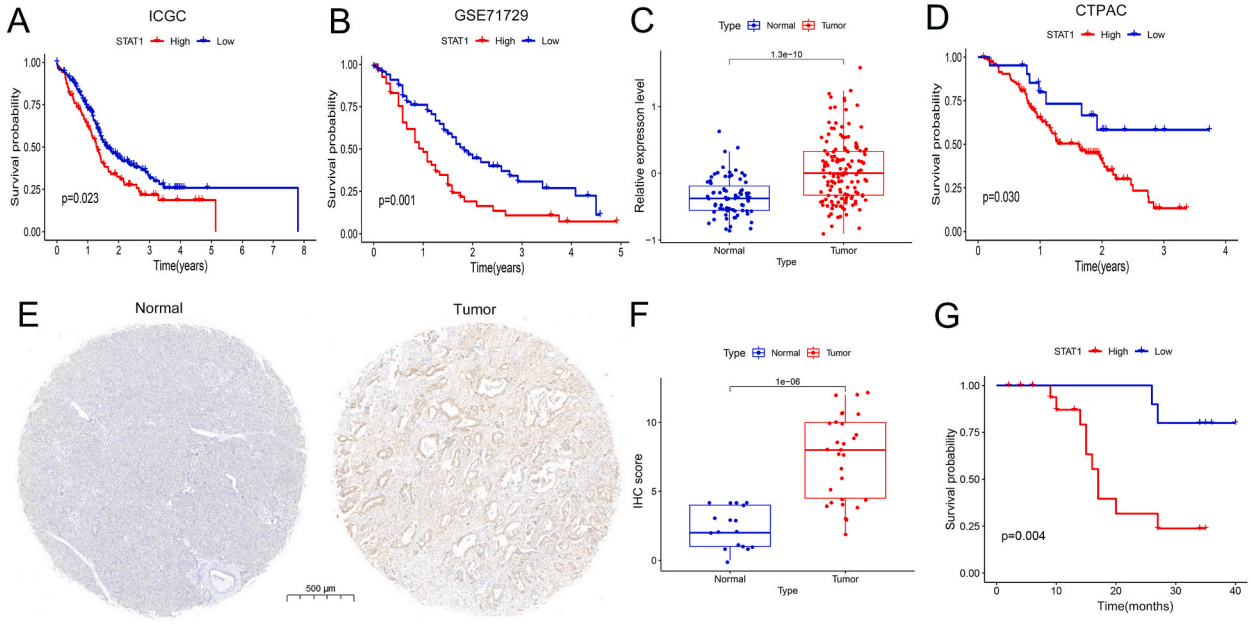

Fig. 4. Validation of the differential expression and prognostic value of the STAT family by multiple datasets. (A) Kaplan–Meier plot for overall survival of 269 pancreatic cancer patients in ICGC dataset according to the different level of STAT1 mRNA. (B) Kaplan–Meier plot for overall survival of 125 pancreatic cancer patients in dataset GSE71729 according to the different level of STAT1 mRNA. (C) The differential expression of STAT1 protein between the normal and tumor tissues in the CTPAC datasets. (D) Kaplan–Meier plot for overall survival of 127 pancreatic cancer patients in dataset GSE71729 according to the different level of STAT1 protein. (E) Representative pictures of IHC staining for STAT1. (F) The differential IHC scores of STAT1 between the normal and tumor tissues. (G) Kaplan–Meier plot for overall survival of 31 pancreatic cancer patients according to the different level of IHC scores. IHC, immunohistochemistry.

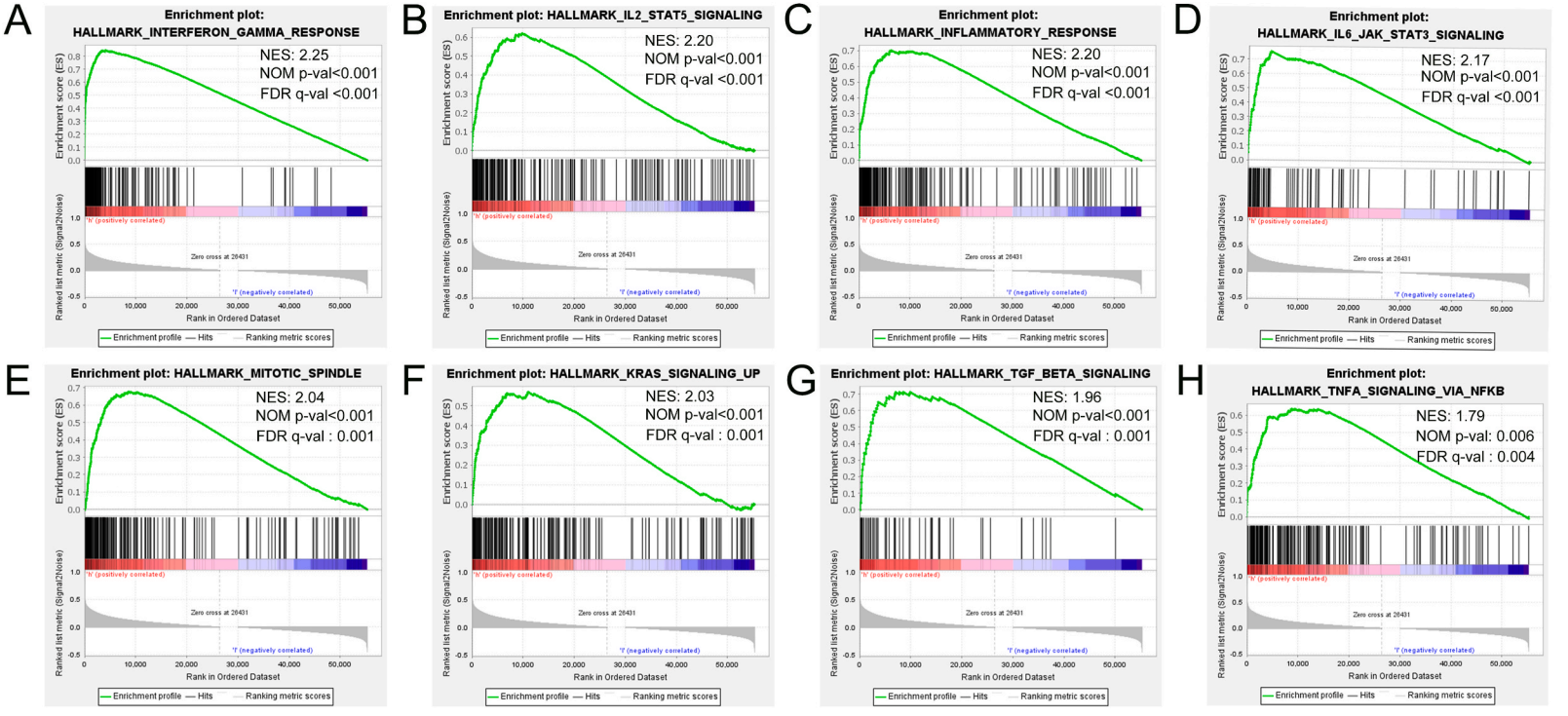

Fig. 5. Gene set enrichment analysis of STAT1 in pancreatic cancer in the TCGA cohort. The results indicated that pathways about interferon gamma response(A), IL-2-STAT5 signaling(B), inflammatory response(C), IL-6-JAK-STAT3 signaling(D), mitotic spindle(E), KRAS signaling up(F), TGF-β signaling(G) and TNFA signaling via NFKB(H) were differentially enriched in the high STAT1 expression group. Gene sets with a normalized P-value <0.01 and false discovery rate (FDR) < 0.01 are considered as significant.

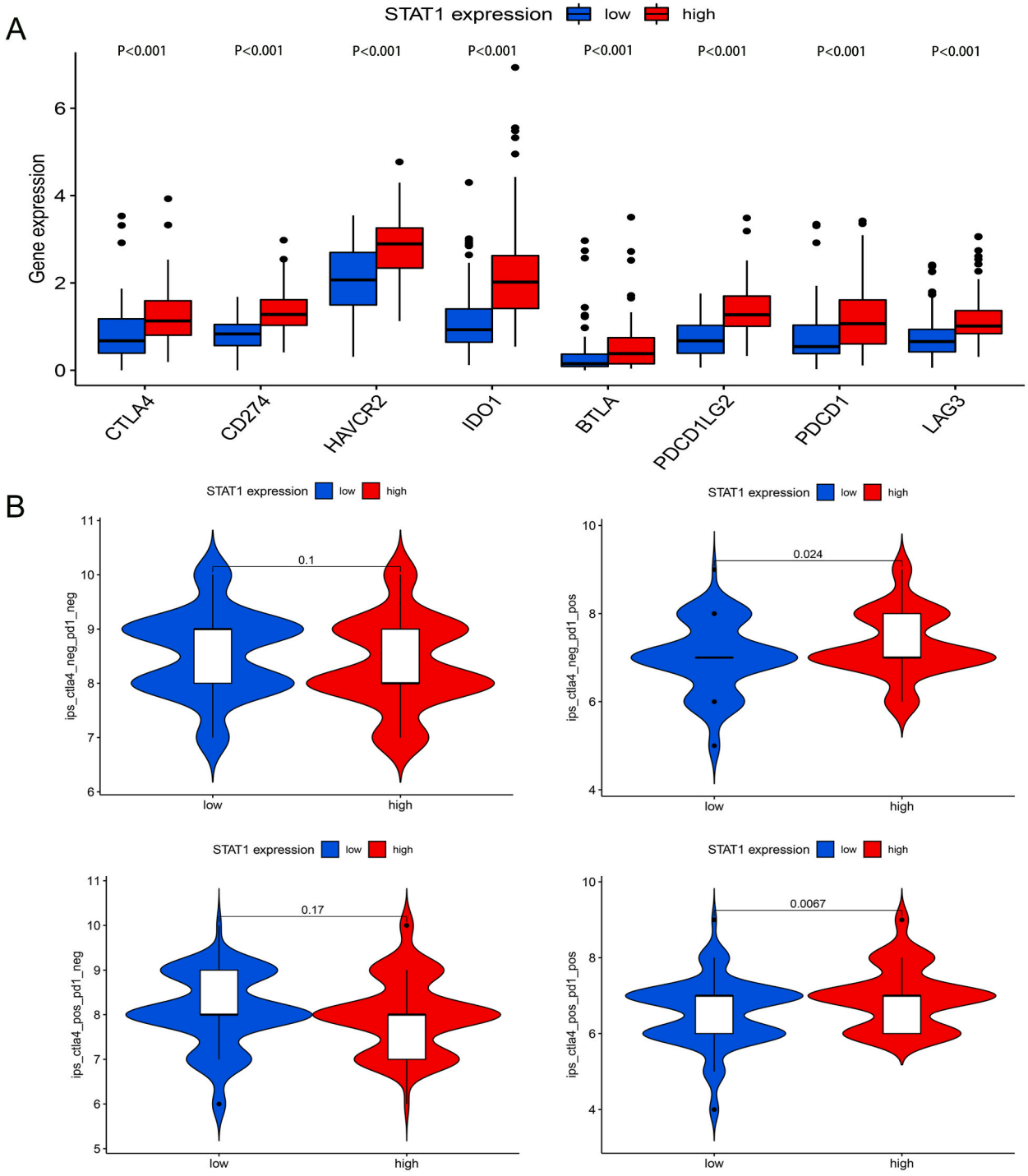

Fig. 6. The association between STAT1 expression and hot immune checkpoints and responses to immunotherapy in the TCGA cohort. (A) The comparison of the hot immune checkpoints abundance between the low and high STAT1 expression. (B) The differential level of immunophenoscore in different STAT1 expression groups; ips_ctla4_neg_pd1_ neg refers to CTLA4-negative response and PD1-negative response; ips_ctla4_neg_pd1_pos refers to CTLA4-negative response and PD1-positive response; ips_ctla4_pos_pd1_neg refers to CTLA4-positive response and PD1-negative response; ips_ctla4_pos_pd1_pos refers to CTLA4-positive response and PD1-positive response.

identified in the low expression group.

4. Discussion

Several researches have suggested the dysregulation of STATs is closely related to the initiation and progression of many tumors including hematologic malignancies and solid tumors [30–33]. STATs not only regulate cancer cell proliferation and differentiation, but also impact cell apoptosis [34–36]. Although the prognostic roles of STATs in certain cancers have been reported, there has been no research about differential expression and roles of STATs in PC. To our best knowledge, this study is the first study to comprehensively explore the transcription levels and roles of the STATs in PC and their association with prognosis, immune infiltration and the efficiency of drugs in patients with PC.

Functional enrichment analysis suggested that STATs participated in the pathways associated with immune response, carcinogenesis and progression, which was consistent with the previous studies [37]. The JAK/STAT pathways could induce the expression of several important molecules participating in tumor and inflammation [38]. Furthermore, a significant association between expression of STATs and the level of TIICs was identified in PC, except STAT6, which comes as no surprise given the fact that STATs are extensively involved in the production of several cytokines reported by numerous studies [8,39].

Using multiple online databases, the differential mRNA levels of STATs were investigated. Only STAT1 mRNA levels were significantly upregulated in PC samples than normal samples in both 7 Oncomine datasets and TCGA cohort at the same time. Meanwhile, PC patients with high expressions of STAT1/4/6 had a worse OS and PFS, while higher expression of STAT5B correlated with a favorable prognosis in PC patients in the TCGA cohort. Paradoxically, the previous study found that inhibiting STAT5b in cancer cells could attenuate tumor angiogenesis, metastases and chemoresistance in PC [14,40]. The possible cause of the contradiction might be the discordant expression of STAT5B between tissues and cell lines. As we found in EMBL-EBI, the STAT5B mRNA level was lower in PC cell lines than other STATs, while STAT1 was still highly-expressed in PC cell lines. STAT1 might be a potential diagnostic and prognostic biomarker and target in PC. Therefore, STAT1 was selected for further analysis and validation. However, the research on

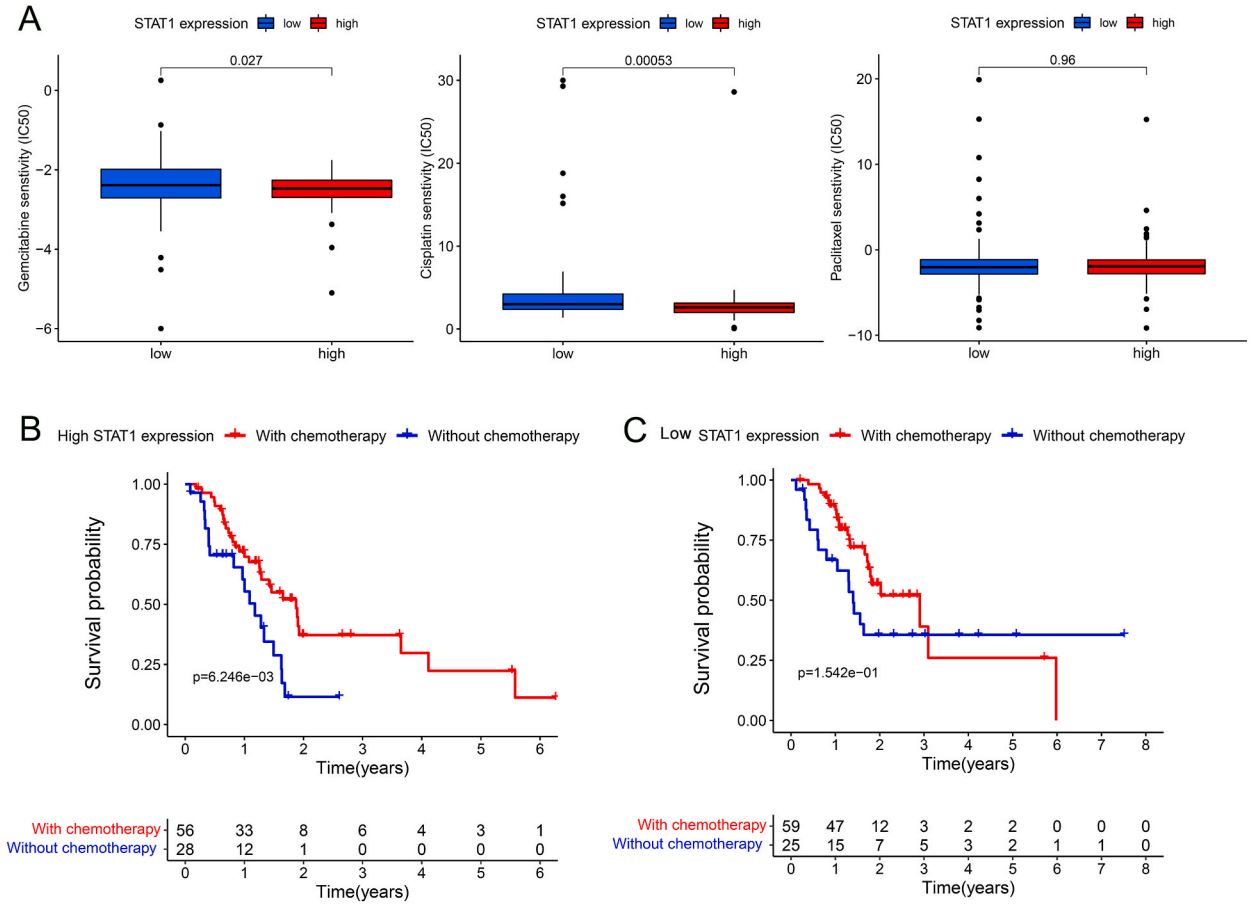

Fig. 7. The association between STAT1 expression and chemotherapy responsiveness in the TCGA cohort. (A) Three common chemotherapeutic responses in high- and low-STAT1 groups. (B) The Kaplan-Meier estimates overall survival in the high STAT1 expression group. (C) The Kaplan-Meier estimates overall survival in the low STAT1 expression group.

STAT1 in PC is limited. Meanwhile, the roles of STAT1 are still controversial in different tumor types [41]. Several studies indicated the decreased STAT1 expression in some cancers and their association with poor prognosis, including breast cancer [42] and colorectal cancer [43], while contradictory results have been found in other cancers, such as Ovarian cancer [44] and esophageal squamous cell carcinoma [45], which reflected the heterogeneity of the tumor. Hence, analyses of two independent cohorts were used to further validate the prognostic value of STAT1 mRNA and showed the results consistent with that in TCGA cohort. Additionally, the differential expression and prognostic performance of STAT1 protein were further verified by the proteome dataset CTPAC and IHC that STAT1 protein was up-regulated in PC and patients with higher STAT1 levels had shorter OS.

Then, GSEA was conducted to identify the pathways enriched in high STAT1 group. The KRAS signaling pathway, a well-known dysregulated pathway in the development and progression of PC, was found in the tumor with higher STAT1 expression. STAT1 has been reported to promote the proliferation, invasion, migration and tumorigenicity in other cancers [41], further supporting the pro-tumor role of STAT1. Moreover, the responses to cytokines, such as IFN-γ, TGF-β and TNF-α, were also enriched in the high STAT1 group, which had been reported to be involved in the regulation of the immune checkpoint expression and the sensitivity to immunotherapy [46,47]. Penafuerte et al. reported that TGF-β antagonist inhibited tumor growth and angiogenesis by inducing STAT1 activation [48]. Besides, studies have found a close association between STAT1 expression and PD-L1 expression in triple-negative breast cancer and ovarian cancer [49,50]. Overexpression of STAT1 in breast cancer cells could recruit the infiltration of myeloid-derived suppressor cells and inhibit the cytotoxicity of the T cells [51]. These findings were consistent with our finding that the expression of immune checkpoints, such as CTLA4, PD-1, PD-L1 and LAG3, were significantly higher in the high STAT group, which could partly reflect roles of the STAT1 in the immune suppression in PC. Furthermore, the STAT expression could predict the sensitivity to PD-1 and PD-1+CTLA-4 blockade.

Accumulating evidence indicated that STAT1 signaling of cancer cells was associated with resistance to chemoradiotherapy. Knockout of STAT1 in the cervical cancer cell impaired sensitivity to radiation and cisplatin and STAT1 expression was identified as one of the biomarkers of cervical cancer patients' responsiveness to chemoradiotherapy [52]. While in PC, STAT1 phosphorylation caused by IFN-γ stimulation improved the gemcitabine sensitivity via inhibition of FOXM1 [13], which is consistent with our findings that chemotherapeutic agents such as gemcitabine and cisplatin were found to have lower IC50 in STAT1 high expression group. Meanwhile, chemotherapy could significantly prolong the survival of the PC patients in the high STAT1 group, not in the low STAT1 group, further supporting the analysis of drug sensitivity.

However, it is worth noting that there are certain limitations in this study. Our study did not investigate the mechanism by which STATs influence the immune infiltration of PC. Further experimental studies are needed to reveal the association between TIICs and STATs and their specific mechanisms in PC. Furthermore, the sample size in our validation cohort was relatively small. A large, prospective, and multicenter validation cohort will be required in further studies.

In conclusion, our findings demonstrated that STATs played an essential role in the development, progression and immune regulation of PC. STAT1 could act as a diagnostic and prognostic biomarker with great potential and participate in immune evasion and progression of PC, which would enable us to select better therapeutic strategies.

Ethics approval and consent to participate

The protocol of this study was approved by the Ethics Committee of Yangzhou Hospital affiliated to Nanjing University of Chinese Medicine.

Availability of data and materials

The data that support the findings of this study are available on request from the corresponding author.

Author contributions

Study concept and design: ZH, YL; Acquisition of data: ZH, XZ, SW, XD, QW, QL, HL, YW, HW, XW, HW, YL; Statistical analysis: ZH, YL; Drafting of manuscript: ZH, XZ, HW, SW; Critical revision of manuscript for important intellectual content: ZH, XZ, HW, SW, XD, QW, QL, HL, YW, XW, HW, YL.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e16150.

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