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Pan-cancer analysis of Sp1 with a focus on immunomodulatory roles in gastric cancer

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Abstract

Background Sp1, a transcription factor, regulates essential cellular processes and plays important tumorigenic roles across diverse cancers. However, comprehensive pan-cancer analyses of its expression and potential immunomodulatory roles remain unexplored.

Methods Utilizing bioinformatics tools and public datasets, we examined the expression of Sp1 across normal tissues, tumors, and immune cells, and screened for pre- and post-transcriptional modifications, including genetic alterations, DNA methylation, and protein phosphorylation, affecting its expression or function. The association of Sp1 expression with immune cell infiltration, tumor mutational burden, and immune checkpoint signaling was also investigated. Single-cell transcriptome data was used to assess Sp1 expression in immune cells in gastric cancer (GC), and findings were corroborated using immunohistochemistry and multiplex immunofluorescence in an immunotherapy-treated patient cohort. The prognostic value of Sp1 in GC patients receiving immunotherapy was evaluated with Cox regression models.

Results Elevated Sp1 levels were observed in various cancers compared to normal tissues, with notable prominence in GC. High Sp1 expression correlated with advanced stage, poor prognosis, elevated tumor mutational burden (TMB), and microsatellite instability (MSI) status, particularly in GC. Significant correlations between Sp1 levels and CD8+T cell and the M1 phenotype of tumor-associated macrophages were further detected upon multiplex immunofluorescence in GC samples. Interestingly, we verified that GC patients with higher Sp1 levels exhibited improved response to immunotherapy. Moreover, Sp1 emerged as a prognostic and predictive biomarker for GC patients undergoing immunotherapy.

Conclusions Our pan-cancer analysis sheds light on the multifaceted role of Sp1 in tumorigenesis and underscores its potential as a prognostic and predictive biomarker for patients with GC undergoing immunotherapy.

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Keywords Sp1, Pan-cancer analysis, Prognosis, Immunotherapy response

Background

Gastric cancer (GC) is the fifth most common malignant tumor worldwide and the fourth leading cause of cancer-related deaths [1, 2]. Despite its high incidence, most patients are diagnosed at an advanced stage due to the lack of clear clinical manifestations, which results in limited treatment options and poor prognosis [3]. At present, surgery, and systemic chemotherapy remain the primary treatments for GC. For advanced GC patients, the median overall survival (OS) after chemotherapy is only 12 months [4]. Given the high incidence and poor short-term survival rate of GC, there is a pressing need to explore alternative treatment methods. Among them, anti-PD-1/anti-PD-L1 therapies have shown impressive efficacy and have significantly prolonged survival, especially in untreated patients with high microsatellite instability (MSI-H) or mismatch repair deficient (dMMR) GC [5]. However, the incidence of MSI-H status in GC ranges from 8 to 25%, limiting its utility as a predictive biomarker for advanced GC immunotherapy [6]. Additionally, multiple clinical studies have evaluated PD-L1 expression levels, and especially the combined positive score (CPS), which contemplates PD-L1 expression on tumor and surrounding immune cells, as predictive biomarkers for immune checkpoint inhibitor (ICI) response. However, reliable threshold values have yet to be established; even with commonly used threshold of 1, 5, and 10, patients who benefit from immunotherapy are not consistently identified [7–9]. Therefore, there is an urgent need to identify reliable predictive biomarkers for immunotherapy to enable precise treatment stratification for GC patients.

Tumor formation is a complex developmental process, and integrating pan-cancer analysis introduces a new dimension to cancer research [10]. This approach allows for the investigation of any gene of interest across multiple cancer types, facilitating the assessment of associations between specific genes and clinical outcomes, as well as the exploration of underlying molecular mechanisms. Resources such as The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO) offer extensive collections of functional genomics datasets from a wide variety of cancers, making them invaluable for comprehensive pan-cancer analysis.

The transcription factor specificity protein 1 (Sp1) is a member of the Sp/KLF family, and encodes a zinc finger protein that specifically binds to GC-rich motifs in various promoters. Sp1 regulates critical biological processes such as stem cell maintenance, embryonic development, cell proliferation, and tissue differentiation [11, 12]. The activity of Sp1 is significantly influenced by post-translational modifications, such as phosphorylation, acetylation, glycosylation, and proteolytic processing. Depending on these modifications, Sp1 can function either as a transcriptional activator or repressor. Previous studies have shown that Sp1 is overexpressed in various types of cancer, including ovarian cancer [12], pancreatic cancer [13], hepatocellular carcinoma [14], glioblastoma [15], lung cancer [16], and breast cancer [17], in association with poor prognosis. As a housekeeping gene, Sp1 can activate or inhibit the transformation of normal cells into cancer cells, thereby influencing cancer progression [18, 19]. For example, Liu et al. demonstrated that Sp1 plays a crucial role in promoting proliferation, migration, and chemotherapy resistance in epithelial ovarian cancer [20]. In addition, studies have shown that Sp1 promotes cancer cell proliferation and inhibits apoptosis [20]. Our previous studies have also indicated that Sp1 promotes the development of GC and is a poor prognostic factor for this disease [21]. However, most current research focuses on the role of Sp1 within tumor cells, with limited reports on its extensive pro-tumorigenic roles, especially its effects on the tumor microenvironment (TME). Recent studies have indicated that Sp1 is a key mediator involved in the epigenetic programming and reprogramming of human papilloma virus (HPV) hosts. Inhibition of Sp1 with plicamycin has been shown to enhance anti-PD-1 immunotherapy by reshaping the TME, suggesting that Sp1 blockade may be a promising treatment option for HPV-related cancers [22]. Despite these findings, there are no relevant reports on the immunomodulatory role of Sp1 in GC. Therefore, using bioinformatics analyses and immunolabeling techniques, this study aims to evaluate the role of Sp1 in the immune microenvironment of GC, and its impact on the efficacy of ICIs.

Methods

Gene mapping and protein structure analysis

Based on the UCSC genome browser on human Dec. 2013 (GRCh38/hg38) assembly (http://genome.ucsc.edu/), the genome location information of Sp1 was obtained. We also applied the "HomoloGene" function of the NCBI (National Center for Biotechnology Information) to conduct conserved functional domain analysis of Sp1 in different species. Additionally, we obtained the phylogenetic tree of Sp1 in different species using the constraint-based multiple alignment online tool of the NCBI (https://www.ncbi.nlm.nih.gov/tools/cobalt/).

Gene expression analysis

We first accessed the online Human Protein Atlas (HPA) database and obtained expression data of Sp1 in different

normal tissues, cancerous tissues, and immune cells. As per retrieval parameters, "low specificity" was defined as "normalized expression (NX)≥1 in at least one tissue/region/cell type but not elevated in any tissue/region/cell type". We used tumor immune estimation resource, version 2 (TIMER2) website (http://timer.cistrome.org/) to investigate the expression difference of Sp1 between cancerous and adjacent normal tissues in different tumors of the TCGA project. We also used "Box Plots" module of the Gene Expression Profiling Interactive Analysis, version 2 (GEPIA2) website (http://gepia2.cancer-pku.cn/#analysis) to acquire box plots of the expression difference of Sp1 between tumor tissues and the corresponding normal tissues of the Genotype-Tissue Expression (GTEx) database. In addition, the violin plots of Sp1 expression in different TNM stages of all TCGA tumors with the online tool HEPIA2. We next compared expression levels of total and phosphorylated Sp1 protein between cancerous and adjacent normal tissues via the UALCAN portal (http://ualcan.path.uab.edu/analysis-prot.html). Available Clinical Proteomic Tumor Analysis Consortium (CPTAC) datasets in the UALCAN portal include six tumors, namely, breast cancer (BRCA), ovarian cancer, colon cancer, renal cell carcinoma (RCC), uterine corpus endometrial carcinoma (UCEC), and lung adenocarcinoma (LUAD).

Patients and specimens

From January 2018 to December 2022, 26 patients undergoing gastrectomy for gastric cancer and 24 advanced GC patients receiving ICIs and chemotherapy in the Affiliated Changzhou Second People's Hospital of Nanjing Medical University. The clinicopathological characteristics of these patients were summarized in supplementary Tables 1 and supplementary Table 2. Cancerous and adjacent normal tissue was collected during surgery or puncturation, and histopathologically confirmed and staged according to the Union for International Cancer Control. Patients' written informed consents and approval from the Ethics Committees of the Affiliated Changzhou Second People's Hospital of Nanjing Medical University (No.2017-C-015-01) were obtained for the use of these clinical materials.

Immunohistochemistry

Tumor sections from patients with advanced GC were incubated in an oven at 55 °C for 20 min, followed by dewaxing by three 5-min washes with xylene, and rehydration 5-min washes in 100%, 95%, and 80% ethanol/distilled water. Samples were then heated at 95 °C for 30 min in 10 mmol/L sodium citrate (pH 6.0) for antigen retrieval, and endogenous peroxidase activity was blocked by incubation in 3% H2O2 for 30 min. After a 30-min blocking step with the universal blocking serum (Dako Diagnostics, Carpinteria, CA), the sections were incubated with anti-Sp1 antibody at 4 °C overnight and washed 3 times with PBS at room temperature. Then a secondary, HRP-conjugated antibody was added for 30 min incubation (Dako Diagnostics). The samples were washed 3 times with PBS and developed using DAB, followed by counterstaining with hematoxylin. Dehydration was performed following a standard procedure, and the slides were sealed with cover slips. Images were scanned with a digital pathology slide scanner (KF-BIO, CHINA).

Sp1 immunostaining signals were then evaluated by two researchers, with the clinical information blinded to them, and scored. Brown cytoplasmic staining for Sp1 was considered positive. The percentage of Sp1-positive cells was scored with the following four categories: 1 (<25%), 2 (25–50%), 3 (50–75%), and 4 (>75%). The staining intensity of positive cells was scored as 0 (absent), 1 (weak infiltration), 2 (moderate infiltration), and 3 (strong infiltration). The final score was the sum of the intensity and the percentage.

Survival analysis

The "survival map" module of GEPIA2 was used to conduct the survival analysis of Sp1 across all TCGA tumors. Cutoff-high (50%) and cutoff-low (50%) values were used as the expression thresholds for splitting the high-expression and low-expression groups of Overall survival (OS) and Disease-free survival (DFS).

Genetic alteration analysis

We investigate the genetic alteration characteristics of Sp1 with the cbioportal website (https://www.cbioportal.org/). The results of the alteration frequency, mutation type and Copy number alteration (CNA) were obtained in the "Cancer Types Summary" module. We also used the "Comparison" module to obtain the data of OS, progression-free survival (PFS), and DFS differences in the TCGA cancer cases with or without Sp1 genetic alteration.

Analysis of tumor behavior states, immune infiltrates, and immune biomarkers

The online tool Sangerbox (http://sangerbox.com/index.html) was used to investigate the correlations between tumor mutational burden (TMB), MSI and Sp1 expression in all types of cancers in TCGA. The Sangerbox platform was further used to assess the correlations between the Sp1 expression and a variety of genes involved in immune checkpoint signaling, such as CTLA4. Spearman's correlation was performed on the data and the *P*-values and partial correlation (cor) values were obtained.

We used the TIMER2 online tool to explore correlations between Sp1 expression and several types of

immune cells, including B cells, CD4+ T cells, CD8+ T cells, dendritic cells, macrophages, and neutrophils in TCGA tumors. The TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, XCELL, MCPCOUNTER, and EPIC algorithms were applied for immune infiltration estimations, especially for CD8+ T cells. Corresponding *P*-values and correlation values were obtained via the purity-adjusted Spearman's rank correlation test. The data were visualized as a heatmap and a scatter plot.

DNA methylation analysis

We also used the SangerBox tool to investigate the correlations between the Sp1 expression and four classical DNA methyltransferases (DNMTs) including DNMT1, DNMT2, DNMT3A, and DNMT3B in all types of cancer. The MEXPRESS web (https://mexpress.ugent.be/) was used to analyze the DNA methylation level of Sp1 of multiple probes in different cancers of TCGA database. The beta value of methylation, the Benjamini-Hochberg-adjusted *P*-value and Pearson correlation coefficient value of each sample were obtained. The promoter region probes were highlighted.

Phosphorylation analysis

We used iPTMnet database (http://proteininformationresource.org/iPTMnet) to analyze the predicted phosphorylation features of the S7, T42, S59, S101, T278, T453, S641, T668, S698, and S702 locus of Sp1. We also investigate the differences in phosphorylation levels of Sp1 between normal tissues and primary tumors, including BRCA, ovarian cancer, colon cancer, RCC, and UCEC, using the CPTAC analysis.

Multiplex immunofluorescence

Multiplex staining was performed on GC sections from patients with advanced GC using a TSA 6-color kit (H-D110061,yuanxibio). The panel of primary antibodies panel included anti-CD8 (#BX50036, Biolynx), anti-CD68 (#BX50031-C3, Biolynx), anti-HLADR (#ab92511, Abcam), and anti-PanCK (#GM351507, Gene Tech). These antibodies were sequentially applied, followed by HRP-conjugated secondary antibody incubation (Cat# DS9800, Lecia Biosystems), and fluorescent tyramide signal amplification. The slides were washed with TBST buffer and heat-treated by microwaving after each TSA application. Nuclei were finally stained with DAPI (D1306, ThermoFisher), and the stained slides were scanned to obtain multispectral images using a Pannoramic MIDI imaging system (3D HISTECH). Images was analyzed using Indica HALO software.

Enrichment analysis of Sp1-related genes

The STRING online tool (https://string-db.org/) was applied to investigate the top 50 experimentally determined Sp1-binding proteins. The main parameters were set as follows: minimum required interaction score ["Low confidence (0.150)"], meaning of network edges ("evidence"), max number of interactors to show ("no more than 50 interactors" in 1st shell) and active interaction sources ("experiments"). The GEPIA2 was used to determine the top 100 Sp1-correlated genes based on the TCGA datasets. Furthermore, we used the "Gene_Corr" module of TIMER2 to supply the heatmap data of the selected genes, which contains the correlation and *P*-value in the Spearman's rank correlation test. The log2 TPM was applied for the dot plot. The *P*-value and the correlation coefficient (R) were indicated. Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to conduct an intersection analysis to compare the Sp1-binding and interacted genes. Then, these two sets of genes were combined and submitted to DAVID for additional functional annotation, such as Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). In this work, we mainly focused on three aspects of GO analysis: biological processes (BP), cellular components (CC), and molecular functions (MF). In addition, we used KEGG analysis to investigate the pathways in which the Sp1-binding and interacted genes were involved.

Gene set enrichment analysis

Gene Set Enrichment Analysis (GSEA) was used to explore the up-downregulations among different pathways associated with Sp1 in STAD. The functional gene set was set to c2.cp.kegg.v7.4.symbols.gmt, the analysis parameters were "No collapse", the number of permutations was set to "1000", the permutation type was set to "Phenotype", and the above files were analyzed by GSEA software (version 3.0). In this study, GESA was used to explore Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways significantly associated with high and low Sp1 expression, and mapped the top five pathways. P-value < 0.05 and FDR < 0.25 were considered statistically significant.

scRNA-seq data analysis

GC single-cell RNA sequencing (scRNA-seq) data (GSE163558) were obtained from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). The data included 10 fresh human tissue samples of six patients, including primary tumors, adjacent non-tumoral samples, and six metastases from various organs or tissues (liver, peritoneum, ovary, lymph node). Data filtering and preprocessing were conducted using the R package "Seurat". The initial screening criteria included: genes expressed in at least three cells; each cell expresses at least 250 genes; the percentage feature set function was used to calculate the percentage of mitochondria content and

rRNA expression, keeping only those cells that expressed between 200 and 5000 genes, and mitochondrial genes comprising less than 15% of the total genome. Following data filtering, samples were merged for further analysis. To address batch effects and integrate different single-cell transcriptome samples, the FindIntegrationAnchors and IntegrateData functions in the Seurat package were employed, identifying 4000 highly variable genes with the FindVariableFeatures function. Then, principal component analysis (PCA) was performed using the RunPCA function. Cell clustering was carried out with the FindNeighbors and FindClusters functions (resolution=0.1, dim=50). Dimensionality reduction was achieved using the UMAP method. Marker genes for each cluster were identified using the FindAllMarkers function (logFC=0.75, min.pct=0.25, p-adj<0.05). According to current classification standards, in this analysis, we classified cells as M1 macrophages based on high expression of TNF and CD86, whereas M2 macrophages were identified based on high expression of VEGFA, VEGFB, IDO1, CD68, CD163, and CCL20.

Results

Sp1 expression analysis

The aim of this study was to investigate the oncogenic role of Sp1 (Supplementary Fig. 1A). The structure of Sp1 is characterized by a Zinc-finger double domain (Supplementary Fig. 1B). As shown in the phylogenetic tree presented in Supplementary Fig. 1C, Sp1 shows relative conservation across various species.

We conducted a comprehensive analysis of Sp1 expression patterns across various tumor tissues, normal tissues, and blood cells. As depicted in Supplementary Fig. 2A, Sp1 shows the highest expression in early spermatids, closely followed by urothelial cells. Utilizing integrated data from the HPA, GTEx, and Functional Annotation of the Mammalian Genome 5 (FANTOM5) datasets, we observed that the expression of Sp1 is most prominent in the esophagus among all normal tissues (Supplementary Fig. 2B). Additionally, Sp1 demonstrated the highest expression in neutrophils, compared to other types of blood cells (Supplementary Fig. 2C).

We further utilized TIMER2 to examine the expression levels of Sp1 in different types of cancers within the TCGA database. Notably, differential expression of Sp1 was observed in BRCA, cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), kidney chromophobe carcinoma (KICH), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and UCEC exhibited significant differences compared to the corresponding normal tissues ($P<0.001$, Fig. 1A).

Additionally, when normal tissues from the GTEx dataset were included as controls, we observed significant differences in Sp1 expression levels between cancerous tissues and adjacent normal tissues across various cancer types, including CHOL, GBM, low-grade glioma (LGG), pancreatic adenocarcinoma (PAAD), STAD, and uterine carcinosarcoma (UCS) ($P<0.01$, Fig. 1B).

The analysis of the CPTAC dataset revealed elevated protein levels of Sp1 in BRCA, colon cancer, and LUAD compared to normal tissues ($P<0.001$). Conversely, total Sp1 protein expression was found to be lower in primary clear cell renal cell carcinoma (ccRCC) ($P=0.009$) and UCEC ($P=0.007$) in relation to matched normal tissues. No significant difference in Sp1 protein levels was observed between ovarian cancer and ovarian tissues (Fig. 1C).

Using the online tool GEPIA2, we identified a significant correlation between Sp1 gene expression and pathological stage of kidney renal clear cell carcinoma (KIRC) and LIHC ($P<0.05$, Fig. 1D), and marginal associations with the pathological stages of lung squamous cell carcinoma (LUSC; $P=0.0829$) and skin cutaneous melanoma (SKCM; $P=0.054$). No significant correlations were found for other cancer types.

Through the examination of cancerous and adjacent normal tissues from 26 GC patients enrolled in our hospital, we validated a significant increase in the protein levels of Sp1 in GC compared to adjacent normal tissues ($P=0.006$, Fig. 1E, F).

Through a comprehensive analysis of gene and protein expression data, we mapped the expression profile of Sp1 across various cancer types.

Survival analysis

We next investigated the correlation between Sp1 gene expression and survival outcomes of cancer patients using GEPIA2. As illustrated in Supplementary Fig. 3A, the median OS was significantly longer in patients with low, rather than high, Sp1 expression in LGG, LIHC, PAAD, and THCA ($P<0.05$). In contrast, KIRC patients with low Sp1 expression exhibited a shorter median OS than those with high Sp1 expression ($P<0.001$). Moreover, high Sp1 expression correlated with poor DFS in LGG, adrenocortical carcinoma (ACC), urothelial bladder carcinoma, and LIHC (Supplementary Fig. 3B). Whereas low Sp1 expression correlated with poor DFS in KIRC patients ($P<0.001$). No significant correlations were observed between OS, DFS, and Sp1 expression in other types of cancers. Our results suggest that Sp1 could serve as a potential biomarker for predicting patients' prognosis. Furthermore, consistent with its expression patterns, the predictive capability and prognostic outcomes of Sp1 vary across different cancer types.

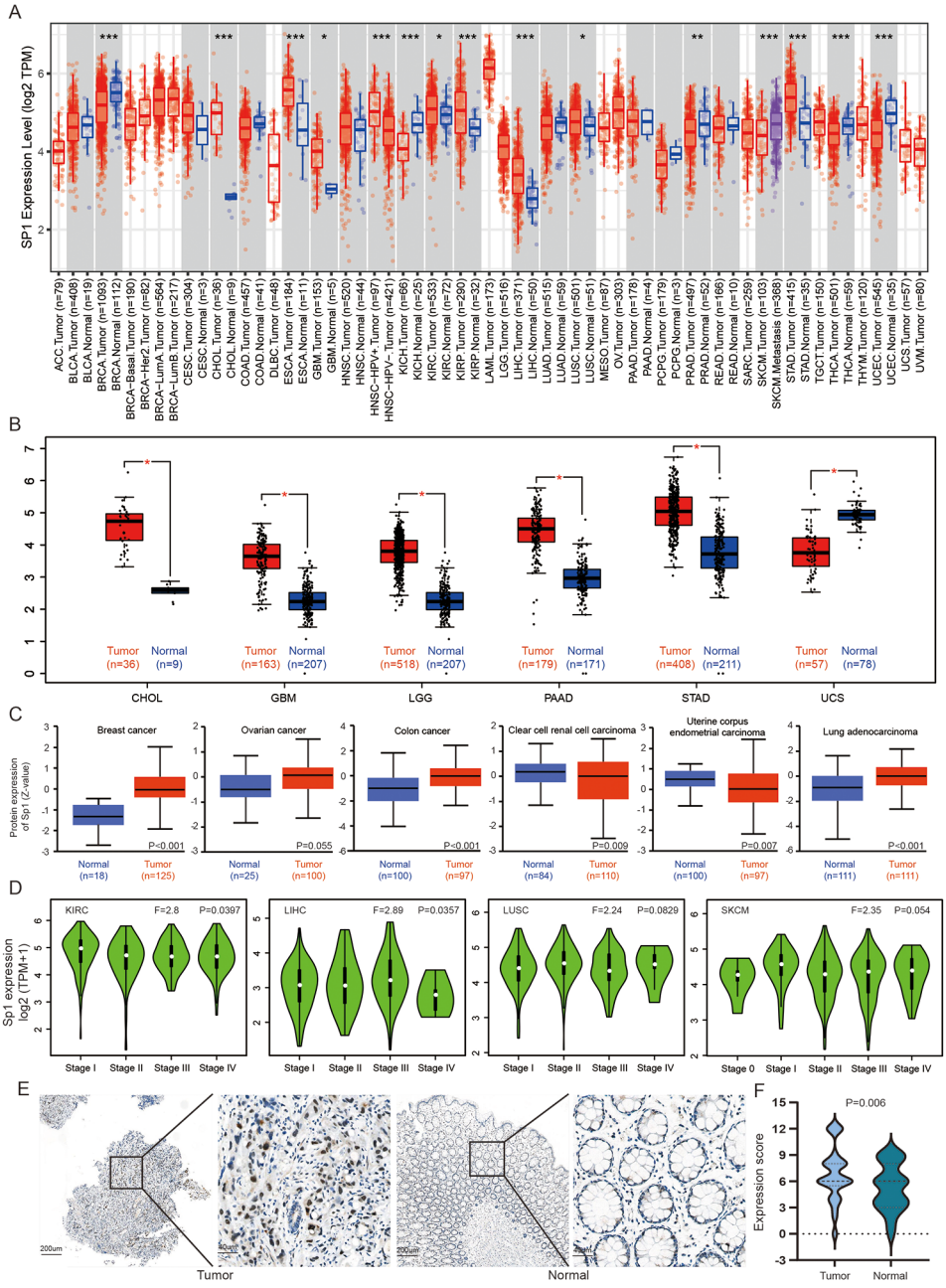

Fig. 1 Expression of Sp1 in different tumor types and correlation with pathological stages. (**A**) TIMER2 analysis of Sp1 expression in different tumor types. (**B**) For CHOL, GBM, LGG, PAAD, STAD, and UCS data in the TCGA project, the corresponding normal tissues were included as controls. (**C**) Protein expression levels of Sp1 in breast cancer, ovarian cancer, colon cancer, ccRCC, UCEC, and LUAD, and matched normal tissues in the CPTAC dataset. (**D**) Analysis of the Sp1 gene expression according to pathological stages (stage I-IV) in KIRC, LIHC, LUSC, and SKCM in TCGA. Log2 (TPM + 1) transformation was applied for normalization. (**E**) Representative immunohistochemistry images of Sp1 expression in GC and adjacent normal tissues. (**F**) Quantification of IHC data from (**E**). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Analysis of Sp1 genetic alterations

We investigated genetic alterations in Sp1 in different types of cancers using the TCGA database (Supplementary Fig. 4). The highest alteration frequency (10.53%), characterized mainly by amplification, was observed in patients with UCS (Supplementary Fig. 4A). In turn, all cases of SKCM and LIHC with an alteration frequency > 1% exhibited mutations in Sp1. Structural variants, defined as large rearrangements within DNA segments, were the predominant source of variation in the Sp1 in UCS. The sites and types of Sp1 genetic alterations, along with post-translational modifications, are listed in Supplementary Fig. 4B. We found that the missense and truncating mutations were the primary

genetic variations. Given that UCS displayed the highest alteration frequency for Sp1, we investigated the correlation between Sp1 alteration status and patient survival. Intriguingly, we found a significant correlation between Sp1 mutational burden and PFS ($P=0.0294$), but not OS, DFS, and disease-specific survival (Supplementary Fig. 4C, $P>0.05$).

DNA methylation analysis

We used SangerBox interface to investigate the correlation between Sp1 expression and four classical DNMTs, DNMT1, DNMT2, DNMT3A, and DNMT3B. As shown in Supplementary Fig. 5, Sp1 expression exhibited a significant correlation with the expression levels of DNMT1, DNMT2, DNMT3A, and DNMT3B expression in THCA, uveal melanoma (UVM), diffuse large B-cell lymphoma (DLBC), LGG, and LIHC (all $R>0.3$, $P<0.001$).

With the online tool MEXPRESS, we further investigated the association between Sp1 DNA methylation and the pathogenesis of various types of cancers using the TCGA database. In patients with GC, we found a positive correlation between Helicobacter pylori infection ($P=0.022$), sample types ($P=0.002$), copy number ($P<0.001$) and Sp1 expression, and a significant negative correlation between Sp1 DNA methylation and Sp1 expression based on multiple probes, sequences for non-promoter regions and promoter regions, such as cg14794577 ($R=-0.339$, $P<0.001$, Supplementary Fig. 6). This suggests that the expression of Sp1 was largely affected by the methylation processes in various cancers, especially in GC.

Sp1 phosphorylation analysis

A list of characterized Sp1 phosphorylation sites is provided in Supplementary Fig. 7A. On the iPTMnet database (https://research.bioinformatics.udel.edu/iptmnet/), S7, T42, S59, S101, T278, T453, S641, T668, S698, and S702 were identified as Sp1 phosphorylation sites supported by the highest confidence data. We investigated differences in Sp1 phosphorylation levels between normal tissues and primary tumors, specifically in BRCA, ovarian cancer, colon cancer, ccRCC, and UCEC, using CPTAC analysis. Results showed that the T42 residue within Sp1 exhibited significantly higher phosphorylation levels in the primary tumor of colon cancer compared to normal colon tissues ($P<0.001$). Conversely, higher phosphorylation levels on T42 were observed in matched normal tissues compared to primary BRCA ($P=0.045$), ovarian cancer ($P=0.003$), and UCEC ($P=0.019$). There were no significant difference in T42 phosphorylation levels between normal kidney tissues and ccRCC ($P=0.134$) (Supplementary Fig. 7B).

Enrichment analysis of Sp1-related genes

To investigate the molecular mechanisms linking Sp1 to tumorigenesis, we identified Sp1-binding proteins and genes correlated with Sp1 expression for pathway enrichment analyses. Utilizing the STRING tool, we identified a total of 50 Sp1-binding proteins, with their interaction network illustrated in Fig. 2A. The GEPIA2 tool was then used to retrieve from TCGA tumor expression data the top 100 genes correlated with Sp1 expression. Among these genes, the highest correlations were observed for ASXL2 ($R=0.78$), ATF7 ($R=0.75$), BAZ2A ($R=0.75$), MAP3K2 ($R=0.75$) and PKN2 ($R=0.75$) (all $P<0.001$). As shown in the heatmap in Fig. 2B, a positive correlation between Sp1 and these five genes exists across various cancer types. An intersection analysis of the two datasets revealed two common members, namely CRREBP and EP300 (Fig. 2C).

We next integrated the two datasets to conduct KEGG and GO enrichment analyses. KEGG results, depicted in Fig. 2D, suggested that pathways such as "viral carcinogenesis" and "pathways in cancer" might contribute to Sp1's impact on tumor pathogenesis. In turn, GO enrichment analysis data revealed that most of these genes are associated with pathways or cellular processes related to gene transcription, including DNA binding, protein binding, transcription factor binding, chromatin binding, histone deacetylase binding, and others (Supplementary Fig. 8).

Using the TCGA pan-cancer dataset, we conducted GSEA which showed that T cell receptor signaling pathway, chronic myeloid leukemia, and small cell lung cancer (SCLC), were predominantly enriched in the Sp1 high-expression group. Conversely, glycerolipid metabolism and olfactory transduction were primarily enriched in the Sp1 low-expression group (Fig. 2E).

Our pathway analysis showed that Sp1 is involved in immune cell regulatory pathways. Since substantial evidence indicates that the host immune system plays a crucial role in inhibiting and promoting tumor growth and metastasis. Understanding Sp1's impact on the immune microenvironment is, therefore, essential for developing more effective cancer treatments. Using the TIMER2 database, we investigated the potential correlations between Sp1 expression and several types of tumor-infiltrating immune cells, including B cells, CD4+ T cells, CD8+ T cells, dendritic cells, macrophages, and neutrophils. The analysis demonstrated highly significant correlations between Sp1 expression and these immune cells in colon adenocarcinoma (COAD), head-neck squamous cell carcinoma (HNSC), and KIRC ($P<0.005$ for all) (Supplementary Fig. 9).

Previous studies demonstrated that Sp1 plays a crucial role in regulating the expression and function of various immune cells, including CD8+ T cells, and macrophages.

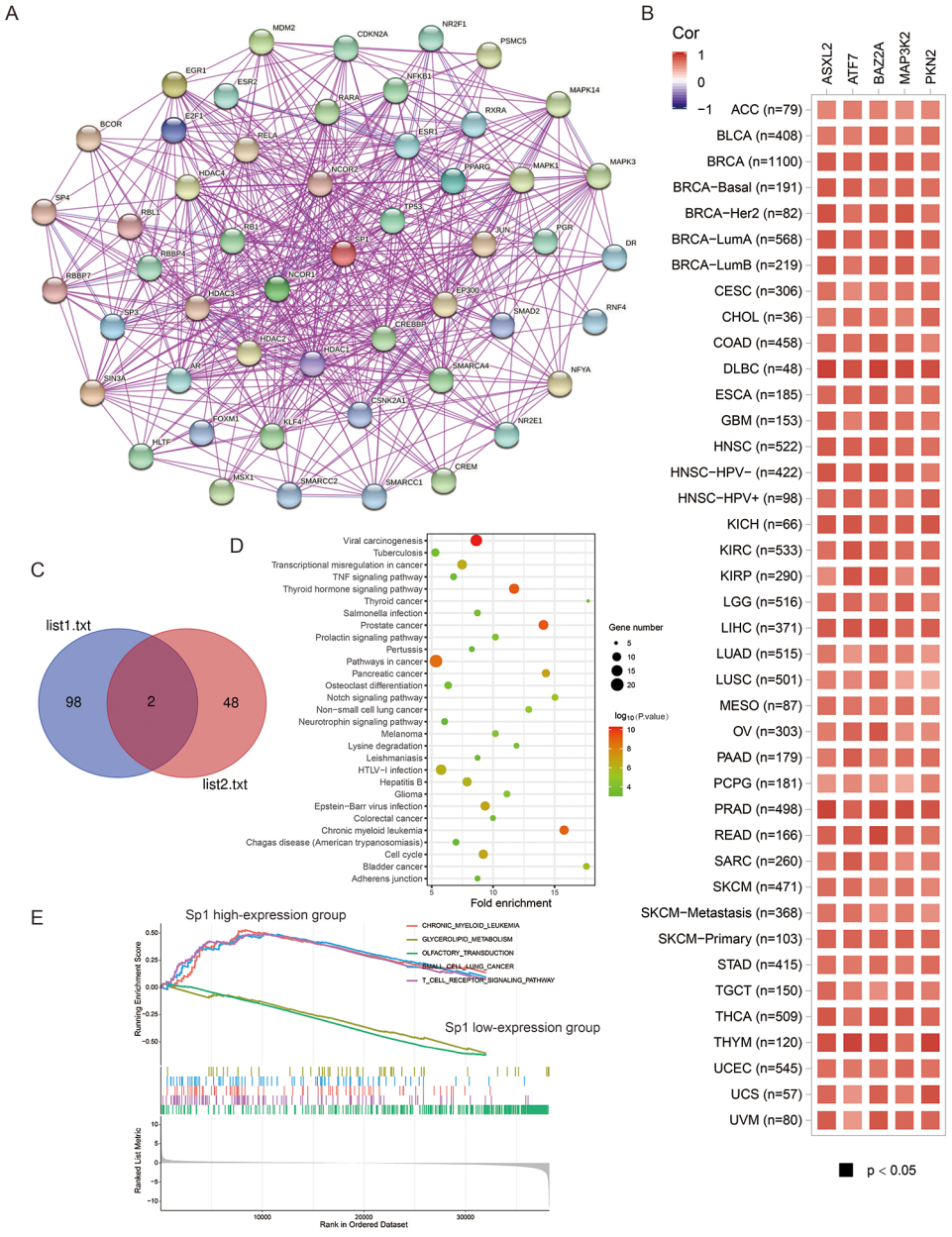

Fig. 2 Enrichment analysis of Sp1-associated genes. (**A**) STRING protein-protein interaction network for 50 Sp1-binding proteins. (**B**) Heatmap depicting pan-cancer expression of the top five genes correlated with Sp1 expression (GEPIA2). (**C**) KEGG pathway analysis was performed based on the Sp1-binding and interacted genes. (**D**) KEGG pathway analysis was performed based on Sp1-binding proteins and Sp1- interacting genes. (**E**) Gene Set Enrichment Analysis (GSEA) according to Sp1 expression in the TCGA pan-cancer dataset

To assess the correlations between CD8+ T cells, M1/M2 phenotype of tumor-associated macrophages (TAMs), and Sp1 expression, we used the EPIC, MCPCOUNTER, and TIMER algorithms (Supplementary Fig. 10A). As illustrated in Supplementary Fig. 10B-E, the three algorithms indicated significantly positive correlations between Sp1 expression and CD8+ T cells in STAD, BRCA-Basal, SKCM, and prostate adenocarcinoma (PRAD) ($P<0.05$ for all). We also detected a positive correlation between Sp1 expression and the M1 phenotype of TAMs in STAD with all three algorithms. The enrichment analysis guided us in identifying the specific pathways and interacting genes through which Sp1 regulates tumorigenesis.

Sp1 expression in single cell transcriptome data

To elucidate the specific role of Sp1 in shaping the TME, we investigated Sp1 expression within various types of immune cells. We next performed scRNA-seq data analysis on a total of 42,968 cells from GC samples contained

in the GSE163558 dataset. To investigate the expression of Sp1 in GC-infiltrating immune cells, we subdivided the immune cell types into eight subclusters, including macrophages, T cells, B cells, natural killer (NK) cells, neutrophils, tregs, mast cells, and plasmacytoid dendritic cells (pDCs) (Fig. 3A). Results revealed that Sp1 expression was most prominent in macrophages and T cells, confirming a strong correlation between Sp1 and these immune cells in STAD. Since Sp1 is generally expressed in macrophage cluster, we further divided macrophage cluster into M1 and M2 subtypes according to cell markers. Notably, Sp1 was expressed in M1 and M2 phenotypes of TAMs (Fig. 3B). Furthermore, compared to norma lgastric tissue samples, within GC tissues Sp1 expression levels were higher in macrophages and monocytes and lower in T cells, with no significant difference for B cells, neutrophil cells, NK cells and pDCs (Fig. 3C).

Value of Sp1 expression for predicting immunotherapy efficacy

We further validated the correlations between CD8+ T cells, M1/M2 phenotype of TAMs, and Sp1 expression in advanced GC patients treated with ICIs and chemotherapy. Representative multiple immunofluorescence images of the detection of CD8+ T cells, M1 phenotype of TAMs (CD68+HLADR+), and M2 phenotype of TAMs (CD68+HLADR-) are shown in Fig. 4A. As shown in Fig. 4B, Sp1 expression was positively correlated with CD8+ T cells ($r=0.409$, $P=0.047$) and M1 phenotype of TAMs ($r=0.432$, $P=0.035$). In contrast, no significant correlation was found between Sp1 expression and M2 phenotype of TAMs ($P=0.350$).

Immunochemistry images depicting different expression levels (low and high) of Sp1 in GC are presented in Fig. 4C. We found that patients achieving partial response (PR) exhibited higher levels of Sp1 in cancerous tissues compared to those achieving stable disease (SD) or progressive disease (PD) (Fig. 4D). Furthermore, Kaplan-Meier analysis showed that patients with high expression of Sp1 had better OS than those with low expression of Sp1 (17.3 vs. 7.8 months; $P=0.004$, Fig. 4E). In the univariate analysis, factors including ECOG PS at ICI initiation, CD8+T cells, M1 phenotype of TAMs, M2 phenotype of TAMs, and Sp1 expression were identified as potential prognostic factors in patients with STAD treated with ICIs (Table 1). Notably, ECOG PS at ICI initiation ($P=0.034$), CD8+T cells ($P=0.033$), M2 phenotype of TAMs ($P=0.015$), and Sp1 expression ($P=0.002$) showed independent prognostic value in the multivariate Cox regression model (Table 1). Results from the

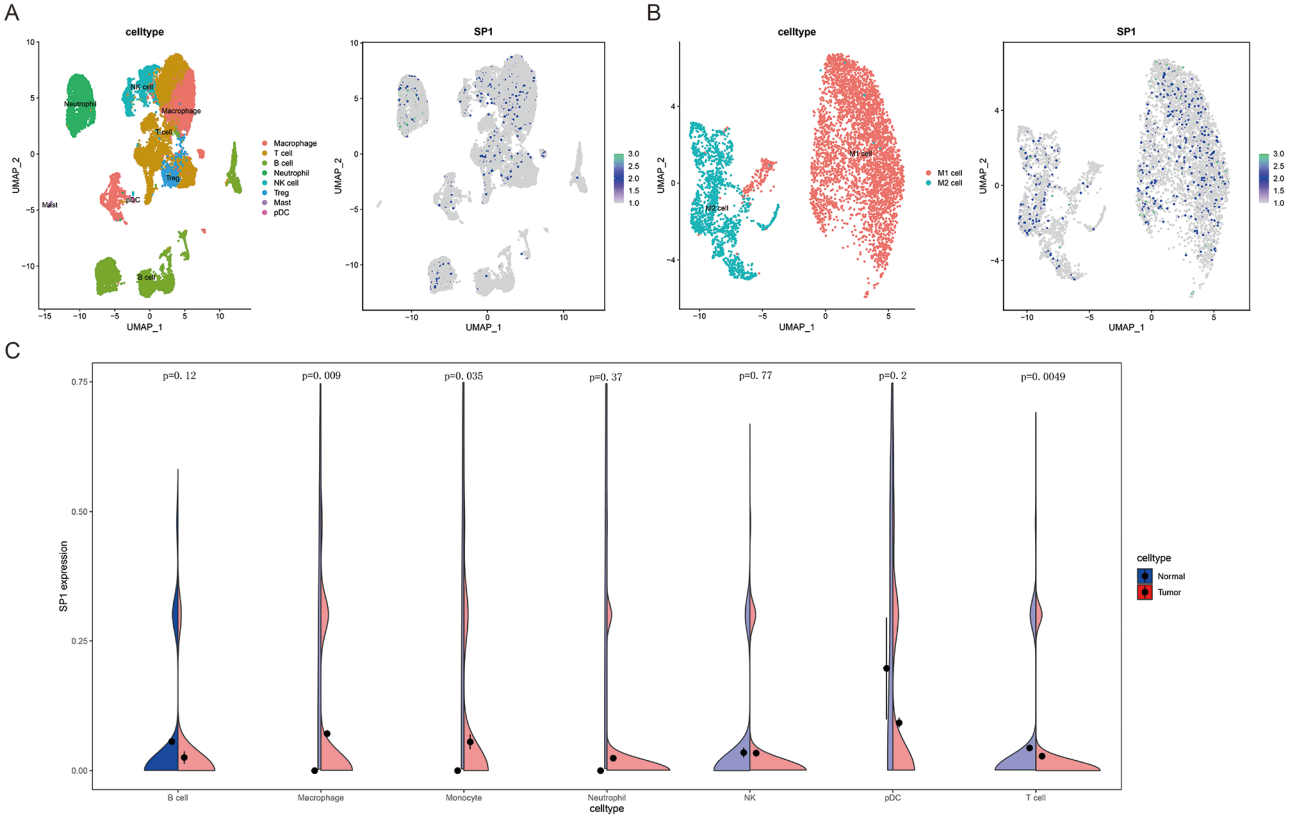

Fig. 3 scRNA-seq analysis of Sp1 expression in GC. (**A**) Immune cell subclustering in the GSE163558 dataset. (**B**) UMAP plot depicting expression of Sp1 in the M1 and M2 phenotypes of TAMs. (**C**) Comparison of Sp1 in different immune cell subclusters between GC and adjacent normal tissue

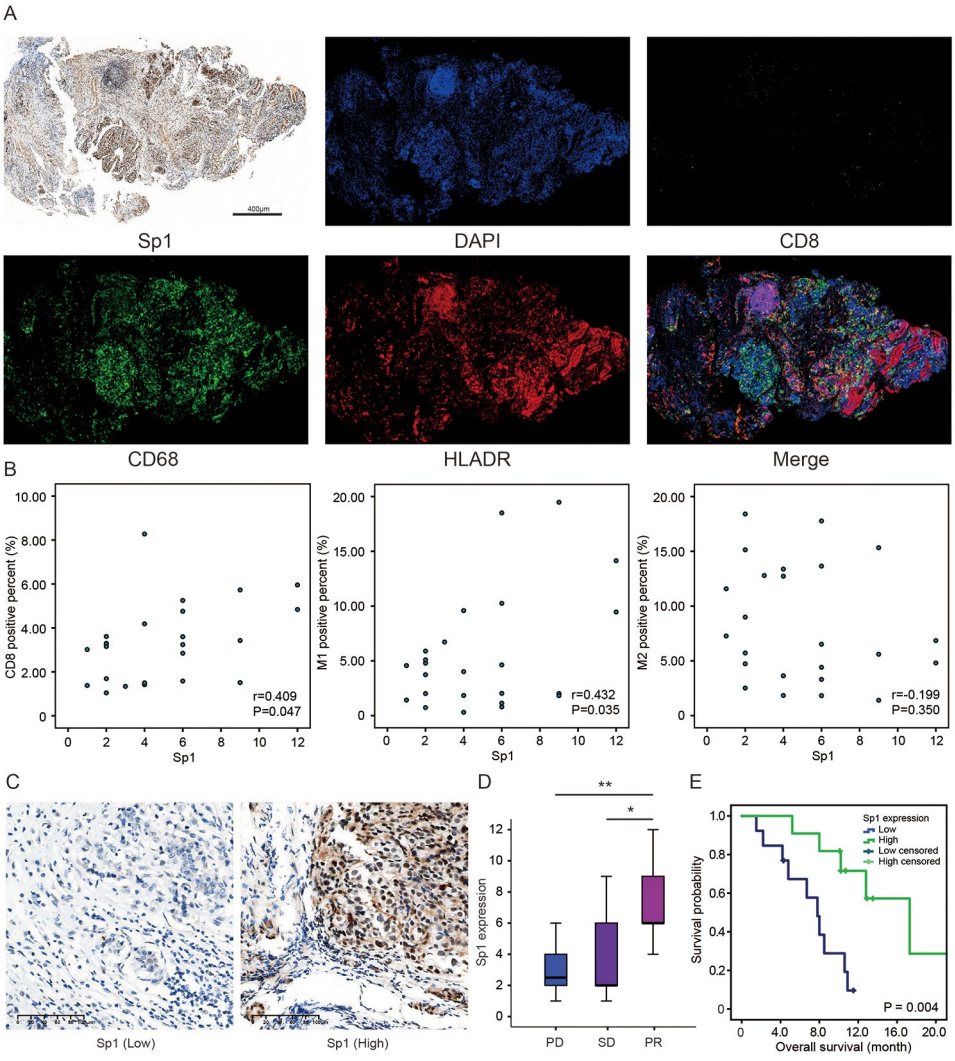

Fig. 4 Sp1 expression correlates positively with immunotherapy efficacy in GC. (**A**) Representative Sp1 immunohistochemistry and simultaneous multiplex immunofluorescence analysis of CD8, CD68, and HLADR expression in GC samples. Scale bars = 400 μm. (**B**) Co-expression analysis of CD8 + T cell, M1 TAM, and M2 TAM markers and Sp1 in GC. (**C**) Representative immunochemistry images depicting different expression levels of Sp1 in GC. (**D**) Sp1 expression levels in relation to immunotherapy outcomes in GC. (**E**) Analysis of overall survival in GC patients based on Sp1 expression

validation cohort suggest that Sp1 protein expression can serve as an independent prognostic factor for patients receiving ICIs.

Association of Sp1 with biomarkers of cancer immunotherapy

We investigated the potential correlation between Sp1 expression and TMB and MSI, two key predictors of the response to cancer immunotherapy, across all types of cancers in the TCGA database. A positive correlation between Sp1 expression and TMB was noted in GC (P=0.0012), COAD (P=0.006), LGG (P=0.013), PAAD (P=0.036), and thymoma (THYM) (P=0.0039). Conversely, we found a negative correlation between Sp1 expression and TMB in BRCA (P=2.9e-11), and THCA (P=1e-06). Meanwhile, Sp1 expression was positively correlated with MSI in COAD (P=1.5e-07), LUSC (P=6.1e-05), rectum adenocarcinoma (READ) (P=0.0013), and UCEC (P=0.001). Conversely, a negative correlation was observed between Sp1 expression and MSI in BRCA (P=2.8e-06), DLBC (P=4.8e-07), HNSC (P=4.9e-09), LGG (P=0.033), PRAD (P=0.00017), SKCM (P=7.4e-07), and THCA (P=0.0029) (Fig. 5B).

ICIs play a pivotal role in the treatment of cancers. Therefore, we investigated the potential correlations between Sp1 expression and several genes involved in immune checkpoint signaling, such as CTLA4 (Fig. 5C). Sp1 expression demonstrated significant correlations with CD200, NRP1, CD200R1, CD276, CD160, and TNFSF15 in most types of cancers (P<0.01). Additionally, we found that Sp1 expression was significantly correlated with most of these genes in GC samples.

Table 1 Univariate and multivariate analysis of prognostic factors in patients with GC treated with ICIs

	Univariate analysis			Multivariate analysis		
Characteristics	**HR**	**95%CI**	***P*-value**	**HR**	**95%CI**	***P*-value**
Age						
≥ 65	1.118	0.392–3.190	0.835			
< 65	Reference					
Sex						
Male	2.018	0.677–6.014	0.207			
Female	Reference					
ECOG PS at ICI initiation						
2	3.241	1.098–9.564	0.033	8.252	1.169–58.264	0.034
0–1	Reference			Reference		
PD-L1 expression						
≥ 1%	0.348	0.118–1.022	0.055			
< 1%	Reference					
Antibiotic use						
Yes	3.346	0.908–12.327	0.069			
No	Reference					
Corticosteroids use						
Yes	1.672	0.590–4.739	0.334			
No	Reference					
CD8+T cells (%)	0.552	0.374–0.817	0.003	0.523	0.289–0.948	0.033
M1 phenotype of TAMs	0.832	0.710–0.975	0.023	1.026	0.789–1.334	0.850
M2 phenotype of TAMs	1.112	1.005–1.231	0.041	1.200	1.036–1.389	0.015
CEA (ng/ml)	1.005	1.000-1.010	0.051			
Sp1						
High expression	0.180	0.049–0.664	0.010	0.069	0.013–0.385	0.002
Low expression	Reference			Reference		

Discussion

This study is, to our knowledge, the first to provide an extensive analysis of the genetic characteristics and predictive value of Sp1 across a spectrum of cancers, with a particular focus on its potential impact on immunotherapy for GC. We utilized genomics, single-cell omics, and GC samples from patient in our hospital. Initially, we identified conserved sequences of Sp1 across various species, suggesting that despite continuous evolutionary divergence, Sp1 remains a crucial factor necessary for fundamental cellular functions, stability, or proliferation. This conservation highlights the importance of Sp1 in maintaining essential biological processes that are invariant across different organisms [23, 24].

Our analysis revealed that compared to matched normal tissues, transcriptional levels of Sp1 were significantly elevated in most cancer types, particularly in tumors of the digestive system, such as CHOL, ESCA, LIHC, PAAD, and STAD. These findings align with previous research, including our own studies in PAAD and LIHC [13, 14, 23, 25–28]. Interestingly, we observed lower Sp1 expression in certain cancers, particularly those associated with hormones, such as BRCA, PRAD, THCA and UCEC. Although Sp1 has been reported to regulate several hormone receptors, which may influence treatment outcomes after endocrine therapies, the specific mechanisms remain unclear and warrant further investigation [29–33].

At the protein level, most expressional variations aligned with the mRNA trends, as verified in our GC cohort through immunohistochemistry. However, the expression pattern of Sp1 protein in BRCA contradicted the corresponding mRNA findings, which may be attributable to post-transcriptional regulation, limited sample size of CPTAC data (only 18 controls), or association with undifferentiated subtypes. Additionally, we observed significant associations between Sp1 expression and clinical stages in several cancer types. Collectively, these findings suggest that Sp1 may play diverse roles at different stages of tumorigenesis across various anatomical sites.

Kaplan-Meier analysis corroborated that elevated Sp1 expression corresponded to poorer OS in LGG, LIHC, PAAD, and THCA, and was associated with adverse DFS in LGG and ACC. Conversely, in KIRC, higher Sp1 expression correlated with better OS and DFS. These prognostic indicator values are indeed supported by both clinical and experimental verification [13, 14, 27, 33–37]. It is worth noting that in THCA, the relationship between Sp1 mRNA expression and OS appeared inconsistent compared to other cancers. The fact that Sp1 has been implicated in modulating both tumor suppressor genes and oncogenes in THCA [35, 38], and displays

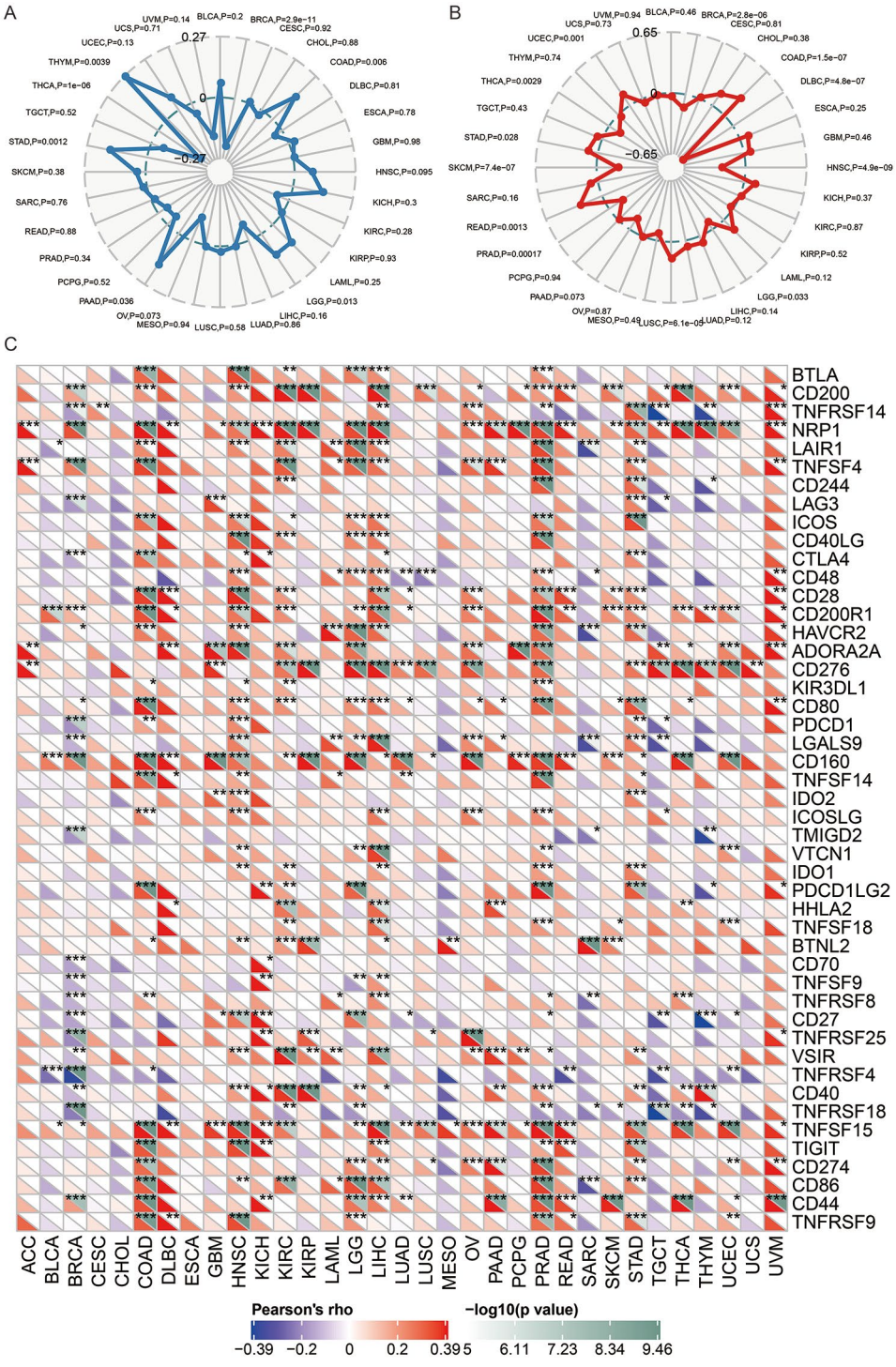

Fig. 5 Association of Sp1 with immune markers in cancer. Correlation between tumor mutational burden (**A**), microsatellite instability (**B**) and Sp1 expression across all types of cancers in the TCGA database. (**C**) Correlations between Sp1 expression and various genes involved in immune checkpoint signaling

distinctive expression patterns [39–41], indicates that the underlying mechanisms are complex and require more in-depth research.

Gene mutations significantly influence the occurrence, progression, and therapeutic response of tumors. Our findings indicate that Sp1 mutations are most prevalent in UCS and may serve as a protective biomarker for patients with this type of cancer. TMB and MSI indices reflect mutational status of the tumor genome and are indicative of the potential efficacy of ICIs. Generally,

tumors characterized by high TMB levels and MSI status exhibit a favorable response to immunotherapy [42–44]. Our results showed that Sp1 up-regulation was strongly associated with TMB and MSI across multiple cancer types. Specifically, Sp1 expression was positively correlated with TMB and MSI in STAD and COAD.

DNA methylation is a chemical modification process that involves the transfer of active methyl groups to specific bases in the DNA chain, catalyzed by DNMTs [45–48]. Typically, cancer cells exhibit a global loss of genetic modifications alongside abnormal methylation at enhancer and promoter regions. These alterations in methylation distribution lead to the inhibition of tumor suppressor gene expression and an increase in proto-oncogene expression, thereby further promoting tumorigenesis. In our study, we utilized the SangerBox platform to investigate the association between Sp1 expression and four classical DNMTs (DNMT1, DNMT2, DNMT3A, and DNMT3B) across different tumors. Our findings revealed significant correlations between Sp1 expression and all four DNMTs in THCA, UVM, DLBC, LGG, and LIHC, compared with matched normal tissues. Interestingly, in GC samples, we observed a significant negative association between Sp1 DNA methylation status, at non-promoter and promoter regions, and gene expression levels. These results suggest that Sp1 may promote tumorigenesis in GC through DNA methylation processes.

KEGG and GO enrichment indicated that the top gene sets most closely associated with Sp1 are significantly related to cancer-associated pathways, particularly those involved in viral carcinogenesis. It is well known that the onset of many cancers can be caused by viral infections, such as HPV leading to cervical cancer, HBV leading to liver cancer, and EBV leading to nasopharyngeal carcinoma, lymphoma, and GC [49–53]. In view of other research findings, though warranting further attention, we are confident that Sp1 plays a key role in cancers caused by viral infections [22, 54–56]. Through screening and integration of Sp1-interacting proteins and the most relevant Sp1-associated genes, we identified two intersecting genes, CREBBP and EP300. CREBBP and EP300 are well-known homologous lysine acetyltransferases frequently mutated in hematological malignancies and have become promising drug targets [57, 58]. However, there is a lack of studies focusing on the mechanism connecting Sp1 and CREBBP/EP300 in different cancers.

Through GSEA analysis focused on GC, we selected the top five pathways. We observed that differentially expressed genes in the high Sp1 expression subgroup upregulated the T cell receptor signaling pathway. It is well known that malignant tumor cells establish a complex TME conducive to their growth and proliferation. The TME encompassing tumor cells and surrounding stromal cells, immune cells, inflammatory cells, secretory factors, and microvessels, conducive to their growth and proliferation. Among these components, immune cells such as CD8+ T cells and macrophages play a crucial role in supervising and eliminating tumor cells while regulating their growth and dissemination [59]. “Cold” tumors, which lack T cell infiltration, exhibit poor responsiveness to immunotherapy compared to “hot” tumors, characterized by abundant T-cell infiltration and favorable responses to immunotherapy [60, 61]. Subsequent analyses of immune infiltration uncovered significant correlations between Sp1 expression and various immune components in various types of cancer (Fig. 12). Our previous studies, along with those of other researchers, have shown that high abundance of tumor-infiltrating CD8+ T cells infiltration is associated with better immune therapeutic outcomes [62–65]. We assessed the correlation between Sp1 expression and the proportion of CD8+ T cells using different algorithms, finding a positive correlation in BRCA-Basal, PRAD, SKCM, and STAD. This result was confirmed in our own STAD cohort. Further, scRNA data supported that immune components were the main altered cluster between tumor and normal tissues. Meanwhile, within tumors Sp1 expression was overall higher in macrophages and monocytes than in T cells within tumors.

High expression of Sp1 in tumor cells has been associated with macrophage infiltration, correlating with poorer prognosis in colorectal cancer [66]. Macrophage Sp1 expression deficiency promoted the transition from M2 to M1 phenotype, inducing apoptosis in lung cancer. Conversely, a transition from M1 to M2 phenotype, which promotes lung cancer growth, is facilitated by HDAC2-mediated Sp1 deacetylation [67]. HDAC2 is also significantly overexpressed in GC and is associated with poor prognosis, but predicts a better outcome of immunotherapy by enhancing CD8+T cell infiltration and cytotoxicity, thus rendering a “hot” tumor status [65, 68, 69]. This suggests that Sp1 may play a similar crucial role in shaping the TME by reprogramming macrophage phenotypic transitions and activating CD8+ T cells in GC. Although we observed a positive correlation between Sp1 expression and the M1-phenotype of TAMs, which suggests a tumor-suppressive effect, recent insights highlight the complexity of macrophage phenotypic transitions. Thus, it is essential to recognize a broader spectrum of macrophage classifications beyond the M1 and M2 extremes [70–73]. Sp1 has been shown to maintain the naïve state of CD8+ T cells [74]. We observed reduced Sp1 expression in T cells within GC samples, potentially indicating transformation toward mature CD8+ T cells. Alternatively, this reduction could reflect a tumor extracellular matrix response mechanism aimed at preventing CD8+ T cells from approaching the parenchymal region

[75]. In addition to the widely studied macrophages and CD8+T cells, the role of monocytes in immunotherapy has gained increasing attention. Monocytes, recruited from the bloodstream into tissues, can differentiate into TAMs or dendritic cells, significantly contributing to the TME. Our single-cell analysis revealed that Sp1 expression in monocytes within gastric cancer tissues is higher than in normal tissues. Elevated Sp1 levels in monocytes enhance their adhesion to epithelial tissues, potentially promoting monocyte recruitment and colonization of tumor tissues via blood vessels. Furthermore, high monocyte infiltration has been shown to improve immunotherapy efficacy in gastroesophageal cancer. Given that monocytes can express PD-L1, influencing gastric cancer outcomes, the strong correlation between Sp1 and PD-L1 suggests that Sp1 expression in monocytes may play a role in immunotherapy efficacy by contributing to CD8+T cell exhaustion through monocyte-related mechanisms.

ICIs play a crucial role in cancer treatment, with immune checkpoint genes serving as important therapeutic targets [76, 77]. In GC, research has demonstrated that Sp1 can bind to the PD-L1 promoter region, contributing to PD-L1 overexpression and thus promoting cancer development [78]. Our study identified multiple immune checkpoints, including CD200R1, CD276, CD160, TNFSF15, and NRP1, that exhibit a positive correlation with Sp1 expression across various tumors. This suggests that Sp1 may represent a novel target for tumor immunotherapy. In GC, the majority of immune checkpoints, such as LAG3, NRP1, TIGIT, and CTLA4, showed a significant positive correlation with Sp1 expression. These checkpoints are known to contribute to the exhaustion of CD8+T-cells, thereby facilitating immune evasion by the tumor. These findings underscore the potential of targeting Sp1 for enhancing the efficacy of immunotherapy in GC.

Additionally, our immunohistochemical analysis of tumor tissue from patients with advanced GC indicated higher levels of Sp1 in cases with PR compared to those with SD or PD. Meanwhile, Kaplan-Meier survival analysis further revealed that patients with high Sp1 expression had improved OS compared to those with low Sp1 expression. Univariate analysis identified ECOG PS and Sp1 expression as potential prognostic factors for GC patients treated with ICIs. Moreover, multivariate Cox regression models confirmed that ECOG PS and Sp1 expression at the onset of ICI treatment independently predicted patient prognosis.

Based on our analysis, we postulate that high Sp1 expression in tumor tissue promotes the maturation and infiltration of CD8+ T cells while concurrently enhancing the expression of immunosuppressive molecules. This dual action results in an increased number of CD8+ T cells, but their functionality remains in a state of exhaustion. The presence of exhausted CD8+ T (Tex) cells is a key driver of tumor immune evasion. Recent studies have categorized Tex cells into ICI permissive and ICI-refractory subsets, highlighting potential mechanisms underlying resistance to immunotherapy [79, 80]. Therefore, patients who respond positively to ICIs may have a higher proportion of reversibly exhausted Tex cells, or a predominance of total CD8+cell infiltration.

There are certain limitations to this study. First, the sample size is limited, which may introduce some bias. Additionally, while we gathered robust evidence in human specimens, we did not conduct molecular-level validation of the specific mechanisms by which Sp1 promotes GC progression and enhances immunotherapy efficacy. We plan to conduct in-depth mechanistic studies which will focus on Sp1's interactions within the TME, particularly its interactions with Tex cells, to obtain more robust evidence.

Conclusions

In summary, this study conducted a comprehensive pan-cancer analysis of Sp1, examining its expression and potential function. The expression of Sp1 was influenced by regulatory factors such as copy number variations, DNA methylation, post-translational modifications and et al. We also found novel potential roles for Sp1 in GC. Patients with high tumor Sp1 expression showed better responses to ICIs and survival benefits. Further analysis revealed a strong positive correlation between Sp1 expression and TMB, as well as infiltration of CD8+T cells and the M1 phenotype of TAMs. This suggests that Sp1, as a pro-oncogene, may promote the reprogramming of multiple oncogenes in tumor cells, leading to the formation of numerous new antigens and enhanced immune cell infiltration. Additionally, high Sp1 expression also promotes PD-L1 expression, contributing to immune escape but paradoxically leading to better immunotherapy efficacy. Therefore, Sp1 could serve as an effective biomarker for predicting the treatment efficacy of ICIs in GC.

Abbreviations

Sp1	Transcription factor specificity protein 1
GC	Gastric cancer
TCGA	The Cancer Genome Atlas
GEO	Gene Expression Omnibus
HPA	Human Protein Atlas
TMB	Tumor Mutational Burden
MSI	Microsatellite Instability
MSI-H	High microsatellite instability
OS	Overall Survival
CPS	Combined Positive Score
ICIs	Immune Checkpoint Inhibitors
TME	Tumor microenvironment
HPV	Human papilloma virus
NCBI	National Center for Biotechnology Information
TIMER2	Tumor immune estimation resource version 2

GEPIA2	Gene Expression Profiling Interactive Analysis version 2
CPTAC	Clinical Proteomic Tumor Analysis Consortium
BRCA	Breast cancer
RCC	Renal cell carcinoma
UCEC	Uterine corpus endometrial carcinoma
LUAD	Lung adenocarcinoma
IHC	Immunohistochemistry
CNA	Copy number alteration
PFS	Progression-free Survival
mIF	Multiplex Immunofluorescence
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
BP	Biological processes
CC	Cellular components
MF	Molecular functions
GSEA	Gene Set Enrichment Analysis
GTEx	Genotype-Tissue Expression
FANTOM5	Function Annotation of the Mammalian Genome 5
CHOL	Cholangiocarcinoma
ESCA	Esophageal carcinoma
GBM	Glioblastoma multiforme
KICH	Kidney chromophobe
KIRP	Kidney renal papillary cell carcinoma
LIHC	Liver hepatocellular carcinoma
STAD	Stomach adenocarcinoma
THCA	Thyroid carcinoma
LGG	Brain lower grade glioma
PAAD	Pancreatic adenocarcinoma
ccRCC	Clear cell renal cell carcinoma
KIRC	Kidney renal clear cell carcinoma
LUSC	Lung squamous cell carcinoma
SKCM	Skin cutaneous melanoma
ACC	Adrenocortical carcinoma
DNMTs	DNA methyltransferases
UVM	Uveal melanoma
DLBC	Diffuse large B-cell lymphoma
UCS	Uterine carcinosarcoma
DFS	Disease-free Survival
SCM	Skin cutaneous melanoma
GSEA	Gene Set Enrichment Analysis
SCLC	Small cell lung cancer
COAD	Colon adenocarcinoma
HNSC	Head-neck squamous cell carcinoma
TAMs	Tumor-associated macrophages
PRAD	Prostate adenocarcinoma
scRNA-seq	Single-cell RNA sequencing
NK	Natural killer
pDCs	Plasmacytoid dendritic cells
mIHC	Multiple immunofluorescence
PR	Partial response
SD	Stable disease
PD	Progressive disease
THYM	Thymoma
READ	Rectum adenocarcinoma
DNMTs	DNA methylation transferases
Tex	Exhausted CD8+ T cell

Supplementary Information

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Supplementary Material 1

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Author contributions
Yang Zhou, Jianhua Chang, Libao Gong, and Junjie Hang designed the study. Yang Zhou, Zhenzhen Luo, Lixia Wu, Xiaoli Zhou, and Junjie Hang performed the experiments. Jinfeng Guo, Lixia Wu, Jun Jie Huang, Qiuhua Duan, and Junjie Hang performed bioinformatic analysis. Jinfeng Guo, Lixia Wu, Daijia Huang, Li Xiao, and Junjie Hang prepared the Figures. Yang Zhou, Qiuhua Duan, Libao Gong, and Junjie Hang collected and analyzed the data. Yang Zhou, Zhenzhen Luo, Lixia Wu, Jianhua Chang, Libao Gong, and Junjie Hang wrote the manuscript. All authors revised and approved the final manuscript.

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

Declarations

Competing interests
The authors declare no competing interests.

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