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RESEARCH ARTICLE

Innate immune checkpoint Siglec10 in cancers: mining of comprehensive omics data and validation in patient samples

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Abstract **Sialic acid binding Ig-like lectin 10 (Siglec10) is a member of innate immune checkpoints that inhibits the activation of immune cells through the interaction with its ligand CD24 on tumor cells. Here, by analyzing public databases containing 64 517 patients of 33 cancer types, we found that the expression of *Siglec10* was altered in 18 types of cancers and was associated with the clinical outcomes of 11 cancer types. In particular, *Siglec10* was upregulated in patients with kidney renal clear cell carcinoma (KIRC) and was inversely associated with the prognosis of the patients. In 131 KIRC patients of our settings, Siglec10 was elevated in the tumor tissues of 83 (63.4%) patients compared with that in their counterpart normal kidney tissues. Moreover, higher level of Siglec10 was associated with advanced disease (stages III and IV) and worse prognosis. Silencing of *CD24* in KIRC cells significantly increased the number of Siglec10-expressing macrophages phagocytosing KIRC cells. In addition, luciferase activity assays suggested that *Siglec10* was a potential target of the transcription factors c-FOS and GATA1, which were identified by data mining. These results demonstrate that Siglec10 may have important oncogenic functions in KIRC, and represents a novel target for the development of immunotherapies.**

Keywords innate immune checkpoint; Siglec10; kidney renal clear cell carcinoma

Introduction

Cancer immunotherapies targeting the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4), programmed death 1 (PD-1) and its ligand 1 (PD-L1), significantly prolong the overall survival (OS) of patients with most subtypes of cancer by restoring tumor-induced immune deficiency in tumor microenvironment [1,2]. However, only a proportion (20%–30%) of patients achieves a durable response [3], and some initial responders eventually develop resistance to these therapies. Moreover, these therapies can cause adverse events involving the heart, skin, gastrointestinal tract, endocrine glands, liver, the central nervous system, and pulmonary and hematologic systems [4,5]. Therefore, novel targets are desired for the development of new immunotherapies.

Siglec10, a member of the sialic acid binding Ig-like lectin (Siglec) family, is an inhibitory receptor expressed by immune cells such as B cells, monocytes, dendritic cells, natural killer (NK) cells, and a small subset of activated T cells [6–9]. It has five extracellular Ig-like domains, a transmembrane region, and a cytoplasmic tail containing two immune receptor tyrosine inhibitory motifs [10]. Siglec10 can bind to vascular adhesion protein-1 (VAP-1) to mediate lymphocyte adhesion to endothelium and modify the inflammatory microenvironment via enzymatic end products [11]. The expression of Siglec10 is increased in a subset of $CD4^+$ T cells, which release soluble CD52 to induce immune suppression by binding to Siglec10 [12,13]. Many tumors overexpress the anti-phagocytic signal factor CD24, whereas tumor-associated macrophages express high level of Siglec10. Siglec10 expression is linked to NK cell dysfunction and inversely associated with the prognosis of patients with

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hepatocellular carcinoma [14]. Interestingly, ablation of either CD24 or Siglec10, as well as blockade of the CD24–Siglec10 interaction, robustly augment the phagocytosis of CD24-expressing human tumors [7].

However, the expression level, prognostic value, and the regulation of *Siglec10* remain obscure in most malignant neoplasms. To address these questions, we conducted a comprehensive analysis of *Siglec10* in eight data sets containing 64 517 patient samples of 33 human caner types and validated them in patient samples of our settings. The results suggested that Siglec10 may play an important role in cancer progression and represents a potential immunotherapeutic target for drug development.

Methods

Expression of *Siglec10* in cancers

The expression of *Siglec10* in tumor and normal tissues was assessed in databases including The Cancer Genome Atlas (TCGA) data in cBioPortal [15], Oncomine database [16], GEO database [17], Genotype-Tissue Expression [18], and Gene Expression Profiling Interactive Analysis (GEPIA) database [19]. The coexpressed genes of *Siglec10* in tumor tissues and the correlations between *Siglec10* and marker genes of monocytes, M1 and M2 macrophages, were determined using these datasets. The association between *Siglec10* expression level and OS of the patients was analyzed by the Kaplan–Meier method and log-rank test by using the Kaplan–Meier Plotter (KM Plotter) [20] and GEPIA databases.

Siglec10 and immune cell infiltration

The association between *Siglec10* expression and immune cell infiltration was determined using TIMER, a comprehensive resource that contains 10 897 samples across 32 cancer types from TCGA and a powerful tool for systematic analysis of immune infiltrates across diverse cancer types to estimate the abundance of immune infiltrates via correlation modules [21].

Regulation of *Siglec10*

The transcription factors that may regulate *Siglec10* expression were predicted using the GCBI online software. The transcription factors that can directly bind to *Siglec10* were identified through Cistrome data browser.

Patient samples of our settings

The study was approved by the research ethics committees of Chinese Academy of Medical Sciences Cancer Hospital and Beijing Chaoyang Hospital Affiliated to Capital Medical University. The diagnosis of kidney renal clear cell carcinoma (KIRC) and brain lower grade glioma (LGG) was confirmed by at least two pathologists. The patients' characteristics are listed in Tables S1 and S2. All cancer samples were collected with informed consent. Tissue samples were taken at the time of surgery and quickly frozen in liquid nitrogen. The counterpart normal controls were normal tissues adjacent to the tumors that were taken with the tumors and were free of tumor cells as confirmed by pathological examination. Total RNA was isolated using TRIZOL reagent (Invitrogen, Frederick, MD, USA), and proteins were extracted using lysates in RIPA buffer.

Immunohistochemistry assay

Immunohistochemistry (IHC) assay was performed to test Siglec10 expression in KIRC patient samples harvested from Beijing Chaoyang Hospital Affiliated to Capital Medical University (Table S2). A tissue microarray containing 90 pairs of KIRC tumor tissues and corresponding nontumor tissues (Table S2) was purchased from Shanghai Outdo Biotech (Shanghai, China; catalog number: HKid-CRC180Sur-01) to show further the expression of Siglec10 in KIRCs. Formalin-fixed and paraffin-embedded tissue specimens were deparaffinized and subjected to a heat-induced epitope retrieval step in citrate buffer solution. The sections were then blocked with 5% bovine serum albumin for 30 min and incubated with an anti-Siglec10 antibody (Clone 5G6, Biolegend; 1:50) at 4 °C overnight, followed by incubation with secondary antibody for 90 min at 37 °C. Detection was achieved with 3,3′-diaminobenzidine (Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) and counterstained with hematoxylin, dehydrated, cleared and mounted as in routine processing. Immunoreactivity score (IRS) was calculated as IRS (0–12) = RP (0–4) $\times$ SI (0–3), where RP is the percentage of staining-positive cells, and SI is staining intensity.

Cell culture

The renal cell cancer (RCC) cell lines 786-O, A498, and ACHN; the human acute monocytic leukemia cell line THP-1; and the human T lymphocyte line Jurkat were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS). The RCC line Caki-2 was cultured in McCoy's 5A medium containing 10% FBS. The erythroid leukemia line K562, the human B cell line KCB200546M, and the human embryonic kidney cell line 293T were cultured in Dulbecco's modified Eagle medium (DMEM) containing 10% FBS (Gibco/BRL, Grand Island, NY, USA), 100 U/mL penicillin, and 100 mg/mL streptomycin.

Human peripheral blood mononuclear cells and macrophages

Human peripheral blood was collected from healthy volunteer donors, and peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Hypaque density gradient centrifugation. The PBMCs were seeded on plastic culture flasks for 2 h, and monocytes were isolated by plastic adherence. The monocytes were treated with macrophage-colony stimulating factor (M-CSF) to derive macrophages, which were further stimulated with 50 ng/mL recombinant human transforming growth factor-β1 (TGFβ1) (R&D Systems, Inc., Minneapolis, MN, USA) and 50 ng/mL recombinant human interleukin-10 (IL-10) (R&D Systems) to obtain M2 type macrophages [7].

Gene expression and luciferase assay

The 293T cells were transfected with plasmids containing *Siglec10* promoter-driven luciferase, constructs containing *c-FOS*, *GATA1*, and *SPIB* coding sequences, or small interfering RNAs (siRNAs) (Table S3). The total RNA of the cells was extracted with TRIZOL reagent (Invitrogen) according to the manufacturer's instruction. The expression of the interested genes was tested by quantitative reverse transcription-polymerase chain reaction (qPCR) by using the primers listed in Table S3. Luciferase activity was measured using the dual luciferase reporter assay system (Promega, Madison, WI, USA).

Flow cytometry

The expression of CD24 on RCC cell lines was determined by flow cytometry by using APC anti-human CD24 antibody (Clone ML5; Biolegend, San Diego, CA). The expression levels of CD206 and CD209 on M0 and M2 macrophages and Siglecc10 on M2 macrophages were analyzed by flow cytometry and Brilliant Violet 421™ anti-human CD206 (Clone 15-2, Biolegend), APC anti-human CD209 (Clone 9E9A8, Biolegend), and PE anti-human Siglec-10 (Clone 5G6, Biolegend) antibodies, respectively.

Assays of phagocytosis of macrophages

The effect of the Siglec10–CD24 axis on the phagocytic activity of the M2 macrophages in RCC cells was tested by transfecting 786-O cells with siRNA for negative control (siNC) or si*CD24*. Forty-eight hours later, the 786-O cells (1×10^5) were labeled with CellTrace™ CFSE Cell Proliferation Kit (Invitrogen) and then co-incubated with the M2 macrophages (5×10^4) in 96-well U-bottom plates for 3 h. The cells were dissociated with TrypLE Express (Invitrogen), collected, stained with PE anti-human CD14 antibody (Clone M5E2, Biolegend), and analyzed by flow cytometry on an LRSFortessa Analyzer (BD Biosciences). Phagocytosis was calculated as the percentage of $CD14^+CFSE^+$ cells among $CD14^+$ cells.

Western blot

For Western blot, proteins were subjected to 8%–15% sodium dodecyl sulfate-polyacrylamide gel, electrophoresed, and transferred onto a nitrocellulose membrane. After blocking with 5% nonfat milk in Tris-buffered saline, the membrane was washed and incubated with the indicated primary and secondary antibodies and detected by Luminescent Image Analyzer LSA 4000 (GE, Fairfield, CO, USA). The antibodies used included mouse anti-β-Actin (#A5441, Sigma, St. Louis, MO, USA; 1:5000) and rabbit anti-Siglec10 (#NBP1-82759, Novus Biologicals, LLC, USA; 1:250).

Statistical analysis

All statistical analyses were conducted using the GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA) software. Statistically significant differences were determined by Student's *t*-test of unpaired data, Fisher's exact test, or one-way ANOVA. The survival curve for each group was estimated by the Kaplan–Meier method and log-rank test. *P* values less than 0.05 were considered statistically significant. All statistical tests were two-sided.

Results

Siglec10 is widely expressed in human cancers

The expression of *Siglec10* in human cancers was analyzed in TCGA, GTEx, and Oncomine (Table S4). In the RNA-seq data of TCGA data sets, *Siglec10* was expressed in most human cancers (Fig. 1A). In patients with breast invasive carcinoma (BRCA), esophageal carcinoma (ESCA), kidney chromophobe (KICH), KIRC, kidney renal papillary cell carcinoma (KIRP), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), and uterine corpus endometrial carcinoma (UCEC), *Siglec10* expression was significantly higher in tumor tissues than in counterpart normal tissues. In patients with colon adenocarcinoma (COAD), liver hepatocellular carcinoma (LIHC), prostate adenocarcinoma (PRAD), and rectum adenocarcinoma (READ), *Siglec10* expression was lower in tumor tissues than in counterpart normal controls. In patients with bladder urothelial carcinoma (BLCA), cholangiocarcinoma (CHOL), head and neck cancer (HNSC), lung adenocarcinoma (LUAD), or lung squamous cell carcinoma (LUSC), *Siglec10* expression in tumor tissues was approximately equal to that in counterpart normal controls (Fig. 1A). *Siglec10* expression was

higher in skin cutaneous melanoma (SKCM)-metastasis compared with that in SKCM (Fig. 1A).

Siglec10 expression was further analyzed using the GEPIA data sets, which contain both TCGA and GTEx data. Compared with that in counterpart normal controls, *Siglec10* expression was remarkably upregulated in glioblastoma multiforme (GBM), KIRC, acute myeloid leukemia, LGG, pancreatic adenocarcinoma (PAAD), and

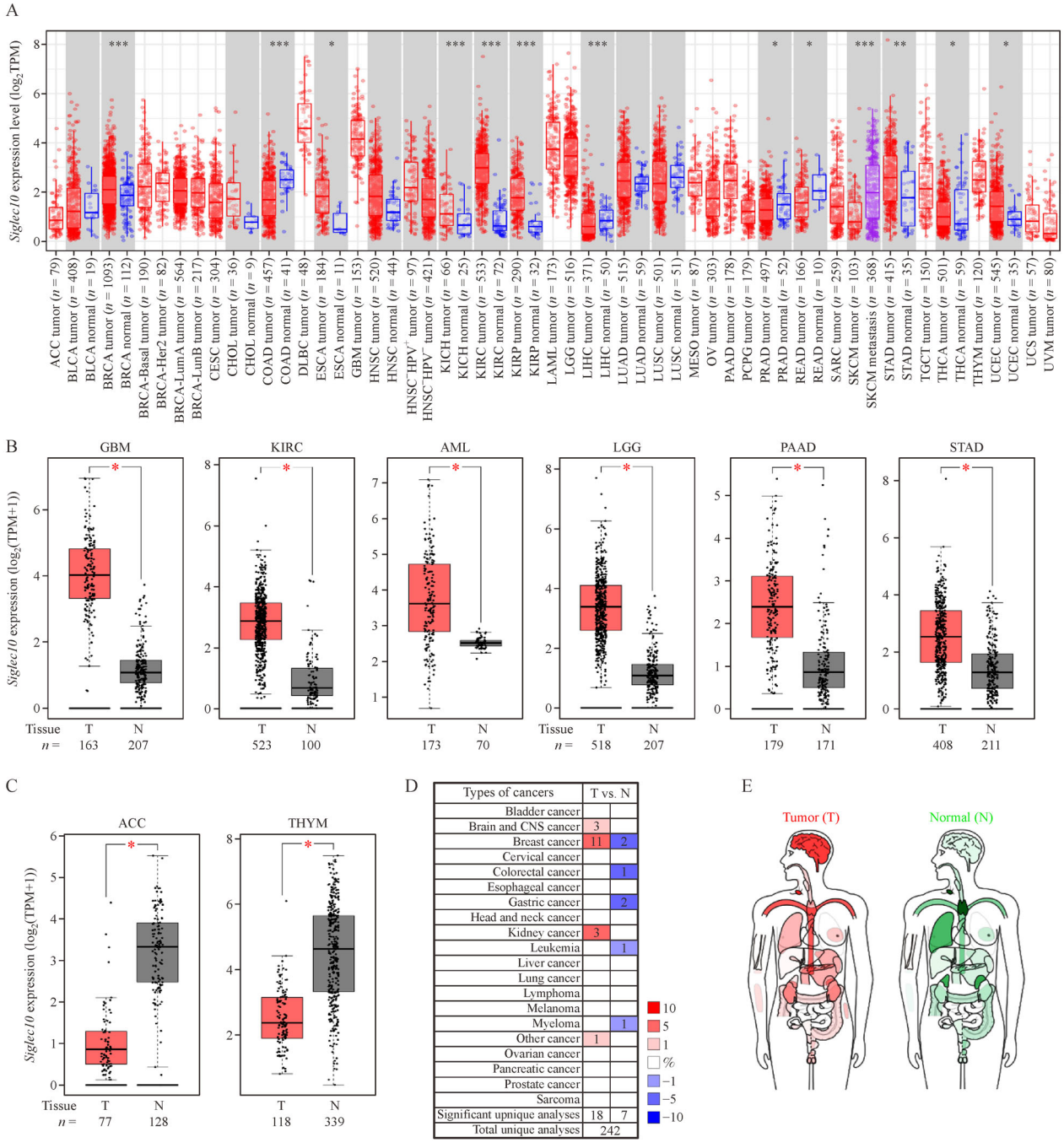

Fig. 1 Expression of *Siglec10* across different human cancer types. (A) *Siglec10* expression levels in different cancer types from the TCGA database were determined by TIMER. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. (B) *Siglec10* expression is significantly upregulated in GBM, KIRC, LAML, LGG, PAAD, and STAD. $*P < 0.05$. T, tumor; N, normal. (C) *Siglec10* is dramatically reduced in ACC and THYM. $*P < 0.05$. The data of (B) and (C), including data from TCGA and GTEx, are from the GEPIA database. (D) Increased (red) and decreased (blue) *Siglec10* expression in data sets with P value < 0.05 compared with normal tissues in the Oncomine database. (E) The body map shows *Siglec10* expression in tumor tissues (red) and normal tissues (green); darker color indicates higher *Siglec10* expression.

STAD (Fig. 1B). In patients with adrenocortical carcinoma (ACC) and thymoma (THYM), *Siglec10* expression was significantly lower in tumor tissues than in counterpart normal tissues (Fig. 1C). In the Oncomine data sets, *Siglec10* expression was upregulated in brain cancer, breast cancer, and kidney cancer but was downregulated in colorectal and gastric cancers, leukemia, and multiple myeloma (Fig. 1D, Table S5). The distribution of *Siglec10* expression in human tissues is shown in a body map (Fig. 1E).

Association between *Siglec10* expression and prognosis of the patients

Using KM Plotter, the potential association between *Siglec10* expression and the OS of the patients was analyzed. Results showed that patients with cervical squamous cell carcinoma (CSCC), esophageal adenocarcinoma, READ, and UCEC, with higher *Siglec10* expression, had longer OS than those with lower *Siglec10* expression (Fig. 2A). By contrast, high *Siglec10* expression was associated with worse prognosis in patients with esophageal squamous cell carcinoma (ESCC), KIRC, testicular germ cell tumor (TGCT), and THYM (Fig. 2B). In the GEPIA database, *Siglec10* overexpression was associated with favorable prognosis in patients with SKCM (Fig. 2C), whereas high *Siglec10* expression was associated with poor outcome in patients with KIRC, LGG, THYM, and uveal melanoma (UVM) (Fig. 2D).

Siglec10 and tumor progression of KIRC and LGG

In the TCGA data sets, higher grades of KIRC and LGG had a higher *Siglec10* expression (Fig. 3A and 3B). In the GEO data set, higher *Siglec10* expression was also seen in higher grades of KIRC (Fig. 3C and 3D). On the basis of the results of gene expression profiling, KIRC was divided into two subtypes, ccA and ccB, which are helpful in discriminating the clinical outcome of KIRC, and patients with the ccA subtype expression profile have a better prognosis than patients with the ccB subtype expression profile [22,23]. *Siglec10* was upregulated in ccB subtype (Fig. 3E), and patients with a higher *Siglec10* expression had a shorter survival time (Fig. 2B). GBM is the highest grade (grade IV), whereas LGG is a lower grade (grade II/III) glioma [24]. GBM exhibited higher *Siglec10* expression than LGG (Fig. 3F), suggesting that *Siglec10* may have a role in brain tumor progression.

Siglec10 is associated with immune cell infiltration

Gene ontology analysis showed that in KIRC and LGG, the coexpressed genes of *Siglec10* were mainly enriched in the immune response, inflammatory response, adaptive immune response, and innate immune response (Fig. 3G and 3H). Compared with the other immune checkpoint receptors, e.g., *PDCD1*, *CTLA4*, and *LAG3*, *Siglec10* exhibited a higher expression level in the tumor tissues of KIRC and LGG (Fig. 3I).

Tumor-infiltrating lymphocytes are considered an independent predicator of tumor progression, treatment response, and clinical outcome [25–27]. The clinical significance of this molecule was revealed using TIMER to explore the correlation between immune cell infiltration and *Siglec10* expression. *Siglec10* expression was positively correlated with the infiltration of B cells, $CD4^+$ T cells, macrophages, dendritic cells, and neutrophils in KIRC (Fig. 4A) and LGG (Fig. 4B).

The relationship between *Siglec10* and the expression of marker genes of cells in tumor microenvironment, e.g., T and B cells, dendritic cells (DC), NK cells, neutrophils, monocytes, M1 and M2 macrophages, tumor-associated macrophages (TAM), type 1 T helper (Th1) and type 2 T helper (Th2) cells, T follicular helper cells, Th17 cells, regulatory T cells, and markers of T cell exhaustion, was analyzed in KIRC and LGG by using TIMER data sets. In these data sets, *Siglec10* had a strong correlation with the marker genes of monocytes, TAMs, and M2 macrophages (Fig. 4C–4E). Similar results were also found in the GEPIA database (Table S6). These findings may unveil the role of *Siglec10* in the regulation of macrophage polarization in KIRC and LGG. In addition, *Siglec10* expression was positively associated with the marker genes of T, B, DC cells, and exhausted T cells in KIRC and LGG (Fig. 4C and Table S7).

Detection of *Siglec10* in patients' samples of our settings

The findings of bioinformatics analysis were validated. Patients' samples were harvested and tested for *Siglec10* expression. In six of the seven patients with LGG (Table S1), *Siglec10* expression was higher in tumor tissues than in normal controls at both mRNA (Fig. 5A) and protein (Fig. 5B) levels. *Siglec10* expression in 131 patients with KIRCs was carefully evaluated via three methods. Results showed that this molecule was overexpressed in 83 patients (63.4%), and its expression was associated with advanced disease stage (stages III and IV) (Table 1). qPCR analyses of 22 KIRC samples revealed that *Siglec10* expression was higher in tumor tissues than in counterpart normal controls ($Siglec10_{tumor}/Siglec10_{normal} > 1$) in 15 patients (68.2%) (Fig. 5C). Western blot assays of 14 samples of the 22 patients revealed that Siglec10 expression was higher in tumor tissues than in normal controls in 10 patients (71.4%) (Fig. 5D). IHC assays were conducted in additional 19 patients. $Siglec10^+$ cells were frequently seen in tumor tissues rather than in counterpart normal controls in 11 patients (57.9%) (Fig. 6A). The IRS of tumor tissues was substantially higher than that of

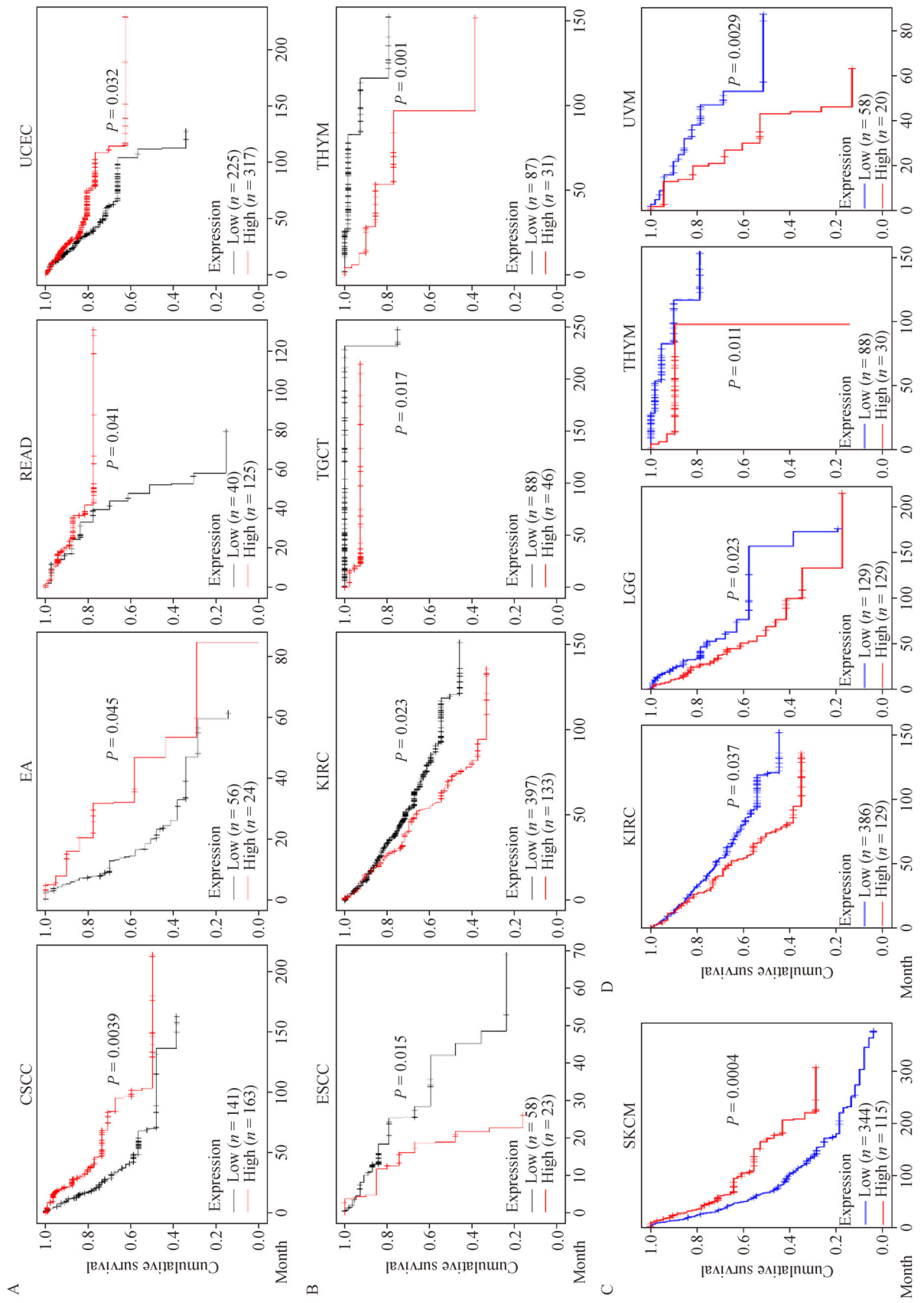

Fig. 2 Overall survival of patients with different types of cancers with high or low *Siglec10* expression levels. (A) High *Siglec10* expression in CSCC, EA, READ, and UCEC is related to better prognosis in KM Plotter data sets. (B) High *Siglec10* expression in ESCC, KIRC, TGCT, and THYM is related to worse outcome in KM Plotter. (C) High *Siglec10* expression in SKCM is related to longer survival in the GEPIA database. (D) High *Siglec10* expression in KIRC, LGG, THYM, and UVM is related to worse clinical outcome in the GEPIA database.

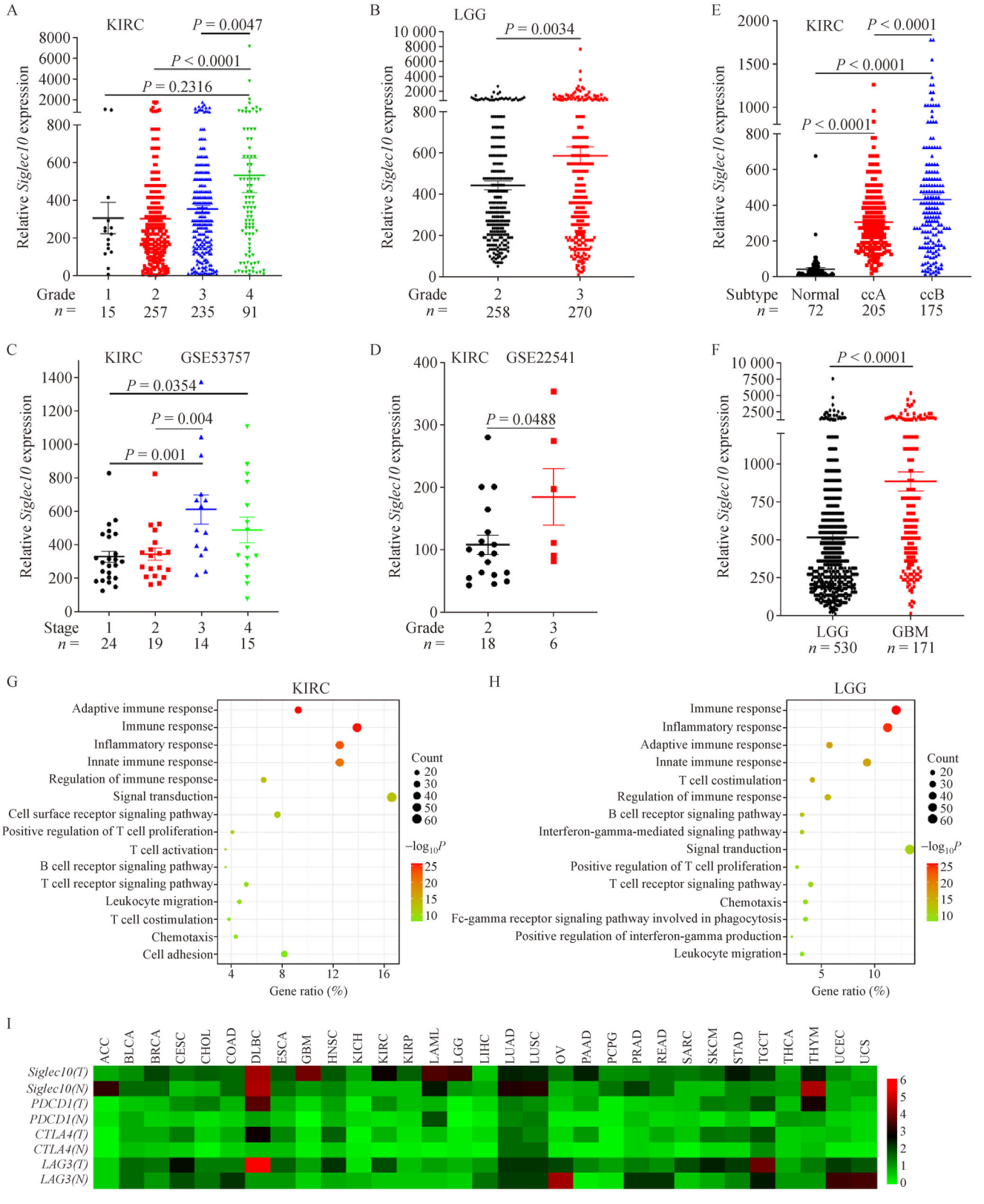

Fig. 3 *Siglec10* expression is related to tumor progression. (A, B) *Siglec10* expression in different grades of KIRC (A) and LGG (B) by analyzing TCGA data sets. (C, D) Correlations between *Siglec10* expression and grades of KIRC in GSE53757 (C) and GSE22541 (D) in the GEO database. (E) The ccB subtype of KIRC has a higher *Siglec10* expression than the ccA subtype. (F) *Siglec10* expression in GBM and LGG. (G, H) GO analysis of the coexpressed genes of *Siglec10* in KIRC (G) and LGG (H). (I) Expression levels of *Siglec10*, *PDCD1*, *CTLA4*, and *LAG3* in different cancer types in the GEPIA database.

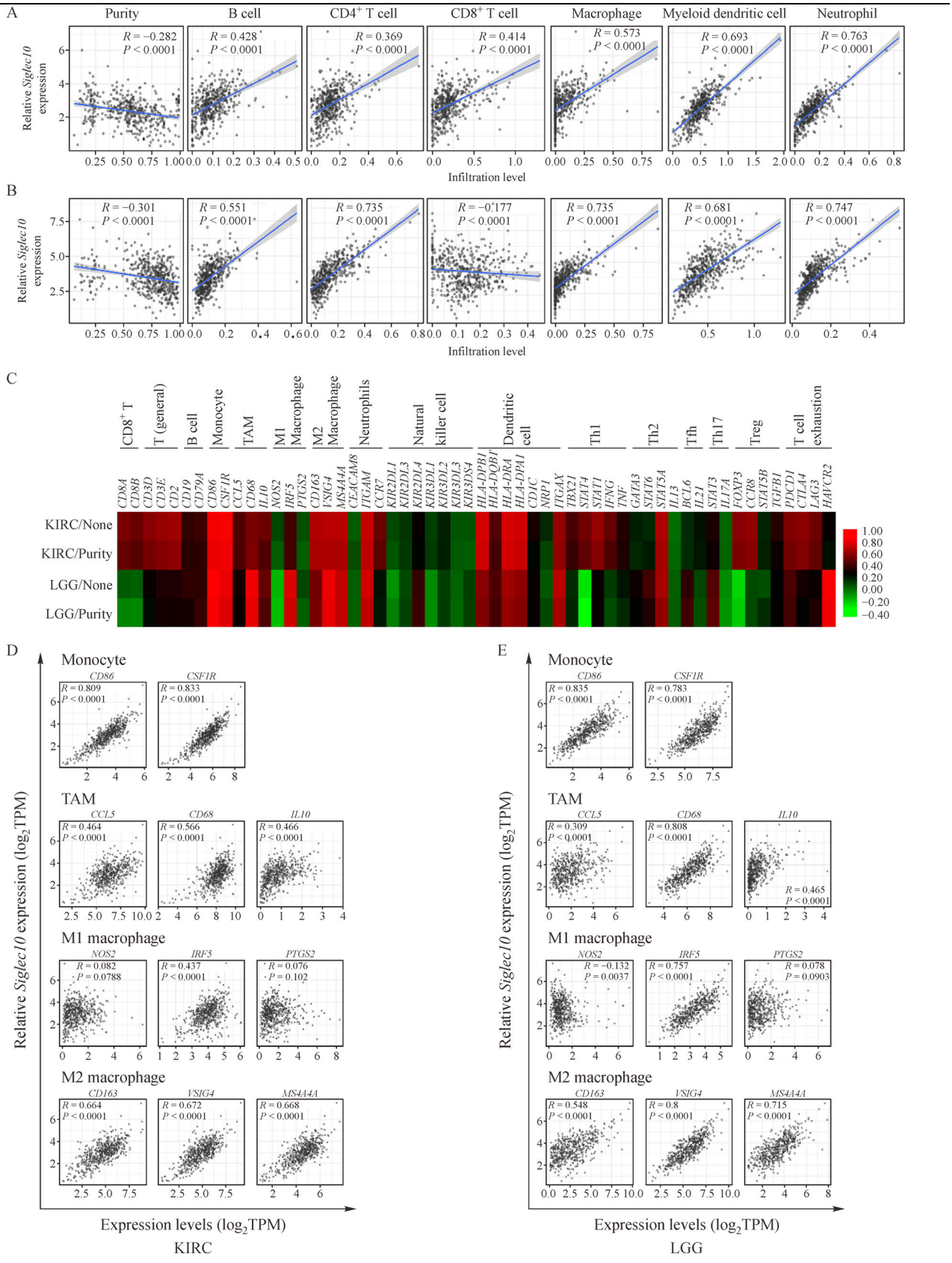

Fig. 4 Correlations between *Siglec10* expression and immune cell infiltration in KIRC and LGG. (A) *Siglec10* expression is negatively related to tumor purity but positively related to infiltrating levels of B cells, $CD4^+$ T cells, $CD8^+$ T cells, macrophages, dendritic cells, and neutrophils in KIRC. (B) *Siglec10* expression is negatively related to tumor purity but positively related to infiltrating levels of B cells, $CD4^+$ T, macrophages, dendritic cells, and neutrophils in LGG. (C) *Siglec10* expression is positively related to the marker genes of monocytes, TAM, M2 macrophages, T cells, B cells, dendritic cells, and exhausted T cells. (D, E) Scatterplots of correlations between *Siglec10* and marker genes of monocytes, TAMs, and M1 and M2 macrophages in KIRC (D) and LGG (E).

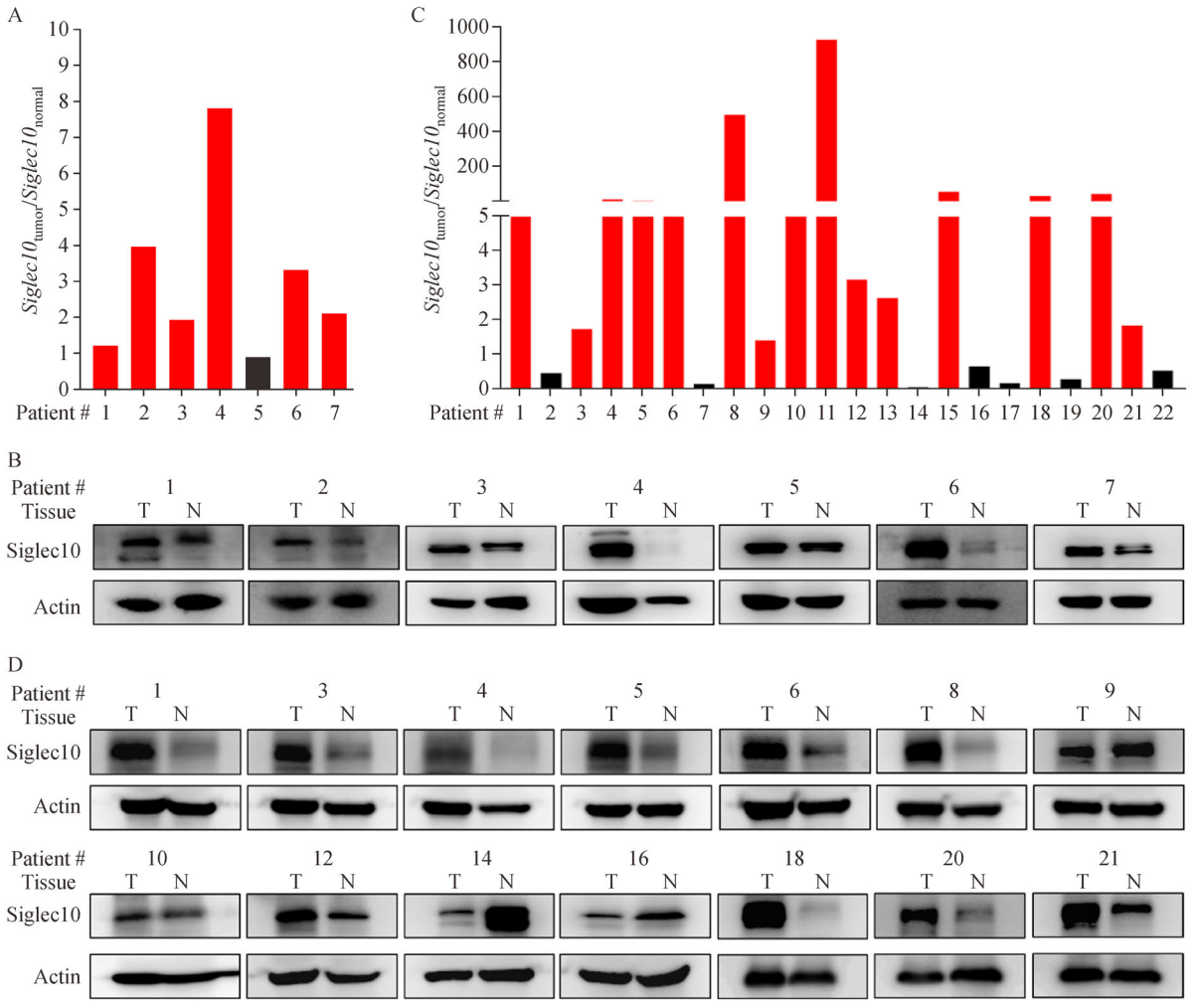

Fig. 5 Detection of Siglec10 in patients' samples by qPCR and Western blot. (A, B) *Siglec10* expression in seven patients with LGG was detected by qPCR (A) and Western blot (B). (C, D) *Siglec10* expression in 22 patients with KIRC was detected by qPCR (C) and Western blot (D).

normal controls (Fig. 6B). IHC analysis of tissue microarray revealed that in tumor tissues, Siglec10 expression was upregulated and IRS increased in 57 of 90 (63.3%) patients with KIRCs (Fig. 6C and 6D). Moreover, high Siglec10 expression was associated with worse prognosis of the patients (Fig. 6E).

Flow cytometry analysis revealed that CD24 was relatively high in ACHN, Caki-2, and 786-O cell lines (Fig. S1A), whereas the M2 type macrophages derived from PBMCs expressed high levels of Siglec10 (Fig. 6G), CD206, and CD209 (Fig. S1B and S1C). Silencing of CD24 by siRNA (Fig. S1D) remarkably increased the percentage of macrophages phagocytosing 786-O cells (Fig. 6H), suggesting that Siglec10 has an important role in promoting immune evasion of KIRC cells.

Potential regulators of *Siglec10*

The potential transcription factors that may have a role in regulating *Siglec10* expression were investigated. From the online GCBI software, 37 transcription factors were found, including AP1, E2F, GATA1, GATA3, HOXA13, SIRT6, SOX4, and others, that may control *Siglec10* expression (Fig. 7A). Using Cistrome data browser, which is a comprehensive database that includes human and mouse ChiP-seq data, 77 transcription factors were found to be able to bind *Siglec10*. The intersection of the two prediction sets was taken, and three transcription factors, c-FOS, GATA1, and SPIB (Fig. 7B), were identified that may have important roles in regulating *Siglec10*.

Whether these three transcription factors can regulate

Table 1 Baseline demographic characteristics of the 131 patients with KIRC

Variable	Number of cases (%)	*Siglec10* expression		
		High, *n* (%)	Low, *n* (%)	*P* values[a]
Total	131	83 (63.4)	48 (36.6)	
Age at diagnosis				0.65
⩽55	54 (41.2)	33 (61.1)	21 (38.9)	
>55	77 (58.8)	50 (64.9)	27 (35.1)	
Gender				0.29
Male	84 (64.1)	56 (66.7)	28 (33.3)	
Female	47 (35.9)	27 (57.4)	20 (42.6)	
Stage				0.04
I	71 (54.2)	42 (59.2)	29 (40.8)	
II	35 (26.7)	21 (60)	14 (40)	
III–IV	19 (14.5)	17 (89.5)	2 (10.5)	
Unknown	6 (4.6)			

[a]*P* values were calculated using a two-sided Fisher's exact test.

Siglec10 expression or not was tested by luciferase assay in 293T cells, which showed a relatively low *Siglec10* level (Fig. 7C). Results showed that ectopic expression of *c-FOS* and *GATA1* increased the luciferase activity driven by the −2000 bp to 0 bp region of *Siglec10* promoter (Fig. 7D). However, exogenous *SP1B* expression was unable to activate *Siglec10* promoter-driven luciferase activity (Fig. 7D). By contrast, silencing of c-FOS and GATA1 by siRNAs remarkably inhibited *Siglec10* expression in Jurkat cells (Fig. 7E).

Discussion

Siglecs belong to the Ig superfamily and have a characteristic N-terminal V-set Ig-like domain [28]. Most members of the Siglec family have the ability to inhibit innate and adaptive immune response to maintain immune homeostasis [29]. Recent studies showed that Siglec7 and Siglec9 are expressed on macrophages, natural killer cells, T cells, and dendritic cells, and can promote immune suppression by interacting with sialic acids on target cells [30]. The Siglec9$^+$CD8$^+$ cytotoxic T cell subset with a high functional capacity and a clonal expansion activity can be inhibited by Siglec9 ligands [31]. Siglec15 can exert diverse functions in osteoclast development, bone resorption, and suppress T cell antitumor immunity through its expression in tumor cells [32]. Siglec10 is mainly expressed on innate immune cells and lymphocytes [12,13], but its role in carcinogenesis remains to be understood. In this study, we performed pan-cancer analysis of *Siglec10* expression. Results showed that the expression of this gene remarkably varied among the 33 types of cancers analyzed herein, i.e., upregulated in some cancers and downregulated in others. Higher *Siglec10* expression was associated with worse prognosis in patients with ESCC, KIRC, LGG, TGCT, THYM, and UVM, but was associated with better outcome in patients with CSCC, EA, READ, SKCM, and UCEC. These results indicated that Siglec10 may have different roles in different types of cancer, and its context-dependent functions should be carefully scrutinized.

Siglec10 may play a vital role in KIRC and LGG because it was overexpressed in these two cancers, and its expression levels were positively associated with disease grades but inversely associated with the clinical outcome of the patients. It was coexpressed with the genes involved in antitumor immunity and represented the most upregulated immune checkpoint among molecules including *PDCD1*, *CTLA4*, and *LAG3* in KIRC and LGG. In KIRC, *Siglec10* expression was positively associated with tumor-infiltrating immune cells, such as B cells, CD4$^+$ T cells, CD8$^+$ T cells, macrophages, dendritic cells, and neutrophils and was linked to macrophage polarization. By contrast, in LGG, *Siglec10* was negatively associated with CD8$^+$ T cell infiltration, possibly due to the decreased CD8$^+$ T cells in LGG compared with that in other types of cancers [33]. Bioinformatics analysis revealed that Siglec10 was elevated in KIRC and LGG as confirmed by the detection of Siglec10 in patients' samples at both mRNA and protein levels. In particular, Siglec10 was elevated in 83 out of 131 (63.4%) patients with KIRC, and knockdown of CD24 enhanced the phagocytic activity of macrophages in KIRC cells, indicating that the Siglec10–CD24 axis plays a role in KIRC pathogenesis by inhibiting innate and adaptive immune systems. Our results also demonstrated the rationale and significance of using online omics resources in investigating specific gene(s) in tumorigenesis.

Siglec10 on the surface of immune cells mediates cancer cells' "don't eat me" signal by interacting with CD24 [7]. In this study, the main molecules that can regulate *Siglec10*

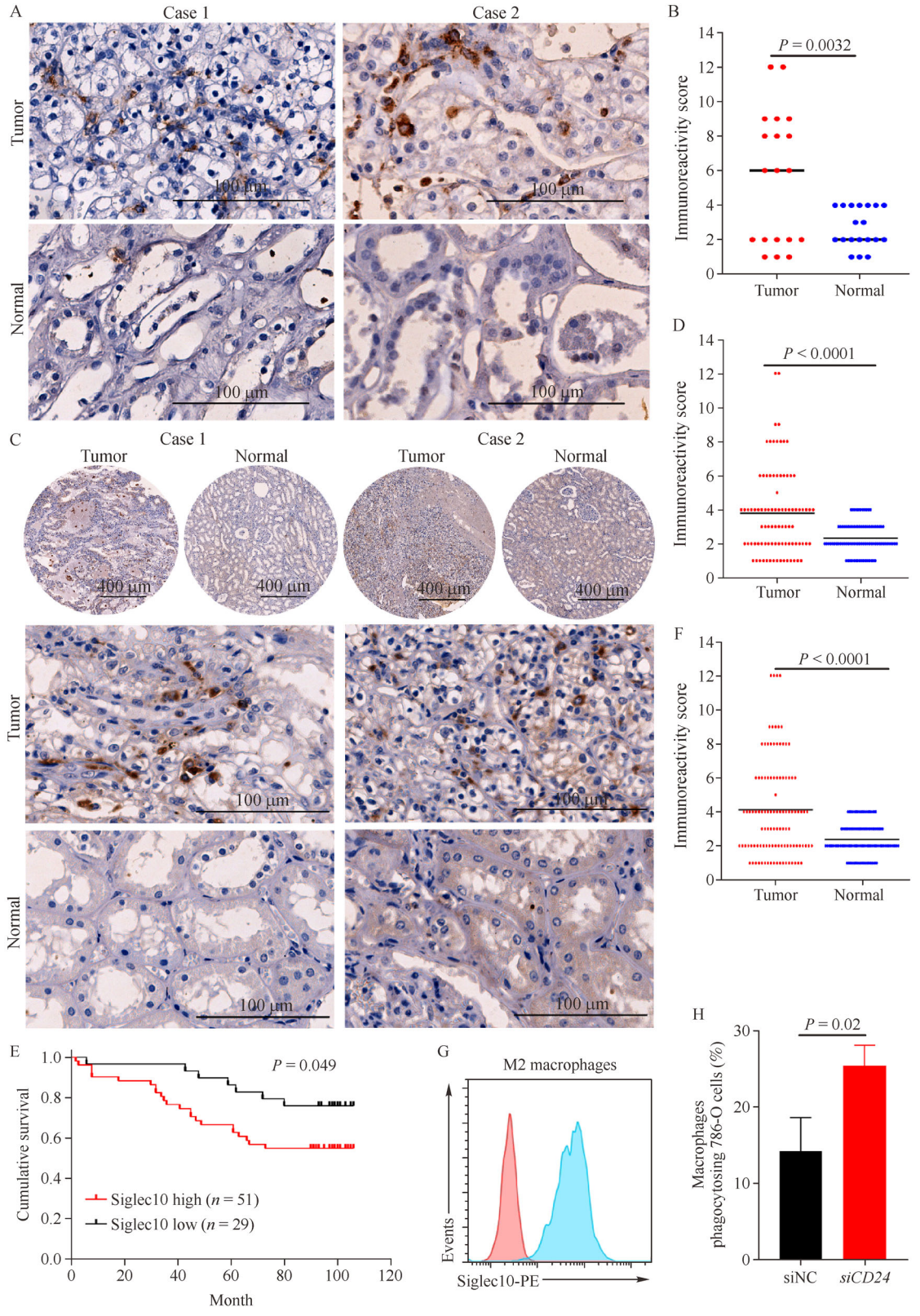

Fig. 6 Detection of Siglec10 in patients' samples by IHC. (A, B) IHC assays were performed using samples of patients with KIRC and an anti-Siglec10 antibody (A). The corresponding immunoreactivity score of Siglec10 in 19 patients was calculated (B). (C, D) IHC assays were performed using KIRC samples on a tissue microarray and an anti-Siglec10 antibody (C). The corresponding immunoreactivity score of Siglec10 in 90 patients was calculated (D). (E) Overall survival of 90 patients with KIRC with a high or a low *Siglec10* expression. (F) Immunoreactivity score of Siglec10 in a total of 131 patients of our settings. (G) *Siglec10* expression (blue) in PBMC-derived M2 macrophages. Red, isotype control. (H) Percentage of macrophages phagocytosing 786-O cancer cells.

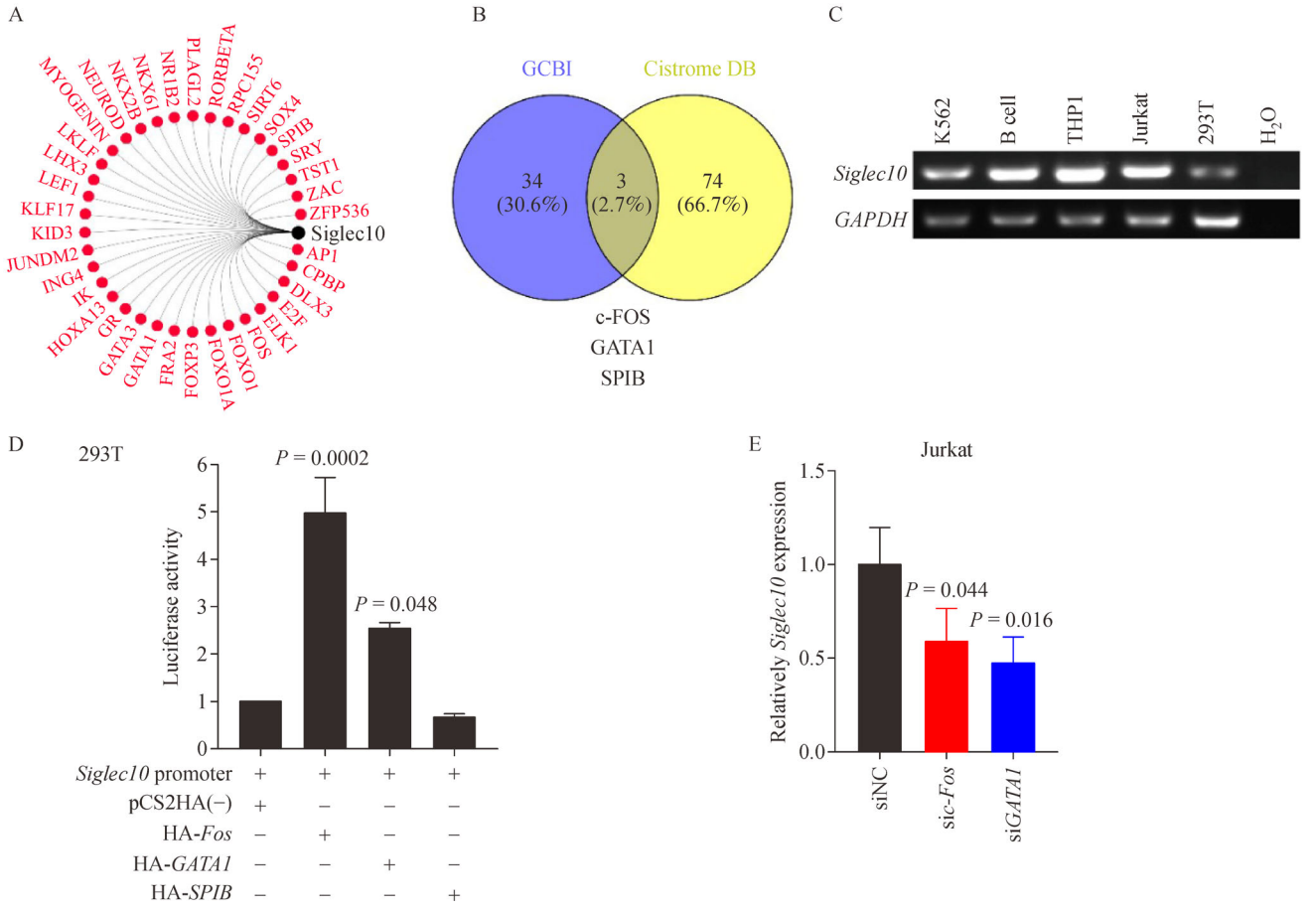

Fig. 7 Potential regulators of *Siglec10*. (A) A total of 37 transcription factors are predicted to be able to regulate *Siglec10* from the GCBI online database. (B) Cross-referencing of the transcription factors in the GCBI database and Cistrome data browser. (C) Expression of *Siglec10* in the indicated cell lines was detected by qPCR. (D) 293T cells were transfected with *Siglec10* promoter–luciferase reporter construct and *c-FOS*, *GATA1*, or *SPIB* genes and assessed by luciferase assays. (E) Jurkat cells were transfected with the indicated siRNAs, lysed 48 h later, and RNA was extracted and used to test the expression of *Siglec10* by qPCR.

were unveiled by systematically analyzing the transcription factors that could regulate this gene. Results suggested that c-FOS, GATA1, and SPIB might modulate *Siglec10* expression because of the presence of transcription factor binding sites in the promoter region of *Siglec10*. Interestingly, the "wet" experiment showed that the ectopic expression of *c-Fos* and *GATA1* but not of *SPIB* was able to upregulate *Siglec10* promoter-driven luciferase activity in 293T cells. Silencing of *c-Fos* and *GATA1* by siRNAs consistently inhibited *Siglec10* expression in Jurkat cells. GATA1 is a transcription factor that plays an important role in erythroid development by regulating the switch of fetal hemoglobin to adult hemoglobin [34]. c-Fos represents a leucine zipper protein that can dimerize with proteins of the JUN family and thereby form the transcription factor complex AP-1 to regulate cell proliferation, differentiation, transformation, and apoptotic cell death [35]. The role of these two transcription factors in regulating Siglec10 and how they affect its downstream molecules to modulate immune system to promote carcinogenesis remain open questions.

In summary, by mining the comprehensive omics data of 64 517 patients, we found that the immune checkpoint *Siglec10* had differential expression patterns in 33 cancer subtypes and was widely expressed in human cancers. In KIRC and LGG, *Siglec10* was substantially upregulated, and its expression level was positively associated with tumor progression but inversely associated with the clinical outcome of the patients. *Siglec10* expression was associated with exhausted T cells and TAMs, suggesting that inhibition of *Siglec10* may boost anticancer immunity in these types of cancers.

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Compliance with ethics guidelines

Chen Zhang, Jiandong Zhang, Fan Liang, Han Guo, Sanhui Gao, Fuying Yang, Hua Guo, Guizhen Wang, Wei Wang, and Guangbiao Zhou declare that they have no conflict of interest. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the *Helsinki Declaration* of 1975 as revised in 2000. Additional informed consent was obtained from all patients whose identifying information is included in this article.

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