Histol Histopathol (2025) 40: 571-584

Histology and Histopathology
From Cell Biology to Tissue Engineering

ORIGINAL ARTICLE Open Access

Expression feature and prognostic function of a novel immune checkpoint Siglec-15 in human colorectal cancer

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Summary. Background. Sialic acid-bound immunoglobulin lectin 15 (Siglec-15) plays an important role in the development of cancer. However, the association between Siglec-15 expression and clinicopathological characteristics of colorectal cancer (CRC) has not been fully investigated.

Methods. In this present study, a number of bioinformatics analyses were performed to provide an overview and detailed characteristics of Siglec-15. Quantitative real-time polymerase chain reaction (qPCR), western blotting and immunohistochemistry analyses were conducted to characterize the expression of Siglec-15 in CRC. Kaplan-Meier survival and Cox regression analyses were performed to identify the prognostic parameters of CRC.

Results. The results of bioinformatics analyses revealed the expression characteristics and prognostic roles of Siglec-15 in CRC. The data of qCPR, western blotting, and IHC analyses demonstrated that the expression of Siglec-15 in CRC tissues was significantly higher than that in non-cancerous tissues. Moreover, the expression level of Siglec-15 in CRC was significantly associated with lymph node metastasis (p=0.001), TNM stage (p=0.001), and overall survival (p=0.026). COX multi-factor analysis indicated that Siglec-15 expression (p=0.023) and tumor differentiation (p=0.003) were independent prognostic factors for CRC.

Conclusions. Collectively, the data suggested that Siglec-15 expression may serve as a novel prognostic factor and Siglec-15 might be identified as an ideal candidate for immunotherapy in CRC treatment.

Key words: Siglec-15, Colorectal cancer, Prognosis, Immunotherapy

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www.hh.um.es. DOI: 10.14670/HH-18-801

Introduction

Colorectal cancer (CRC) is the third most common cancer in humans and the second leading cause of cancer-related mortality worldwide (Mattinzoli et al., 2022). China is currently undergoing a cancer transition, with a dramatically increasing burden of gastrointestinal cancer, and the incidence of CRC has been persistently increasing (Yang et al., 2023). Over the last two decades, numerous therapeutic strategies have been developed for CRC treatment, including surgical resection, standard chemotherapy, and adjuvant therapies (Pan et al., 2020). Furthermore, the widespread use of immunotherapy has led to revolutionary management of CRC (Guo et al., 2023). Monoclonal antibodies (mAbs) that target key immune checkpoints, such as programmed death-1 (PD-1) and cytotoxic T-lymphocyte antigen 4 (CTLA-4), have demonstrated remarkable clinical activity in CRC patients (Jin et al., 2022; Vegivinti et al., 2023). Although deficient mismatch repair/microsatellite instability-high (dMMR/MSI-H) is a potential biomarker for predicting response to treatment with immune checkpoint inhibitors (ICIs) in CRC, some patients with dMMR/MSI-H tumors do not benefit from immunotherapy, let alone mismatch repair proficient/micro satellite stable (pMMR/MSS) cases (San-Roman-Gil et al., 2023). Therefore, identifying new biomarkers that

may be effective therapeutic targets for predicting the prognosis of patients with CRC is crucial.

Sialic acid-bound immunoglobulin lectin 15 (Siglec-15) has recently been found to play an important role in immunomodulation. Chen et al., who first reported the role of the PD-1/PD-L1 pathway in cancer immunotherapy, described the upregulation of Siglec-15 in tumor cells and tumor-infiltrating immune cells, leading to significant immunosuppression in the tumor microenvironment (TME) (Wang et al., 2019). Wang et al. revealed that Siglec-15 expression in macrophages could inhibit the proliferation of antigen-specific T cells, leading to tumor development (Sun et al., 2021). Several studies have reported high expression of Siglec-15 in several types of solid tumors; however, its expression is limited in normal tissue (Hao et al., 2020; Shafi et al., 2022). In our previous study, we prepared a novel Siglec-15-antibody (S15-4E6A) that promoted tumor inhibitory activity against lung adenocarcinoma (LUAD) by modulating macrophage polarization (Xiao et al., 2022). However, studies reporting the clinicopathological characteristics of Siglec-15 in CRC are limited.

In this study, several bioinformatics databases were searched and consulted to investigate Siglec-15 expression in the TME and its characteristics in immunomodulation. We then enrolled CRC tissues and analyzed the expression of Siglec-15 at both mRNA and protein levels. Furthermore, we examined the association between Siglec-15 expression and the clinicopathological data in the CRC cohort. Finally, we evaluated the prognostic significance of Siglec-15 expression in CRC patients.

Materials and methods

Bioinformatics consultations of Siglec-15 expression

The GeneCard database (http://www.genecards.org) was used to determine the overall Siglec-15 expression. The Human Protein Atlas (HPA) database was used to describe the expression characteristics of Siglec-15 (http://www.proteinatlas.org/). The Gene Expression Profiling Interactive Analysis (GEPIA) database was used to investigate the expression levels of Siglec-15 in CRC (http://gepia.cancer-pku.cn/). The TCGA database was used to confirm the general expression characteristics of Siglec-15 (https://cancergenome.nih.gov). The UALCAN database was used to investigate the expression features of Siglec-15 in colon cancer (COAD) and rectal cancer (READ), respectively (https://ualcan.path.uab.edu/analysis.html). The Kmplot database (http://kmplot. com/analysis/) was used to determine the prognostic function of Siglec-15.

Bioinformatics analyses of Siglec-15 in immunomodulation

The 'Gene' module of the TIMER1.0 database was employed to analyze the relationship of Siglec-15 with different levels of immune cell infiltration (https://cistrome.shinyapps.io/timer/). The P-values and correlation coefficients were corrected for purity. The tumor Immune Dysfunction and Exclusion (TIDE) databases were used to obtain response data for immune checkpoint inhibitor treatment (http://tide.dfci.harvard.edu/). The LinkedOmics database was used to screen for differential genes related to Siglec-15 in CRC, and data were analyzed using Pearson's correlation coefficients and demonstrated using volcano plots and heat maps (http://linkedomics.org/login.php). The GeneMANIA database was used to examine gene co-expression and physical interactions (http://www.genemania.org).

Tissue samples collection

Ten fresh CRC tissue samples (six colon cancer and four rectal cancer) and the corresponding noncancerous tissue samples were collected from the Department of Pathology of the Fourth Affiliated Hospital of Nanjing Medical University from May 2021 to December 2022. Simultaneously, 90 formalin-fixed, paraffin-embedded CRC and corresponding noncancerous tissue samples (each pair of CRC and corresponding noncancerous samples from the same patient) were collected from Alenabio Biotech (Xi'an, China) (Han et al., 2017). Important clinicopathological information on CRC cases, including sex, age, tumor size, tumor location, histological type, tumor differentiation, CEA level, metastasis status, TNM stage, and overall survival, was obtained from raw data along with the tissue microarray (TMA) product. Clinical staging was performed according to the latest revision of the American Joint Committee on Cancer/International Union against Cancer TNM staging system (Shi et al., 2015). Written informed consent was obtained from all patients included in this study. Ethical approval to perform This study was approved by the Human Research Ethics Committee of the Fourth Affiliated Hospital of Nanjing Medical University.

One-Step qPCR test

Total RNA was extracted from 10 frozen CRC tissues and matched non-cancerous tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's guidelines. Total RNA extraction and one-step Quantitative Real-time Polymerase Chain Reaction (qPCR) were performed as previously described (Luo et al., 2018). The primers used for qPCR were as follows: Siglec-15, 5'-TTT GAG CCA GAT GAA CCC CC-3' (forward), 5'-CAG GGA GCT CCG AAA TG GTT-3' (reverse); GAPDH,5'- TGC ACC ACC AAC TGC TTA GC -3' (forward) and 5'- GGC ATG GAC TGT GGT CAT GAG -3' (reverse).

Western blotting analysis

Total protein was isolated from three CRC tissue

samples, loaded, separated on 8% Tris-glycine SDS gels, and transferred to nitrocellulose membranes. The membranes were first incubated with self-made S15-IgG (S15-4E6A) and then detected using an ECL kit and autoradiography with an X-ray film. GAPDH was used as an internal control. This protocol has been described previously (Mao et al., 2019a).

Immunohistochemistry (IHC) analysis

Tissue microarray (TMA) was produced by Alenabio Biotech (Xi'an, China), and IHC analysis was performed as previously described (Mao et al., 2019b). Tissue sections were incubated with polyclonal rabbit anti-Siglec-15 antibody (abcam, ab198684, 1:100) in TBS containing 1% bovine serum albumin for 1h. The Siglec-15 immunostaining score was simultaneously evaluated by two independent pathologists based on the intensity and percentage of positive cells. Staining intensity was scored as 0 (negative), 1 (weakly positive), 2 (moderately positive), and 3 (strongly positive). The percentage of Siglec-15-positive cells was also scored according to four categories as follows: 1 was given for 0% to 10%, 2 for 11% to 50%, 3 for 51% to 80%, and 4 for 81% to 100%. The final staining score was defined as the product of intensity and percentage scores. The degree of Siglec-15 staining was quantified as follows: samples with a final score of >4 were considered to have low expression, whereas those with a final score of <4 were considered to have high expression.

Statistical analysis

Statistical analyses were performed using STATA software (version 18.0; Stata Corporation, College Station, TX, USA). The expression of Siglec-15 mRNA in fresh CRC tissues and the corresponding non-cancerous tissues was normalized to that of GAPDH and analyzed using the Wilcoxon non-parametric signed-rank test. The relationship between Siglec-15 expression and clinicopathological factors was analyzed using the chi-squared test. The survival rate was determined using the Kaplan-Meier method. Univariate and multivariate analyses were conducted using Cox proportional hazard regression models to screen and identify potential prognostic factors. The significance level for statistical analysis was set at $p<0.05$.

Results

Bioinformatic analyses of Siglec-15 expression

The GeneCard database demonstrated that Siglec-15 was mainly expressed in the cell membrane (confidence=5), nucleus (confidence=2), and extracellular space (confidence=2) (Fig. 1A). The Human Protein Atlas (HPA) database presents the typical location of Siglec-15 as the nucleoplasm (Fig. 1B,C). The GEPIA database revealed the upregulated expression of Siglec-15 in 33 types of solid tumor tissues (Fig. 1D). Moreover, Siglec-15 expression was positively associated with the CRC TNM stage (F=4.23, Pr=0.00593) (Fig. 1E). The TCGA database confirmed the general expression characteristics of Siglec-15 (Fig. 2A). The UALCAN database revealed upregulated Siglec-15 expression in COAD and READ (Fig. 2B,C). Moreover, the Kmplot database showed that elevated Siglec-15 expression implied a poor prognosis in human CRC in terms of both recurrence-free survival (RFS, p=0.0092) and overall survival (OS, p=0.00045) (Fig. 2D,E).

Bioinformatics information of Siglec-15 in immuno-modulation

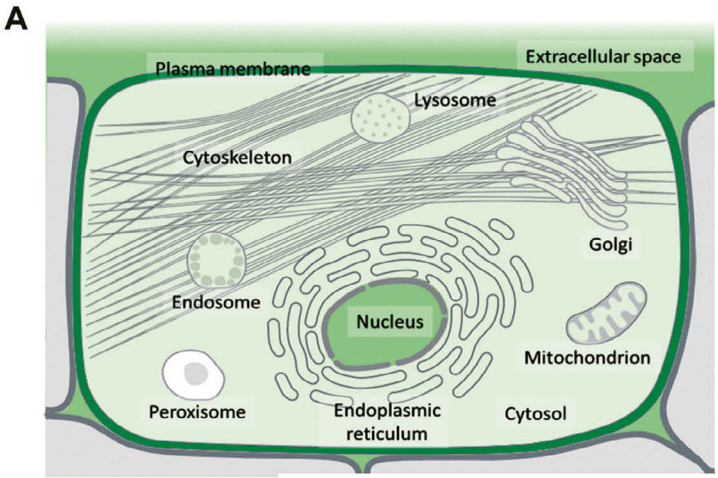
TIMER was used to determine whether Siglec-15 was related to immune infiltration. High expression of Siglec-15 was positively correlated with six immune cell types (B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells) and negatively correlated with tumor purity in LUAD (Fig. 3A). Based on the TIDE database, the area under the ROC curve (AUC) of Siglec-15 expression was used to assess its potential role as a predictive biomarker of immunotherapy response (Fig. 3B). Siglec-15 co-expression data from LinkeDomics showed that 19828 genes from 379 samples were positively or negatively correlated with Siglec-15 (Fig. 4A). The top 50 genes positively and negatively associated with Siglec-15 expression were identified (Fig. 4B,C). The information from GeneMANIA illustrated that Siglec-15 was mainly associated with the interaction between DAP12 and the innate immune system (Fig. 4D).

Siglec-15 expression was elevated in CRC

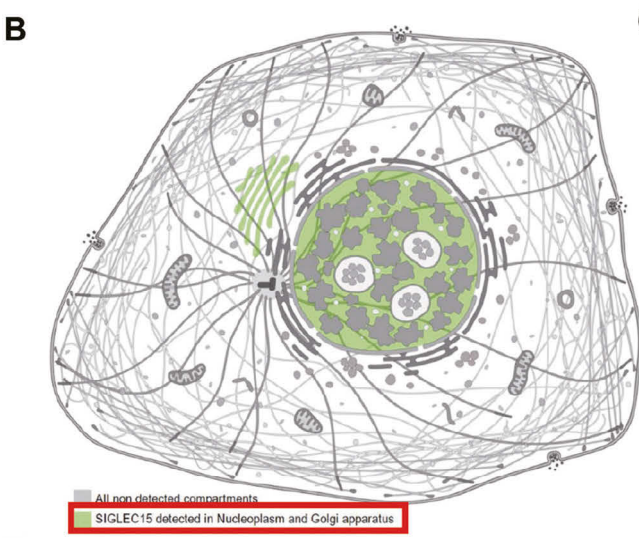
Total RNA was extracted and subjected to one-step qPCR to detect Siglec-15 mRNA expression in the CRC tissues. When normalized to GAPDH, the means of Siglec-15 mRNA in CRC and corresponding non-cancerous tissues were 4.651±1.66 and 2.886±1.28, respectively (p=0.0159). Siglec-15 expression was 1.6-fold higher in CRC samples than in non-cancerous tissue samples (Fig. 5A). Western blotting was performed to confirm the qPCR results. The results showed the same trend for Siglec-15 protein expression in CRC; Siglec-15 expression was significantly higher in cancer tissues than in non-cancerous tissues (Fig. 5B).

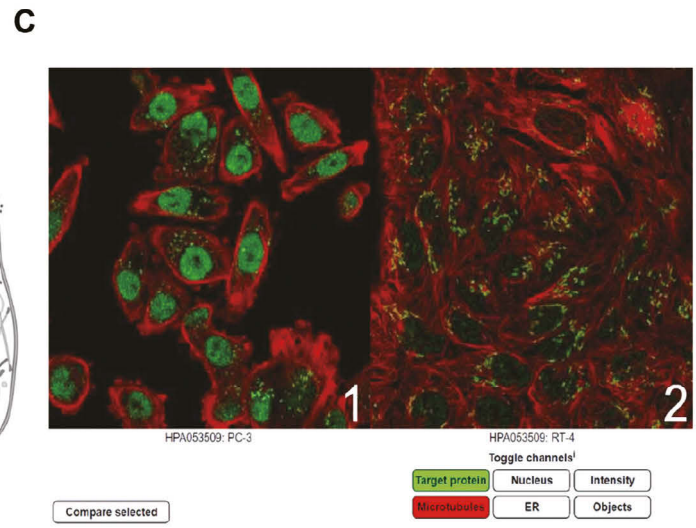
Evaluation of Siglec-15 protein expression by IHC analysis

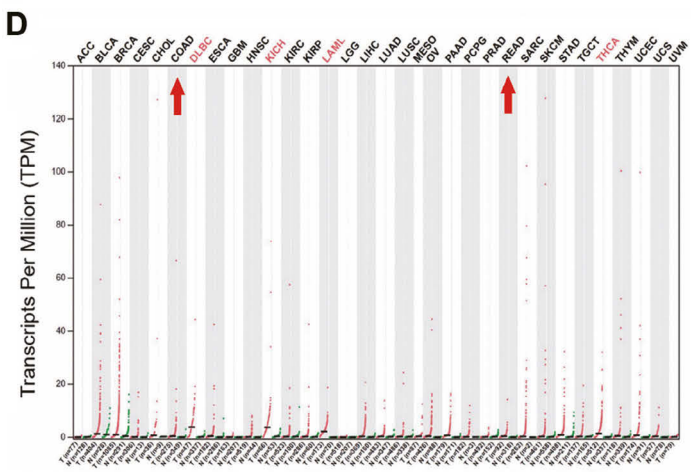
IHC analysis of TMA was performed to further evaluate the expression of Siglec-15 in CRC. In this cohort, high Siglec-15 expression was detected in 32 (35.6%) of 90 CRC tissues, compared to only 11 (12.2%) of the matched non-cancerous tissues. The results showed statistical significance ($p<0.01$) using the chi-square test analysis and were in line with the Siglec-



Compartment	Confidence
plasma membrane	4
nucleus	2
extracellular	2
golgi apparatus	1
lysosome	1
cytosol	1
endosome	1
endoplasmic reticulum	1
mitochondrion	1
cytoskeleton	1







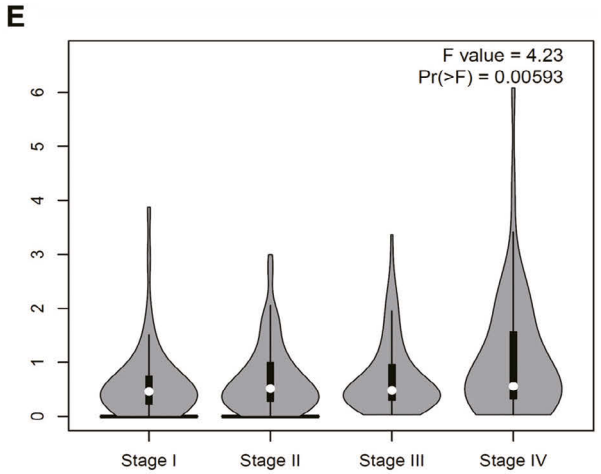


Fig. 1. A. Genecard database showed the major expression location of Siglec-15. Three dominant subcellular localizations of Siglec-15 were cytoplasm membrane, nucleus and extracellular (expression confidence >1, marked by a red frame). **B, C.** HPA database showed the typical location of Siglec-15 protein in was nucleoplasm. Target protein was marked by green fluorescence. **D.** GEPIA database demonstrated the expression of Siglec-15 in various human cancer tissues (red dots) and corresponding non-cancerous (green dots). Red arrow indicates colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ). **E.** GEPIA database showed that siglec-15 expression was positively associated with CRC TNM stage (F value=4.23, Pr=0.00593).

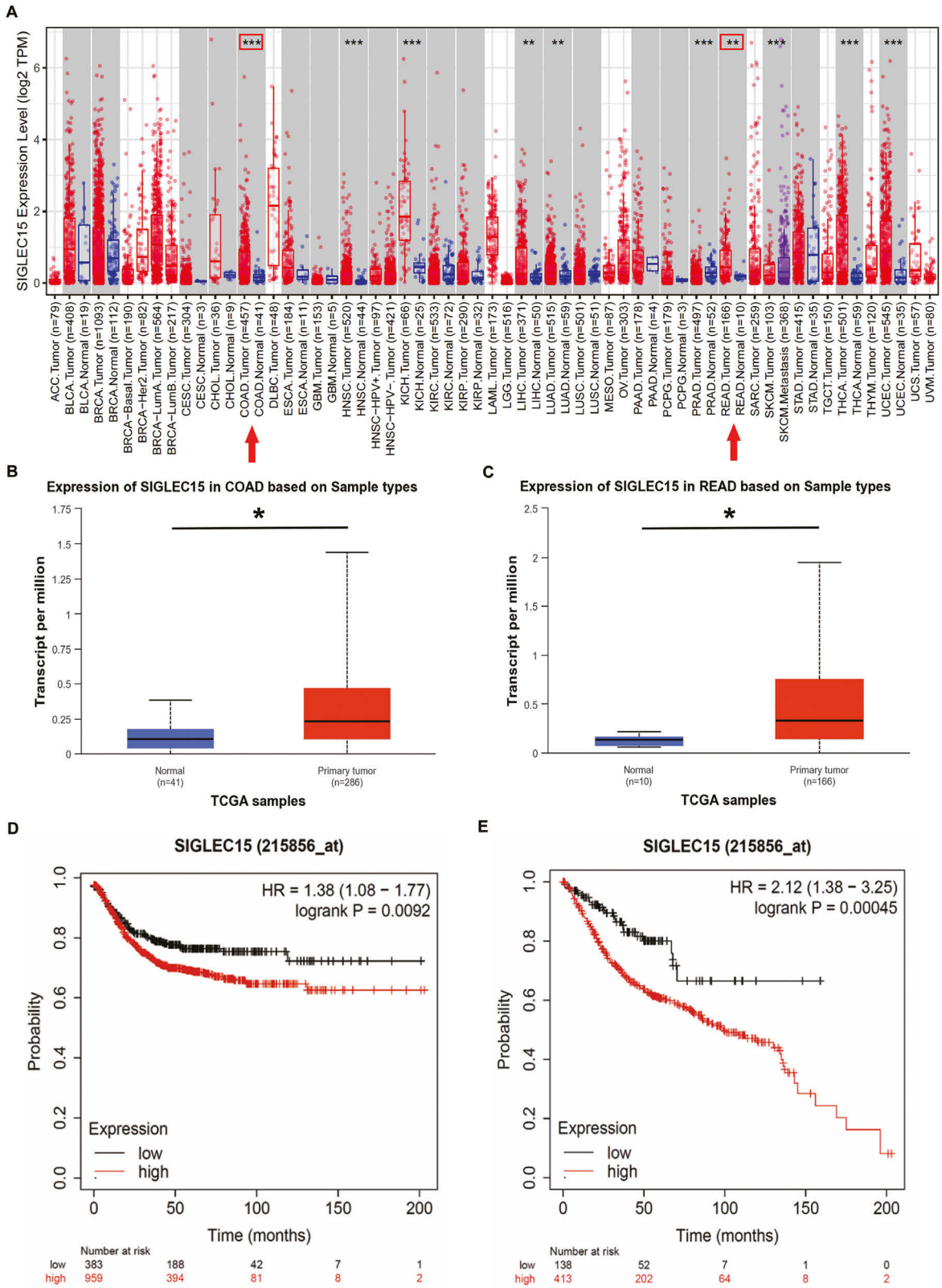


Fig. 2. A. TCGA database confirmed the general expression characteristics of Siglec-15 in 34 types of human cancers. Red frame indicates that the expression of Siglec-15 in colon adenocarcinoma (COAD) and rectal adenocarcinoma (READ) was significantly higher than that in corresponding non-cancerous. **p<0.01, ***p<0.001. **B, C.** UALCAN database further demonstrated the up-regulated expression of Siglec-15 in COAD and READ, respectively. *p<0.05. **D, E.** Kmplot database showed that elevated Siglec-15 expression implied a poor prognosis in human CRC in both recurrence-free survival (RFS, p=0.0092) and overall survival (OS, p=0.00045).

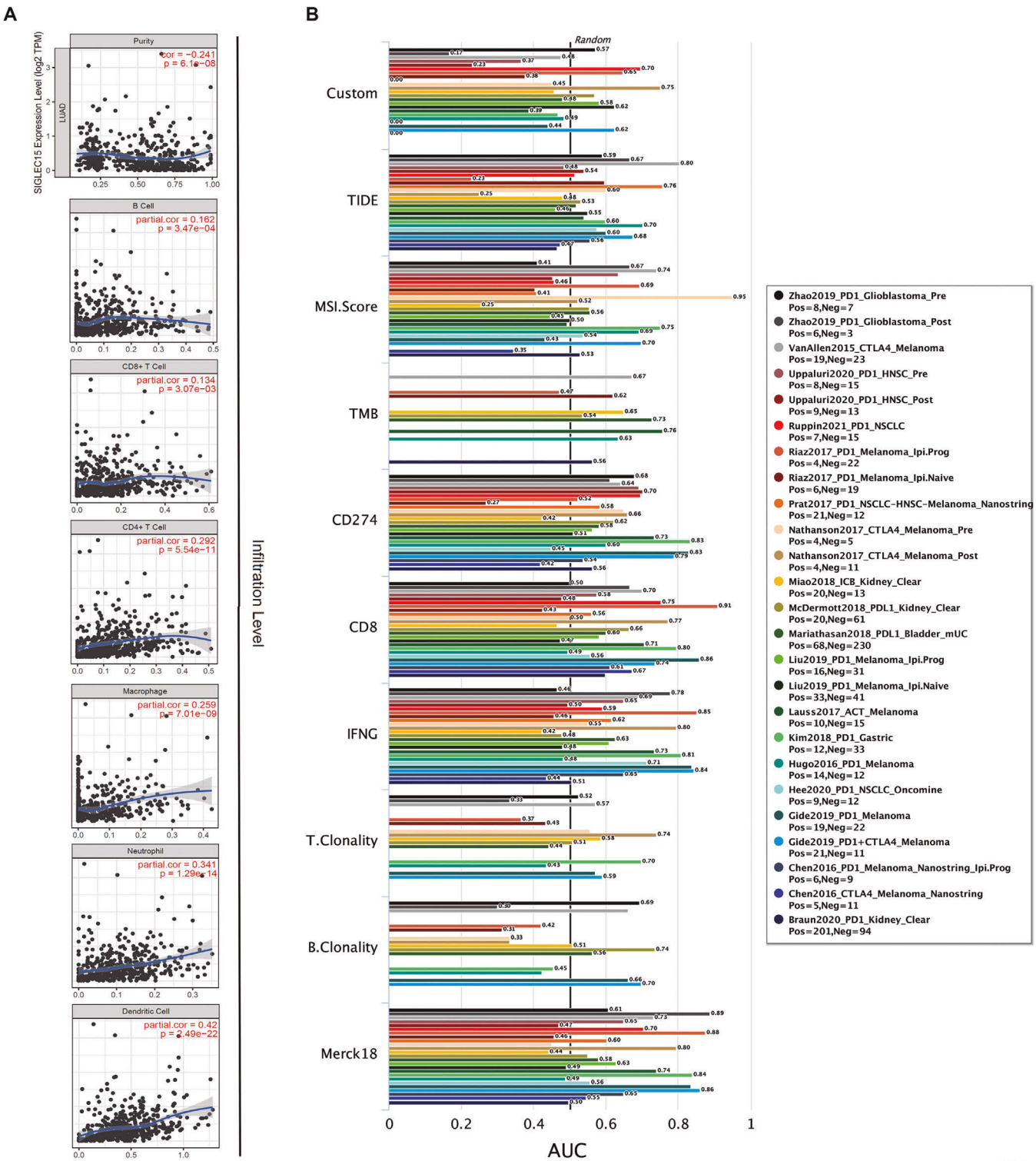


Fig. 3. A. TIMER database elucidated that Siglec-15 expression was positively related to six immune cells (B cells, CD8$^+$ T cells, CD4$^+$ T cells, macrophages, neutrophils and dendritic cells) and negatively correlated with tumor purity in LUAD. **B.** The area under the ROC curve (AUC) of TIDE database indicated that the Siglec-15 expression was depicted to assess its potential role as a predictive biomarker for immunotherapy response.

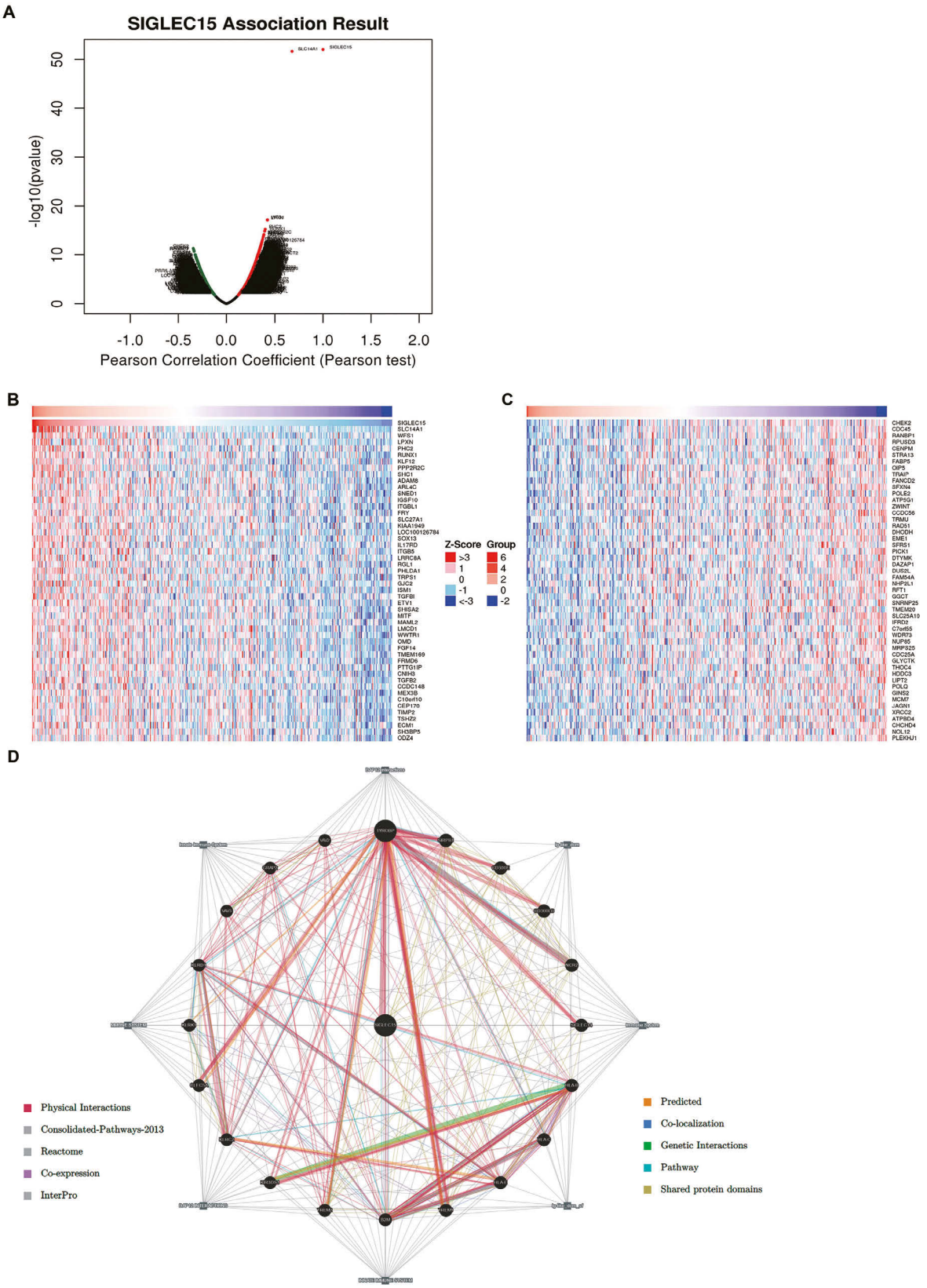


Fig. 4. A. Linkedomics database showed the Siglec-15 coexpression data in which 19828 genes from 379 samples were positively or negatively correlated with Siglec-15. **B, C.** Top 50 genes that were positively and negatively associated with Siglec-15 expression were demonstrated respectively. **D.** GeneMANIA database illustrated that Siglec-15 was mainly associated with DAP12 interaction, innate immune system.

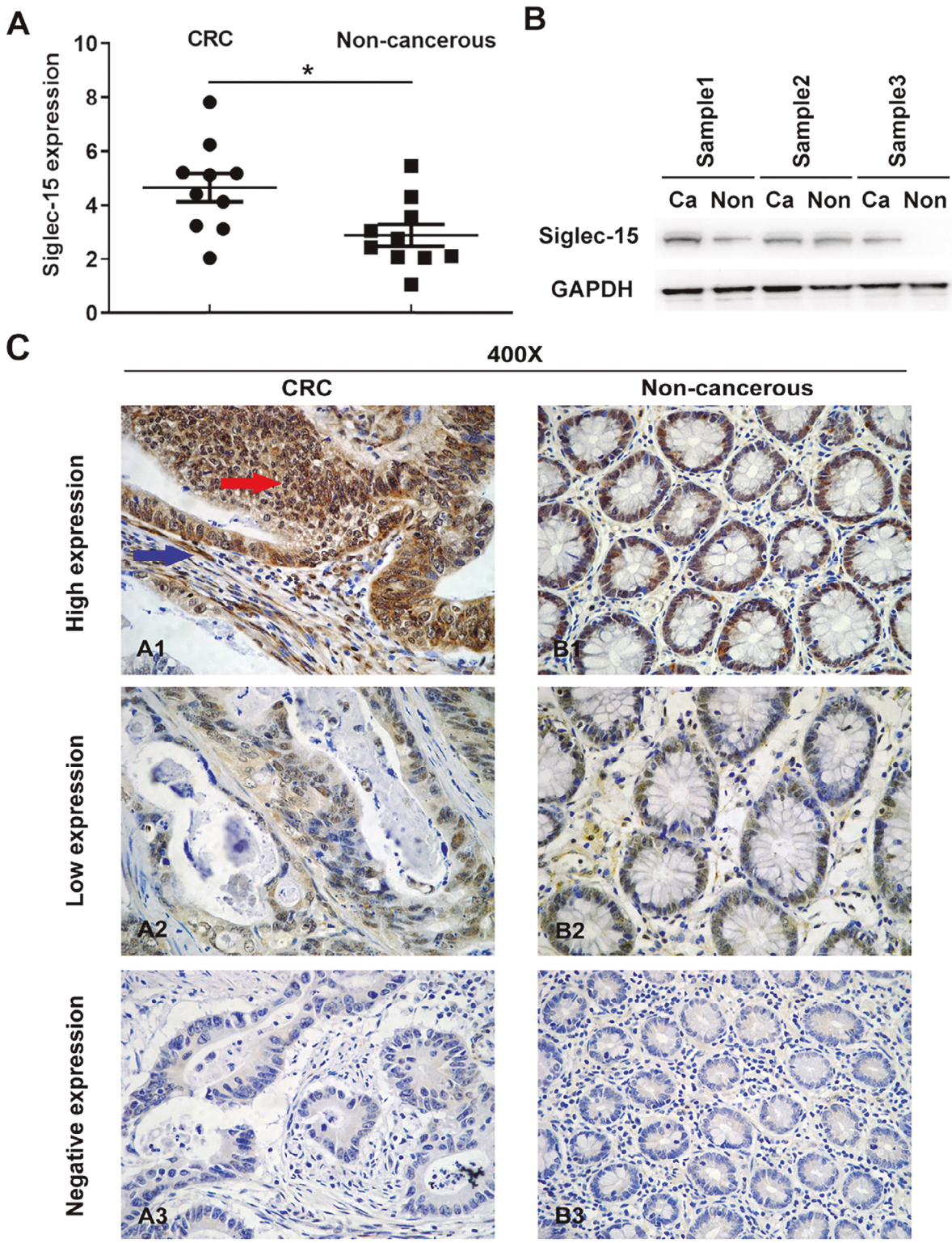


Fig. 5. A. One-step quantitative real-time polymerase chain reaction (qPCR) test was employed to evaluate the expression of Siglec-15 mRNA in CRC tissues and noncancerous tissues. When normalized to GAPDH, the expression of Siglec-15 mRNA in CRC tissues (4.651±1.66) was significantly higher than that in matched noncancerous tissues (2.886±1.28) ($t=2.663$, $df=18$, $p=0.0159$). *$p<0.05$. **B.** Western blotting analysis confirm the results obtained from qPCR test. In three CRC cases, the Siglec-15 protein expression was significantly higher in cancer tissues than that in non-cancerous tissues. Ca: CRC tissue samples; Non: non-cancerous tissue samples. **C.** Representative images of Siglec-15 protein expression in CRC tissues and corresponding noncancerous tissues with tissue microarray (TMA). C1, C2, and C3: high, low and negative immunohistochemical (IHC) staining of Siglec-15 protein in CRC tissue samples. C4, C5, and C6: high, low and negative IHC staining of Siglec-15 protein expression in noncancerous tissue samples. Red arrow showed the cytoplasm staining while blue arrow showed the stromal cells staining of Siglec-15. x 400.

15 levels previously evaluated in fresh CRC samples using qPCR and western blot analyses. Positive staining was mainly localized in the nuclei of CRC cells and in the cytoplasm and stromal cells in some cases. Representative staining of Siglec-15 in CRC is shown in Figure 5C. Specifically, we demonstrated the positive expression of Siglec-15 in macrophages in the tumor microenvironment (Fig. 6).

Relationships between Siglec-15 expression and clinicopathological items

Relationships between high Siglec-15 expression and several important clinicopathological parameters are shown in Table 1. High Siglec-15 expression was significantly associated with lymph node metastasis (p=0.001), TNM stage (p=0.001), and overall survival (p=0.026) (Table 1).

Survival Analysis

Survival analyses were performed to identify prognostic factors in this CRC cohort. In univariate analysis, three elements, including Siglec-15 expression (p=0.007), tumor differentiation (p=0.007), and distant metastasis (p=0.038), were significantly correlated with the overall survival rate of patients with CRC.

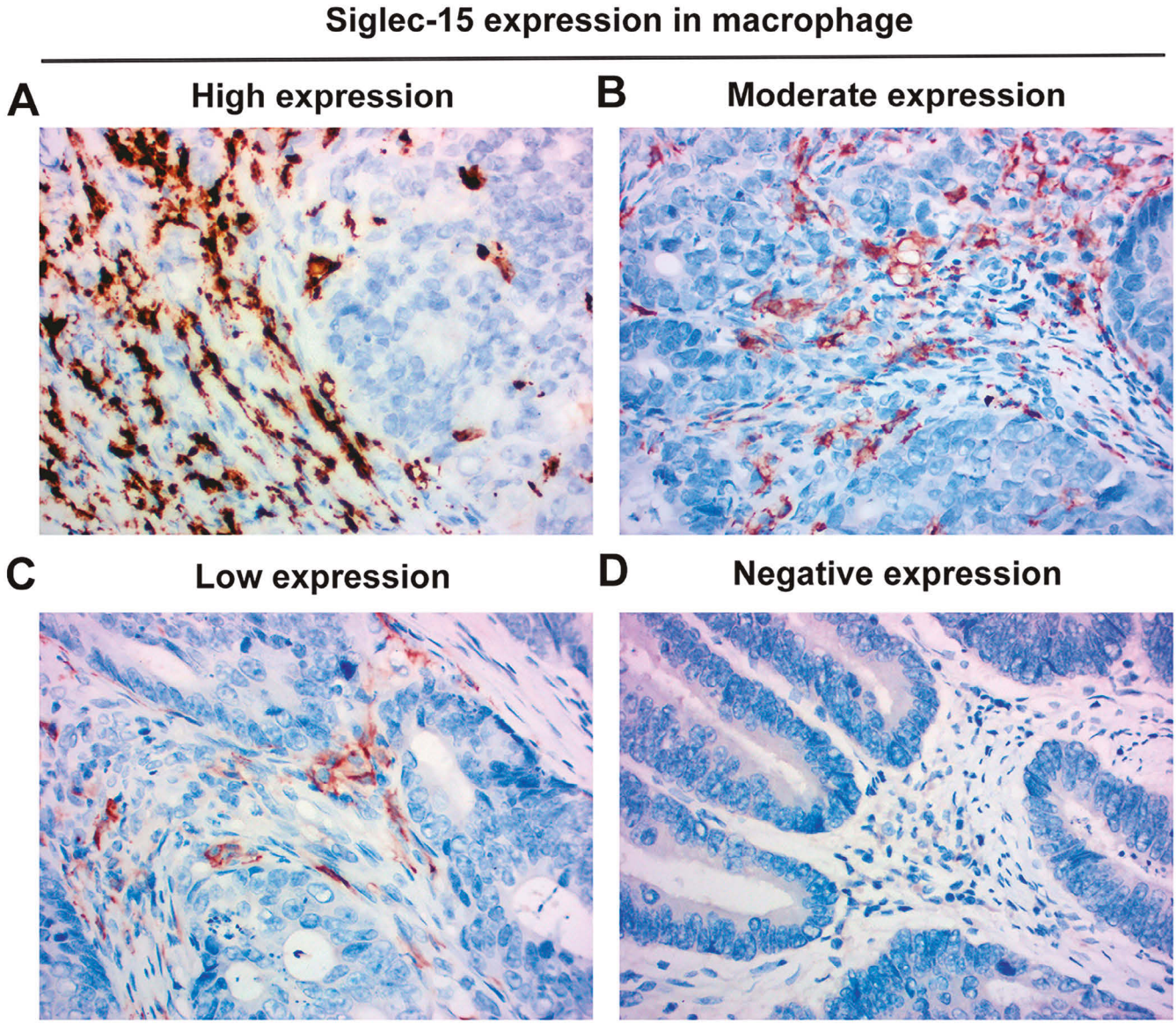


Fig. 6. Representative images of Siglec-15 protein expression in macrophages in CRC tumor microenvironment. **A.** High immunohistochemical (IHC) staining of Siglec-15 protein in macrophage. **B.** Moderate IHC staining of Siglec-15 protein in macrophage. **C.** Low IHC staining of Siglec-15 protein in macrophage. **D.** Negative IHC staining of Siglec-15 protein in macrophage. x 400.

Multivariate analysis was performed to validate Siglec-15 expression (p=0.023) and tumor differentiation (p=0.003) as independent prognostic factors in this CRC cohort (Table 2). Kaplan-Meier survival curves showed that CRC patients with high Siglec-15 expression or poor tumor differentiation had significantly unfavorable survival outcomes (Fig. 7A-C).

Discussion

Although ICIs treatment has achieved tremendous success in cancer management, unavoidable drug resistance and unsolved non-responders have prompted researchers to explore novel checkpoints to overcome the ineffectiveness of therapies targeting the current PD-1/PD-L1/CTLA4 axis (Atkins et al., 2023; Zhang et al., 2023). Recently, Siglecs and their interacting sialoglycans were identified as novel immune checkpoint axes that promote immune evasion and facilitate cancer development (Egan et al., 2023; Schmassmann et al., 2023). Unlike most Siglecs, Siglec-15 has only one IgV and one IgC2 domain, exhibiting high homology with B7 family members. Siglec-15 has been described as a novel immune suppressor that shows mutually exclusive expression with PD-L1, suggesting that in non-responders to anti-PD-1/PD-L1 therapy, treatments targeting Siglec-15 may be beneficial (Kang et al., 2020; Murugesan et al., 2021). In our previous study, we developed a Siglec-15 antibody (S15-4E6A) that exerted a tumor-inhibitory role in lung adenocarcinoma and a modulatory function in macrophage polarization in the tumor microenvironment (Xiao et al., 2022). However, the expression profile of Siglec-15 in CRC, particularly its prognostic characteristics, has not yet been fully elucidated.

In the present study, we first performed serial bioinformatic analyses to explore the expression features of Siglec-15. The GeneCard database revealed the

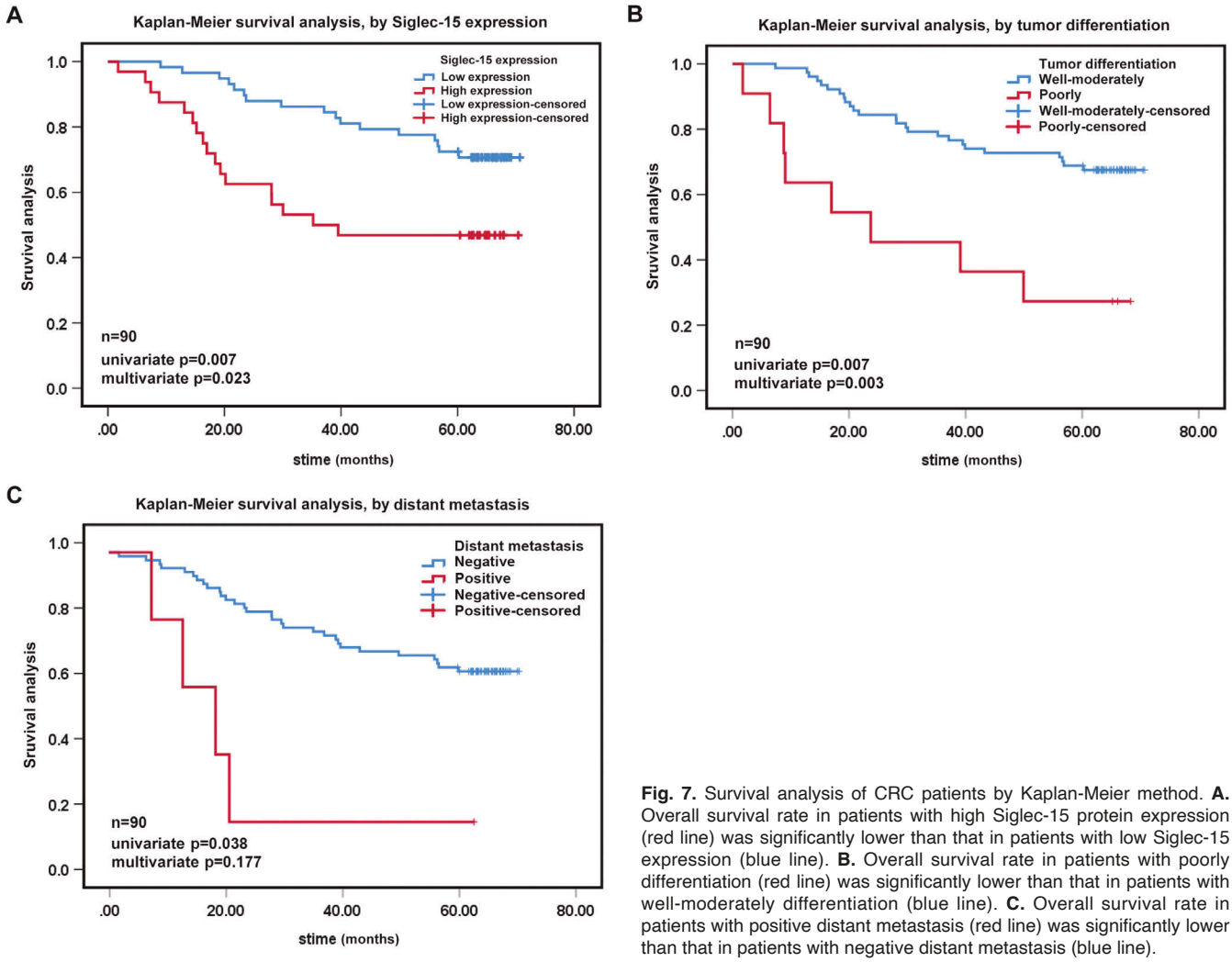


Fig. 7. Survival analysis of CRC patients by Kaplan-Meier method. **A.** Overall survival rate in patients with high Siglec-15 protein expression (red line) was significantly lower than that in patients with low Siglec-15 expression (blue line). **B.** Overall survival rate in patients with poorly differentiation (red line) was significantly lower than that in patients with well-moderately differentiation (blue line). **C.** Overall survival rate in patients with positive distant metastasis (red line) was significantly lower than that in patients with negative distant metastasis (blue line).

subcellular location of Siglec-15, the HPA database showed the expression location of Siglec-15 protein, and the GEPIA database indicated upregulated Siglec-15 expression in CRC. The TCGA database confirmed the general expression characteristics of Siglec-15, and the UALCAN database demonstrated the detailed expression of Siglec-15 in COAD and rectal READ. The KMPlot database was used to determine the prognostic value of the Siglec-15 expression. The TIMER database showed that Siglec-15 was positively associated with certain types of immune cells, including B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells. The TIDE database illustrates the potential role of Siglec-15 as a predictive biomarker of immunotherapy responses. The LinkeDomics and GeneMANIA databases provided further information on the co-expression data and the potential interaction of Siglec-15. The above bioinformatics data highlight the expression, prognosis, and immunoregulatory qualities of Siglec-15, validating the results of previous studies on Siglec-15 in human cancer (Chen et al., 2023; Huang et al., 2023).

Clinical CRC tissue samples were collected and subjected to qPCR, western blotting, and IHC to detect Siglec-15 expression at both mRNA and protein levels. qPCR analysis of 10 CRC samples revealed a remarkably upregulated level of Siglec-15 mRNA expression in CRC tissues compared to that in non-cancerous tissues. Western blot analysis of the three CRC samples confirmed that the protein expression of Siglec-15 was elevated in CRC tissues. IHC analysis of 90 CRC cases further proved that the protein expression of Siglec-15 in CRC TMA was higher than that in non-cancerous tissues. Similarly, Quirino et al. reported positive Siglec-15 expression in gastric cancer (Quirino et al., 2021), and Li et al. reported that Siglec-15 was overexpressed across cancers in a pan-cancer analysis (Li et al., 2020). Our data are consistent with the results of previous studies describing the differential expression of Siglec-15. Moreover, high Siglec-15 protein expression significantly correlated with important clinical attributes, including lymph node metastasis and TNM staging. The above information also agrees with previous studies that described the oncogenic behavior of Siglec-15 in cancer development, such as the promotion of liver cancer cell migration (Liu et al., 2021) and the acceleration of the progression of clear renal cell carcinoma (Yang et al., 2021).

Regarding the prognostic role of Siglec-15, Zhao et al. reported that Siglec-15 expression has significant advantages in OS (Zhao et al., 2022). Chen et al. reported that high levels of Siglec-15 in tumor tissues were positively correlated with poor survival in glioma patients (Chen et al., 2023). Jiang et al. conducted a meta-analysis that summarized 13 observational studies consisting of 1376 patients and showed that elevated baseline Siglec-15 expression significantly correlated with poor OS (Jiang et al., 2022). In the present study, univariate survival analysis was used to screen several clinical parameters that significantly correlated with the overall survival of patients with CRC, including Siglec-15 expression, tumor differentiation, and distant metastasis. Multivariate analysis identified Siglec-15 expression and tumor differentiation as independent prognostic factors for OS in this CRC cohort. The Kaplan-Meier curve showed that patients with CRC with high Siglec-15 expression and poor tumor differentiation had a statistically unfavorable OS. Our current data are consistent with the results of the aforementioned studies, which revealed the prognostic role of Siglec-15 in solid tumors.

However, several issues remain to be resolved.

Table 1. Correlation of Siglec-15 expression with clinicopathological characteristics of CRC patients.

Groups	No.	Siglec-15		χ^2	p value
		+	%		
Gender					
Male	57	19	33.3	0.335	0.563
Female	33	13	39.4		
Age (years)					
≥60	61	19	31.1	1.605	0.205
<60	29	13	44.8		
Tumor size (cm)					
≥5	41	16	39.0	0.396	0.529
<5	49	16	32.7		
Tumor location					
Colon	45	19	42.2	1.975	0.160
Rectum	43	12	27.9		
Ileocecal junction	2	1			
Histological type					
Adenocarcinoma	82	31	37.8	1.549	0.213
Mucinous carcinoma	7	1	14.3		
Signet ring cell carcinoma	1	0	0		
Tumor differentiation					
Well	1	1	100	1.773	0.412
Moderately	76	27	35.5		
Poorly	11	4	36.4		
Unknown	2				
Serum CEA level (ng/ml)					
≥15	10	5	50.0	0.620	0.431
<15	57	21	36.8		
Unknown	23				
Lymph node metastasis					
Positive	35	21	60.0	14.935	0.001*
Negative	55	11	20.0		
Distant metastasis					
Positive	5	2	40.0	0.046	0.831
Negative	85	30	35.3		
TNM stage					
Stage I, II	52	10	19.2	14.324	0.001*
Stage III, IV	38	22	57.9		
Overall survival					
Alive	56	15	26.8	4.976	0.026*
Dead	34	17	50.0		

*$p<0.05$

Table 2. Univariate and multivariate analysis of prognostic factors for overall survival in 90 CRC cohort.

	Univariate			Multivariate		
	HR	p value	95% CI	HR	p value	95% CI
Siglec-15 expression						
High *versus* Low	2.53	0.007*	1.29-4.97	2.34	0.023*	1.12-4.88
Gender						
Male *versus* Female	1.80	0.132	0.84-3.86			
Age (years)						
≥60 *versus* <60	0.95	0.891	0.46-1.95			
Tumor size (cm)						
≥5 *versus* <5	1.54	0.213	0.78-3.06			
Tumor location						
Colon *versus* Rectum *versus* Ileocecal junction	1.02	0.957	0.53-1.95			
Histological type						
Adenocarcinoma *versus* Non-adenocarcinoma	0.28	0.190	0.04-1.89			
Tumor differentiation						
Poorly *versus* Moderately-Well	3.15	0.007*	1.37-7.22	2.94	0.003*	1.53-8.28
Serum CEA level (ng/ml)						
≥15 *versus* <15	1.12	0.754	0.56-2.23			
Lymph node metastasis						
Positive *versus* Negative	1.40	0.321	0.716-2.776			
Distant metastasis						
Positive *versus* Negative	3.05	0.038*	1.07-8.75	2.20	0.177	0.070-6.93
TNM stage						
Stage I-II *versus* Stage III-IV	0.58	0.115	0.30-1.14			

* $p<0.05$

Although Siglec-15 implied poor overall survival in CRC in the present study, Lu et al. reported that the expression of Siglec-15 did not have prognostic significance in survival for CRC, either in DFS or OS (Lu et al., 2023), and Fudaba et al. reported that Siglec-15 expression on macrophages was a favorable prognostic factor for primary central nervous system lymphoma (Fudaba et al., 2021). Jiang et al. also showed that high Siglec-15 expression predicted a significantly better DSS in a previous meta-analysis (Jiang et al., 2022). Therefore, we conclude that these inconsistent or even conflicting results may be due to differences in the tumor types, antibodies used, or experimental protocols. The detailed characteristics of Siglec-15 in human cancer warrant further investigation. In addition, we only provided OS data, but failed to demonstrate other survival data, such as PFS or DFS. We did not explore the PD-L1 expression in CRC, as Siglec-15 might work individually of the PD-1/PD-L1 pathway in TME. Moreover, the original TMA data failed to collect some important clinical items for CRC patients, including MSI-status, Ras/Braf -status, which are of great importance for formulating the strategy of CRC treatment. In future studies, we will focus on data collection and experiments design more elaborately and carefully.

In conclusion, this study investigated the expression of Siglec-15 in both bioinformatic databases and private clinical samples. Differential Siglec-15 expression was observed in CRC, and elevated Siglec-15 expression correlated with several malignant phenotypes of CRC, including lymph node metastasis and TNM stage. Moreover, high Siglec-15 expression is associated with unfavorable OS in patients with CRC. Siglec-15 may be a novel biomarker of CRC, and targeting Siglec-15 may provide a promising strategy for CRC management.

Acknowledgements. We thank Professor. Yuan Mao from the Department of Oncology, The Fourth Affiliated Hospital of Nanjing Medical University for the support and instruction.

Author contribution. YZ and WH designed the study. GL, LZ and RD collected the tissue samples. GL and YF performed the PCR and WB experiments. JY and LX performed the IHC analysis. LZ, YF, and RD performed the statistics. GL and WH drafted the manuscript. YZ and WH supervised the study. All authors read and approved the final manuscript.

Funding. This work is supported by the grants from the National Natural Science Foundation (82073389).

Declaration of competing interest. The authors report no conflict of interest.

Data Availability. The data used to support the findings of this study are available from the corresponding author upon request.

Ethics Approval and Consent to Participate. Written informed consent was obtained from all the patients included in this study. Ethical approval was approved by the Human Research Ethics Committee of the Fourth Affiliated Hospital of the Nanjing Medical University (20230425-K121).

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Accepted July 30, 2024