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SELE gene as a characteristic prognostic biomarker of colorectal cancer

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Abstract

Objective: To investigate the expression and clinical value of the E-selectin gene (*SELE*) in colorectal cancer (CRC).

Methods: Using gene expression profiles and clinicopathological data for patients with CRC from The Cancer Genome Atlas, and tumor and adjacent normal tissues from 31 patients with CRC from Xianyang Central Hospital, we studied the correlation between *SELE* gene expression and clinical parameters using Kaplan–Meier and Cox proportional hazards regression analyses.

Results: Higher expression of *SELE* was significantly associated with a poorer prognosis and shorter survival in patients with CRC. The median expression level of *SELE* was significantly higher in CRC tissues compared with healthy adjacent tissue. Cox regression analysis showed that the prognosis of CRC was significantly correlated with the expression of *SELE*. Immunohistochemical analysis also showed that positive expression of E-selectin increased significantly in line with increasing TNM stage.

Conclusion: This study confirmed that *SELE* gene expression is an independent prognostic factor in patients with CRC.

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Introduction

Colorectal cancer (CRC) is a major threat to human health, with about two million new cases of CRC worldwide in 2018.[1] CRC is the third most common malignant tumor globally, accounting for 690,000 deaths, and the fourth most common malignant tumor in China. The incidence of CRC is increasing rapidly in the eastern developed region of China, accounting for 18.6% of the total number of cases worldwide. The 5-year survival rate of CRC in China is also significantly lower than that in developed countries in Europe and North America, mainly due to the difficulty in early diagnosis and the presence of distant metastases. Early diagnosis, prevention, and treatment of metastases are therefore key to improving the prognosis and survival rate of patients with CRC. Gene testing aids targeted therapy, and the prompt selection of targeted drugs with specific efficacy represents an economic saving for the patient, as well as avoiding delays with disease treatment.

The prognostic values of *KRAS* and *BRAF* gene[2] mutations have remained controversial. In addition, the *SELE* gene encodes E-selectin, which is found in cytokine-stimulated endothelial cells and is thought to be responsible for the accumulation of leukocytes at sites of inflammation by mediating the adhesion of cells to the vascular wall. Its structure includes lectin- and epidermal growth factor-like domains followed by short consensus repeat domains containing six conserved cysteine residues. E-selectin is part of the selectin family of cell adhesion molecules, which are glycoprotein sugar chains or sheath sugars found on the surface of white blood cells and tumor cells. Selectin ligands include saliva-acidized Lewis oligosaccharides (sLeX and sLeA) and other molecules with similar structures,[3] and the interaction between selectin and these ligands and the subsequent adhesion response have been shown to play an essential role in tumor invasion and metastasis.[4] Numerous studies[5–7]have shown that sLeX and sLeA antigens are expressed on the surface of tumor cells, and can combine with selectins on the surface of vascular endothelial cells to mediate the adhesion of tumor cells to endothelial cells. Stronger expression is therefore associated with more intense adhesion and a greater ability to form metastatic foci *in vitro*.[8,9] Studies[4,10]have shown that selectin and ligand-mediated adhesion play an essential role in tumor cell metastasis. An in-depth study of selectin expression will therefore aid the early diagnosis of CRC, evaluation of its prognosis, and the search for new therapeutic targets. The current study analyzed the prognostic role of *SELE* in CRC based on the analysis of data from The Cancer Genome Atlas (TCGA) database.

Materials and Methods

Transcript profiling of SELE *gene*

We analyzed data for patients with colon cancer (TCGA-COAD) and rectal cancer

(TCGA-READ) from the TCGA database[11] (https: //portal.gdc.cancer.gov/). We successively selected patients with adenomas and adenocarcinomas, with quantitative transcript data suitable for assessing gene expression. Finally, we chose Fragments per kilobase of exon model per million mapped fragments (FPKM) as the workflow type to plot single gene expression. After downloading and collating the data, we used the R package "limma" to extract the data and obtain the mean value for any repeated genes. The "Clinical" option was selected to extract clinical data.

Expression of SELE *gene using correlation function by Gene Expression Profiling Interactive Analysis (GEPIA)*

GEPIA[12] contains genotype–tissue expression and TCGA data (http: //gepia.cancer-pku.cn/). We evaluated the expression of *SELE* and other characteristic genes in CRC (*TP53, ERCC1, KRAS, NRAS, BRAF, SMAD4, APC, SOX9, MRC1, MSH2, NDRG4, BMP3, VIM, KDR, EGFR, TFPI2*) using the correlation function of GEPIA.

Immunohistochemical validation

Study subjects. We collected information on patients with colon cancer treated at Xianyang Central Hospital in Shaanxi between January 2019 and June 2019. Patients with postoperative pathological information including tumor size, differentiation degree, TNM stage, and immunohistochemical detection of E-selectin were followed-up from postoperative discharge for 4 to 6 months, until January 2020. The pathology was reviewed independently by three pathologists without access to the clinical data. The study was approved by Xianyang Central Hospital ethics committee (Approval number 20190037), and all patients signed informed consent.

Immunohistochemistry. Ethanol, phosphate-buffered saline (PBS), 3% H_2O_2, 10% normal goat serum, 0.01 M citrate buffer, xylene, and antibodies were provided by Maxim Biotechnology Co. Ltd. (Fuzhou, China). DAB chromogenic solution (Catalog number AR1022) was purchased from Boster Biological Technology Co. Ltd. (Wuhan, China). Rabbit anti-E-selectin antibody (1:500; Catalog number-1273R) was purchased from Beijing Biosynthesis Biotechnology Co. Ltd. (Beijing, China). Horseradish peroxidase-conjugated Affinipure goat anti-rabbit IgG (1:2000; Catalog number sa00001-2) was purchased from Proteintech (Wuhan, China).

Paraffin-embedded tissue slices were dewaxed and subjected to antigen repair using citrate buffer solution in a pressure cooker with air-injection for 2 minutes, and cooled to room temperature. Endogenous peroxidase activity was blocked by incubation at room temperature with 3% H_2O_2 for 10 minutes, followed by the addition of 5% bovine serum albumin (BSA) to the slices for 30 minutes at room temperature to block non-specific antigen reactions. The primary antibody was then added to the working solution at a dilution of 1:500, 1% BSA, incubated overnight at 4°C, and then washed three times with PBS for 3 minutes each. Each section was then incubated with two drops of horseradish peroxidase-conjugated Affinipure goat anti-rabbit IgG (dilution ratio 1:2000, 1% BSA dilution) for 30 minutes at room temperature, rinsed three times in PBS for 3 minutes each, and DAB color was developed, followed by hematoxylin staining and covering with a neutral resin seal. The slides were observed and photographed under a light microscope. E-selectin-positive signals were indicated by brownish

yellow color. Less than 10% of positive cells was considered as a negative result, and $\geq$10% was considered positive.

Statistical analysis

Data were analyzed using SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA) and R (version 3.6) software (www.R-project.org), and graphs were produced using GraphPad Prism (version 5.0) (GraphPad Prism Inc., La Jolla, CA, USA). We carried out single-gene data analysis using R (www.R-project.org) and the Bioconductor package[13] (www.bioconductor.org). We analyzed *SELE* expression using *t*-tests and Kruskal–Wallis tests. Clinicopathological correlation analysis was conducted using logistic regression analysis, and survival was evaluated by Kaplan–Meier analysis and log-rank test. Multivariate analysis was carried out using a Cox proportional risk regression model, and Pearson's correlation analysis was used to assess correlations between genes and related factors. A P-value <0.05 was considered significant.

Results

SELE *gene expression in CRC and adjacent healthy tissues*

Data for 31 patients with colon cancer (18 men, 13 women; median age 72 years, range 46–88 years), including four cases of metastasis, treated at Xianyang Central Hospital between January 2019 and June 2019 were collected, according to a random number table. Seventy-two samples including carcinoma and healthy adjacent tissue were available. We compared *SELE* expression levels between CRC and healthy tissues (Figure 1a). Compared with healthy adjacent tissues, the median level of *SELE* gene expression was significantly higher in the tumor samples ($P< 0.001$). Matched pair analysis between CRC and healthy tissues (Figure 1b) in the same patient showed that *SELE* was significantly overexpressed in CRC samples ($P < 0.001$).

Kaplan–Meier survival analysis

We initially identified 475 patients with colon or rectal adenoma/adenocarcinoma from TCGA database (https: //portal.gdc.cancer.gov). After excluding patients with missing data, we obtained 332 samples with enough data to conduct survival analysis (average follow-up 2.8 years, longest 12 years). After analyzing and collating the data, we obtained survival times and survival status and evaluated the correlation between OS and *SELE* gene expression (Figure 1c). Survival gradually declined over time. We also divided cases into low and high *SELE* expression according to the median value (0.51), then assessed survival according to high or low *SELE* expression. The 5-year OS rate was significantly higher in the low-expression group (0.704, 95% confidence interval [CI] 0.577–0.860) compared with the high-expression group (0.526, 95% CI 0.385–0.718) ($P=0.017$). Patient survival time could thus be predicted according to the expression level of the *SELE* gene, with patients with low expression levels more likely to survive for >5 years, and patients with high levels more likely to survive for <5 years.

Clinical correlation analysis

We investigated *SELE* gene expression in relation to clinical stage using Kruskal–Wallis and logistic regression analyses (Figure 1d). There was no significant correlation between CRC stage and *SELE* gene expression.

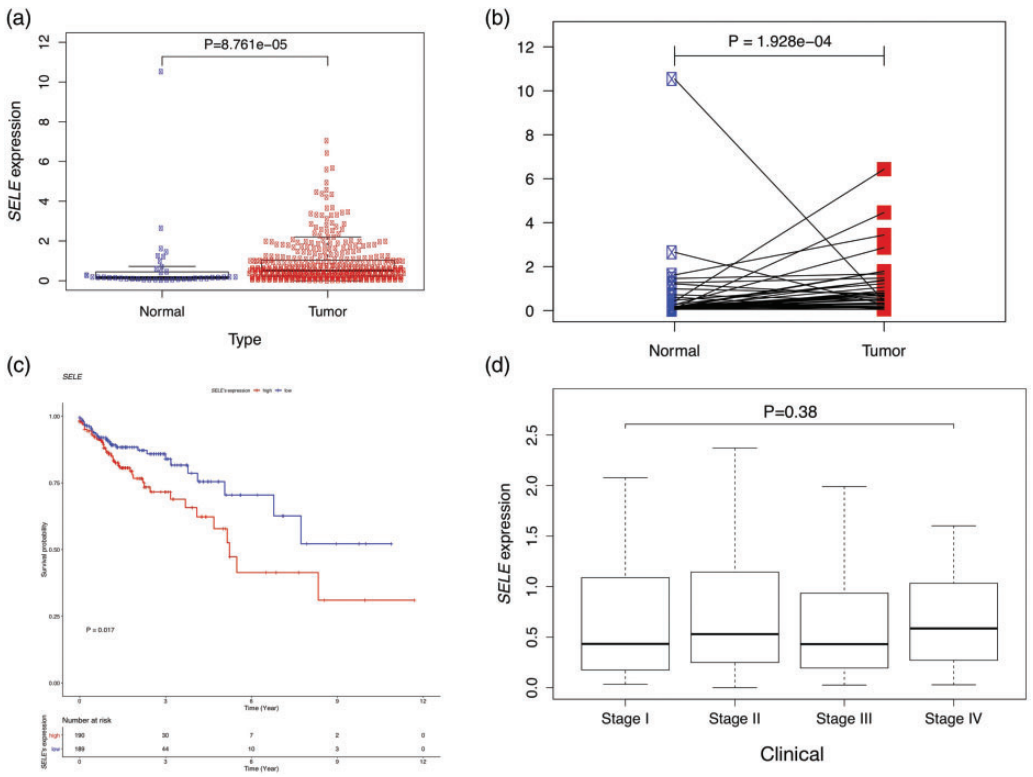

Figure 1. Differential expression of E-selectin gene (*SELE*) in relation to survival and clinical stage in patients with colorectal cancer (CRC). (a) Scatter plot of *SELE* gene expression in CRC and healthy adjacent tissues. (b) *SELE* expression in paired CRC and healthy adjacent tissues in the same patient. (c) Survival analysis in relation to *SELE* gene expression based on The Cancer Genome Atlas data. (d) Differential expression of *SELE* gene in patients with different stages of CRC (Kruskal–Wallis test).

SELE *gene expression had stronger prognosis prediction ability than TNM stage or other clinical features in CRC*

We analyzed *SELE* gene, age, sex, clinical stage, primary lesion, increased involvement of adjacent tissues (T), distant metastasis (M), and regional lymph node involvement (N) as independent factors related to clinical survival using the survival package in single-factor Cox regression analysis (Table 1). Univariate analysis identified *SELE* gene expression, age, clinical stage, primary tumor, increased involvement of adjacent tissues (T), distant metastasis (M), and regional lymph node involvement (N) as independent prognostic factors (all $P < 0.05$). However, sex had no significant effect on the prognosis of CRC.

We then carried out multivariate Cox regression analysis using the R package "survminer" (Figure 2). This indicated that *SELE* gene expression ($P = 0.0067$) and age ($P = 0.0014$), but not clinical stage or TNM stage, were significantly and independently correlated with a prognosis of CRC.

Association between SELE *and key genes in CRC*

We also analyzed the correlations between expression levels of *SELE* and mismatch

Table 1. Univariate and multivariate Cox analyses of clinical parameters in relation to overall survival.

	Univariate Cox analysis				Multivariate Cox analysis			
Variable	HR	95%CI		P value	HR	95%CI		P value
Age	1.029	1.005	1.055	**0.017**	1.040	1.015	1.066	**0.001**
Sex	1.126	0.668	1.899	0.655	0.995	0.582	1.699	0.984
Stage	2.502	1.850	3.382	**<0.001**	1.614	0.672	3.875	0.283
T	2.926	1.742	4.917	**<0.001**	1.453	0.769	2.748	0.249
M	5.226	3.065	8.911	**<0.001**	1.699	0.517	5.587	0.382
N	2.175	1.608	2.941	**<0.001**	1.353	0.800	2.286	0.258
SELE	1.287	1.062	1.559	**0.009**	1.312	1.077	1.597	**0.0067**

HR, hazard ratio; CI, confidence interval.

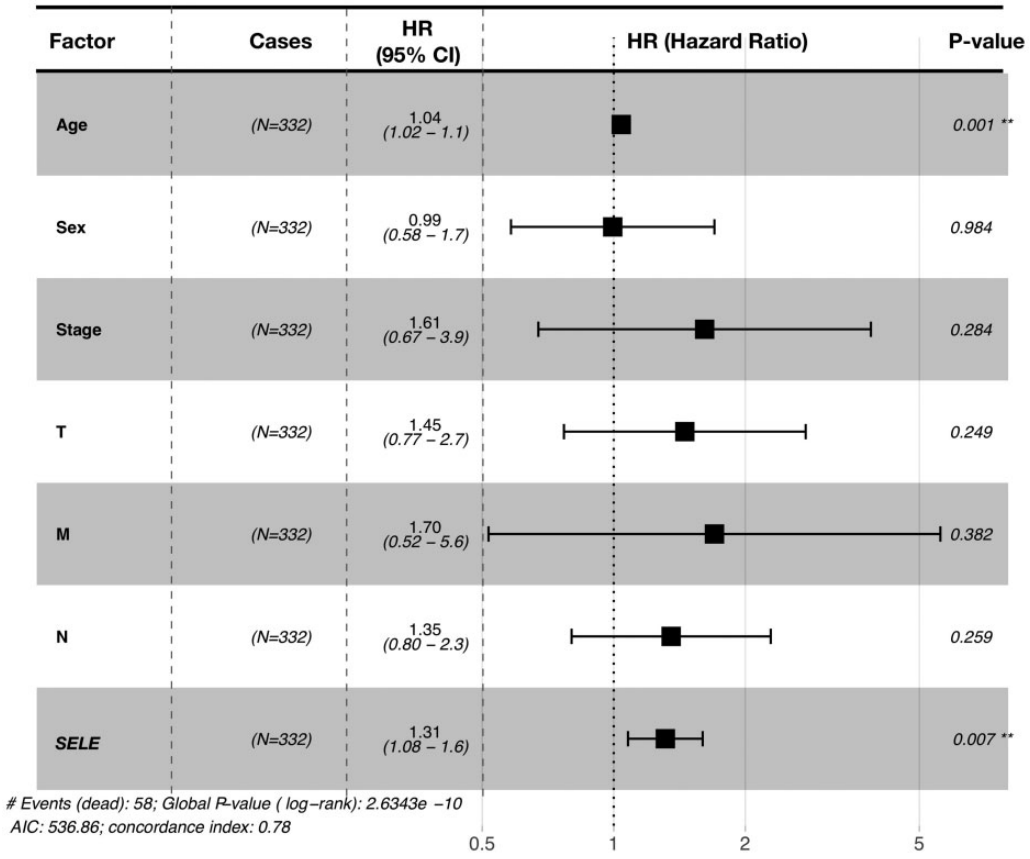

Figure 2. Multivariate analysis of clinicopathological factors affecting overall survival in patients with colorectal cancer (**$P < 0.05$).
AIC, Akaike information criterion.

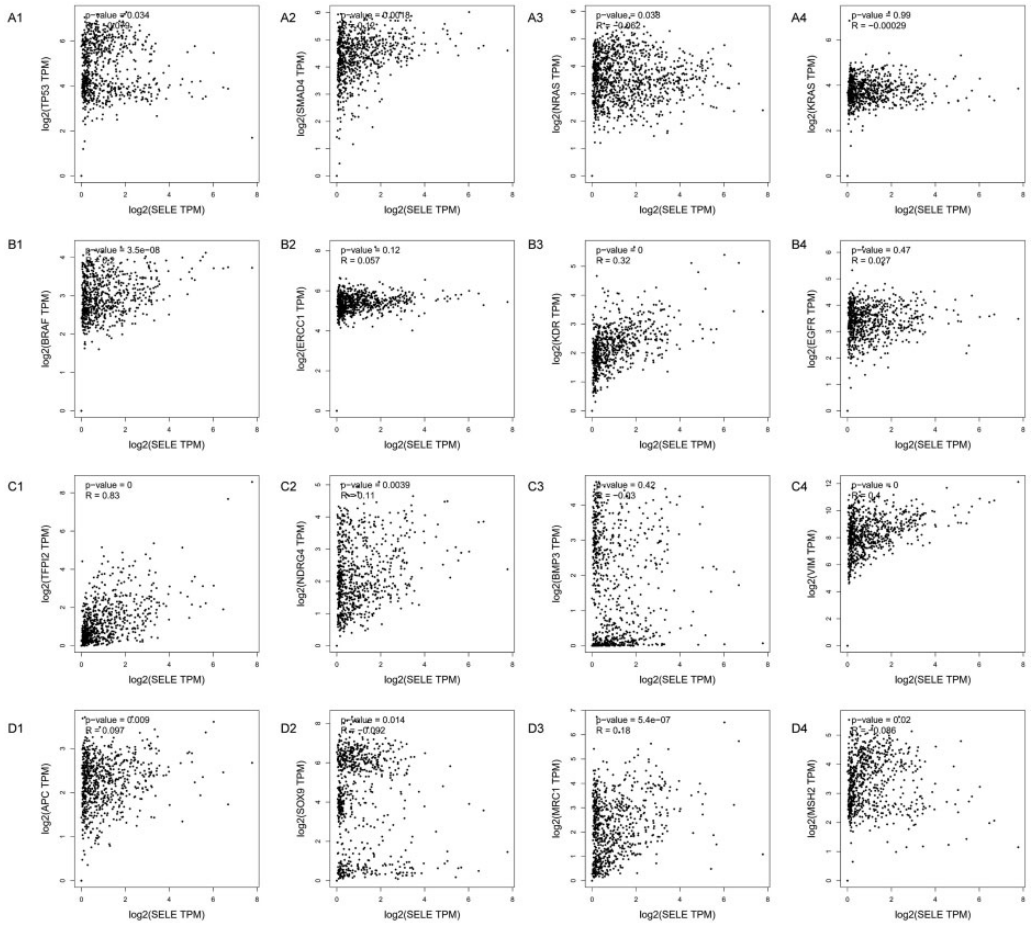

Figure 3. Correlations between *SELE* and key genes in colorectal cancer. *SELE* gene expression was significantly correlated with expression of *MSH2*, *NRAS*, *BRAF*, *KDR*, *SMAD4*, *APC*, *TP53*, *NDRG4*, *MRC1*, *VIM*, *TFPI2*, and *SOX9* genes ($P < 0.05$).
TPM, transcripts per million kilobases.

repair genes using the correlation analysis function in the GEPIA database. We analyzed the correlations between expression levels of *SELE* and *MLH1, MSH2, MSH6*, and *PMS2* mismatch repair genes separately (Figure 3). *SELE* was significantly correlated with *MSH2* expression ($P<0.05$). The American Cancer Society recommends targeted therapy and chemotherapy as the two main drug therapies for CRC. Based on the NCCN guidelines of the United States,[14] experts in the field strongly recommended that all patients diagnosed with stage IV metastasis should undergo *RAS* (*KRAS*, *NRAS*) and *BRAF* gene-status detection in cancer tissues. We therefore analyzed the expression levels of *SELE*, *RAS* (*KRAS, NRAS*), and *BRAF* genes,[15] and expression of the vascular endothelial growth factor receptor gene (*KDR*), *ERCC1*, and *UGT1A1*28/*6* gene polymorphism.[16] *SELE* was significantly correlated with expression of *NRAS BRAF*, and *KDR*[17] ($P<0.05$). We also

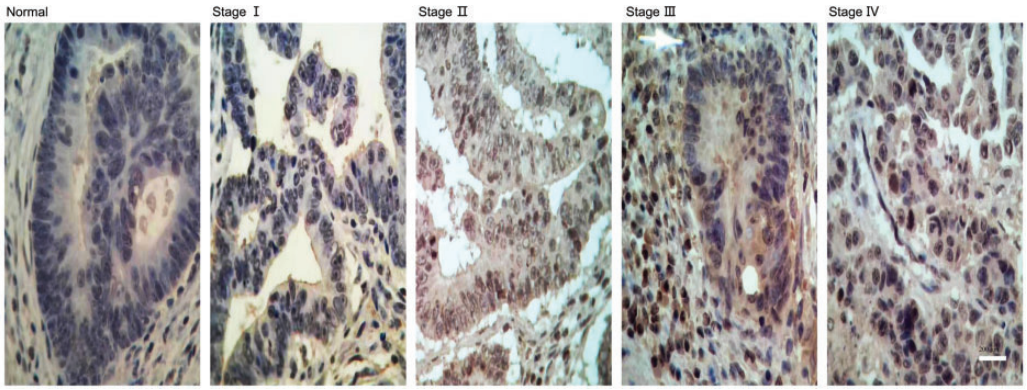

Figure 4. Positive E-selectin signals were detected in the cytoplasm and/or cell membrane, presenting as brownish-yellow granules, in cancer cells and vascular endothelial cells. The incidence of E-selectin positivity was significantly higher in cancer cells compared with healthy intestinal mucosal glandular epithelial cells ($P < 0.05$). E-selectin expression increased significantly with increasing TNM stage ($P < 0.05$).

analyzed the correlations between *SELE* expression and expression of a series of CRC-related genes including *SMAD4, APC, TP53, NDRG4, MRC1, BMP3, VIM, TFPI2,* and *SOX9*. *SMAD4, APC, TP53, NDRG4, MRC1, VIM, TFPI2,* and *SOX9* were significantly correlated with *SELE* gene expression (all $P<0.05$).

Immunohistochemical validation

Positive E-selectin staining indicated by brown-yellow granules was located in the cytoplasm and cell membrane in both cancer cells and vascular endothelial cells, but not in the negative control group (0.1 mol/L PBS) (Figure 4). The incidence of positive E-selectin expression was significantly higher in cancer cells compared with healthy intestinal mucosal glandular epithelial cells ($P<0.05$, rank sum test). The incidence of positive E-selectin expression, calculated according to the product of the number of positive cells and staining intensity, changed significantly with increasing TNM stage ($P<0.05$). We also analyzed the correlations between E-selectin and clinical features and prognosis, which confirmed the result of TCGA analysis. *SELE* gene expression levels were higher in CRC compared with healthy adjacent tissues, and increased with increasing stage, with a higher *SELE* expression level indicating a worse prognosis.

Discussion

We analyzed transcript profiling and related clinical data for patients with CRC in TCGA database, to investigate the correlation between *SELE* gene expression and prognosis in CRC. We showed that *SELE* gene expression levels were significantly higher in CRC tissues compared with adjacent healthy tissues. We also analyzed survival, clinicopathological, and *SELE* gene expression data in relation to CRC using TCGA database. Univariate Cox regression analysis suggested that *SELE* gene expression levels differed significantly between colon cancer tissues in relation to tumor size, invasion depth, and distant metastasis, with higher expression associated with increased tumor differentiation degree, lymph node metastasis, and clinical T, N, and M stages. These results suggest that high expression of the *SELE* gene may

promote malignant biological behavior in colon cancer.

Various factors affect the prognosis of CRC. The current study showed that the poorer prognosis in patients with high expression of *SELE* was associated with shorter survival, determined by multiple regression and Kaplan–Meier analyses. The value of *SELE* gene expression was independent of T, N, and M stages and other clinical data, indicating that is was an essential prognostic factor in CRC. The application of anti-tumor drugs significantly down-regulated *SELE* expression levels in different colon cancer cell lines.[18] Overall, these results suggest that *SELE* may act as an oncogene.

Myeloid cells include macrophages, neutrophils, acidic granulocytes, mast cells, and dendritic cells. Myeloid cells exist in peripheral circulating blood and are recruited into tumor tissue to promote angiogenesis in the tumor microenvironment.[19] Granulocytes, myeloid cells, tumor cells, and vascular endothelial cell membranes all secrete E-selectin. In addition, considering the crucial role of tumor angiogenesis in tumor development, cancer cells have been shown to secrete inflammatory cytokines such as interleukin-1β and tumor necrosis factor-α, to induce *SELE* at sites of distant metastasis, and inhibit the anti-tumor adaptive immune response.[20] The current results showed that increased expression of *SELE* led to a poorer clinical outcome and shorter survival.

This study provided conclusive evidence for increased expression of *SELE* as a clinical adverse prognostic factor in CRC. Survival analysis and univariate and multivariate Cox regression analyses showed consistent results, implicating *SELE* as an oncogene for CRC, as supported by other related studies.[10] Numerous studies[21–23] confirmed that *SELE* was strongly linked to the generation and invasion of CRC in small samples. Other researchers[24] detected preoperative serum E-selection and CA19-9 in 152 patients with CRC and 28 healthy volunteers, and showed that patients with higher serum expression levels of E-selection and CA19-9 had a significantly lower 5-year overall survival rate. Researchers have also used E-selectin as a marker of hematogenous metastasis and prognosis in CRC. *SELE* expression was analyzed in 202 patients with different clinical stages of colon cancer with lymphatic vessel invasion and intestinal perforation treated with oxaliplatin-assisted chemotherapy, using sequence analysis.[25] Positive results occurred in patients with phase II and III colon cancer, suggesting that clinicians could use *SELErs3917412* as a predictor for intestinal cancer. Although some studies found that the average level of selectin expression was significantly higher in CRC patients than in healthy subjects, E-selectin expression levels decreased in a stepwise manner in patients with liver metastasis, patients with lymph node metastasis, and patients without metastasis.[10] The current study analyzed transcriptome profiling and clinically relevant data for CRC from TCGA database, which provided more reliable results compared with the above small-sample study.

The possible signaling pathways underlying these mechanisms are not clear. Some studies suggested that the interaction between HT29 colon cancer cells expressing *DR3* and *SELE* induced and activated the phosphoinositide 3-kinase (PI3K)/Akt pathway,[26] which is the mechanism of activation of colon cancer cells. *DR3* stimulation increased the viability of colon cancer cells by activating the PI3K/nuclear factor-kappa B pathway. *SELE* gene expression was closely related to tumor-related circulating endothelial cells in 55 healthy donors and 81 metastatic tumors[27] (including colon and rectal cancers). Our results also indicated that *SELE* might be a biomarker of poor prognosis and survival in patients with

CRC. The mechanism may involve Toll-like receptor,[28] JAK-STAT,[29] and transforming growth factor-β pathway pathways,[30] pathways in cancer,[31] and Nod-like receptor signaling[28] and other signaling pathways related to CRC.[32] In addition, through the GEPIA database, our results confirmed that *SELE* expression was significantly correlated with the expression of other CRC-related genes, further supporting the scientific value of *SELE* gene expression in CRC.

Although our study showed encouraging results, there were some limitations. The study was mainly based on bioinformatics methods and immunohistochemical validation in a small cohort. More external verification is therefore required before clinical application. In addition, further studies are needed to clarify the detailed mechanism of *SELE* in CRC.

In summary, our study confirmed the importance of *SELE* and *SELE*-related genes in the occurrence and development of CRC, thus laying the foundation for further research into the mechanism of *SELE* in CRC.

Declaration of conflicting interest

The authors declare no conflict of interest.

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