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Original Article

A Comprehensive Prognostic Analysis of Slit2 in Pan-Cancer

Dongliang Guo[1], Huihui Wu[1], Sumin Zhang[2], Tianyi Li[1], Xiangyu Liu[1], Kang Ding[2], Yuanyuan Ge[2]

[1]Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China

[2]Deparment of Colorectal Surgery, Nanjing Hospital of Chinese Medicine Affiliated to Nanjing University of Chinese Medicine, Nanjing, Jiangsu, China

[*]Corresponding Author: Dr. Yuanyuan Ge

Abstract:

Background: Previous studies have partly explored the role of axon guidance protein family member slit guidance ligand 2(*Slit2*) in tumorigenesis and development. However,the expression of *Slit2* in pan-cancer still unclear.In this paper, we firstly study the expression of *Slit2* in pan-cancer and the performance of prognosis, clinical features, immunity, etc.

Methods: Data were obtained from the Cancer Genome Atlas (TCGA), the Genotype-Tissue Expression (GTEx) databases, and the University of California Santa Cruz (UCSC) Xena.We analyzed the expression of the *Slit2* gene in 33 tumors and adjacent normal tissues, using TCGA_GTEx samples, TCGA samples, and paired samples from the TCGA database.Next, we employed the Kaplan–Meier method, including univariate Cox regression analysis and Kaplan–Meier curves to identify 33 tumors in which *Slit2* gene expression correlates with prognosis, as per clinical analysis.For cancers like bladder urothelial carcinoma (BLCA) and colon adenocarcinoma (COAD), we constructed nomogram models to validate the prognostic significance of *Slit2* in cancer.Subsequently, we analyzed the association of *Slit2* with immune cell infiltration.Furthermore, we utilized the receiver operating characteristic (ROC) curve to assess the diagnostic value of the *Slit2* gene.Participated in and conducted functional enrichment analysis to investigate potential signaling pathways associated with *Slit2*.

Results: Our study reveals that *Slit2* is differentially expressed in various tumors and correlates with clinical outcomes and the immune microenvironment.The result suggests that *Slit2* may influence the tumor development and progression through diverse pathways.

Conclusion: In summary, *Slit2* may be a potential prognostic biomarker in pan- cancer.

Keywords: *Slit*, pan-cancer, prognostic analysis, TCGA,gene expression

1. Introduction

Nowadays, cancer is ranked as the second most common cause of death after cardiovascular diseases[1]. Despite remarkable progress in the treatment of certain tumors, most patients still have a dismal prognosis. The continuous development and application of pan-cancer biomarkers have the potential to transform treatment options, especially for tumors lacking effective targeted therapies. Genes play a crucial role in how cancer affects humans [2]. It is an excellent target to be the new focus of treatment.

Slit proteins, belonging to the axon guidance molecule family, and their Roundabout (Robo) transmembrane receptors, are essential for cellular migration. The Slit protein family, consisting of Slit1, Slit2, and Slit3, regulates various cellular processes including axon guidance, with Slit2 also serving as a secreted component of the extracellular matrix.[3]

Slit2 is also detected in other tissues, such as tumors and lymphoid organs[4][5]. To our knowledge, there is currently no pan-cancer analysis of Slit2. It is necessary to explore the role of Slit2 in pan-cancer. Previous studies indicated that Slit2 inhibits cell migration to prevent tumor growth through numerous different mechanisms such as the Robo1 pathway [6–8]. Our study aims to investigate the role of Slit2 in pan-cancer and its relationship with the immune microenvironment, and to preliminarily explore the mechanism of action of Slit2 in tumors.

In this project, we utilized specific data to compare the expression of Slit2 in different tumors with that in normal tissues. Our results demonstrated that Slit2 was differentially expressed in tumors, and Survival analysis revealed that high expression of Slit2 had poor prognoses in some cancers except acute myeloid leukemia (LAML). Immune infiltration analysis suggested a correlation between Slit2 and various immune cells. In addition, enrichment analysis suggested that Slit2 might be involved in the development of tumors. Collectively, Slit2 can influence the prognosis and immune microenvironment, and provide a novel biomarker and a potential therapeutic target.[9,10]

Materials and Methods

Data Collection

We procured the gene expression profiles and clinical data of 33 tumors from The Cancer Genome Atlas (TCGA, https://portal. gdc.cancer.gov/).We also obtained the TCGA-GTEx dataset, which includes TCGA and normal samples, from the University of California Santa Cruz (UCSC) Xena(https://xenabrowser.net/datapages/).Immuno histochemical images of both normal human tissues and tumor tissues were sourced from the Human Protein Atlas (HPA, https://www.proteinatlas.org/). Additionally, we used the Gene Expression Profiling Interactive Analysis database (GEPIA2, http://gepia2.cancer-pku.cn/) to identify the 100 most relevant genes associated with *Slit2* in the TCGA datasets.It is important to note that this study adhered to the established guidelines of TCGA and UCSC, thereby exempting the need for ethical approval and informed consent from the patients.

Expression Analysis of *Slit2*

The mRNA expression levels of *Slit2* in normal and tumor tissues were respectively assessed in TCGA-GTEx samples, TCGA samples, and TCGA paired samples. Furthermore, we examined the protein abundance of *Slit2* in normal and tumor tissues using the HPA database.

Prognosis Analysis

We used Kaplan-Meier analysis and the log-rank test to assess the relationship between *Slit2* expression and clinical outcomes, including overall survival (OS), progression-free interval (PFI), and disease-specific survival (DSS) in various cancer types within the TCGA cohort.Following this, we depicted survival curves that demonstrated a significance level of $p < 0.05$.Additionally, for tumors where *Slit2* expression influenced prognosis, we generated receiver operating characteristic (ROC) curves.

Analysis of *Slit2* Expression and Clinical Features

The association between *Slit2* expression and several critical clinical parameters, such as T stage, N stage, and pathologic stage, was explored in malignancies where *Slit2* exerts an influence on prognosis.

Nomogram Models Development and Evaluation

In our current investigation, univariate Cox regression analysis for overall OS was conducted in tumors demonstrating a prognostic impact of *Slit2*, encompassing OS, PFI, and DSS. We selected tumors with a p-value of less than 0.05 and a sample size over 400 to establish respective nomogram models.This method was effective and efficient in forecasting individual patient OS.Following this, we used calibration curves to assess the accuracy of the nomogram predictions over 1-year, 3-year, and 5-year intervals.

Analysis of Immune Infiltration

In our thorough analysis of tumors in the TCGA database, we employed the Tumor Immune Estimation Resource 2.0 (TIMER2.0, http://timer.cistrome.org/) to examine the relationship between *Slit2* expression and various immune cell subtypes, including B cells, macrophages, CD4+ T cells, and CD8+ T cells, which are vital in tumor occurrence and development.

Functional Enrichment Analysis and Protein-

Protein Interaction Network Analysis

The top 100 genes related to *Slit2* with the most similar expression patterns were obtained from the GEPIA2 database. Gene ontology (GO) analysis, encompassing biological pathways (BP), cellular components (CC), and molecular functions (MF), as well as Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, were conducted on the *Slit2*-related genes to further elucidate the potential functions of *Slit2*. Additionally, a protein-protein interaction (PPI) network was constructed using the 100 *Slit2*-related genes in the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://cn.string-db.org/), with a minimum required interaction threshold of 0.4.

Gene Set Enrichment Analysis and Differential Expression Analysis

In our investigation, the R package clusterProfiler (4.2.1) was employed to carry out Gene Set Enrichment Analysis (GSEA) on the *Slit2* gene in tumors with varying levels of expression levels [11].The adjusted p-value (<0.05), normalized enrichment score (|NES| > 1), and false discovery rate (FDR) q value (<0.25) were utilized to discern differences in functional enrichment between the two phenotypes.The DESeq R package [12] was utilized to conduct a differential expression analysis of *Slit2* in the context of cancers where its expression may impact prognosis.

Statistical Analysis

To compare the differences between two groups, the study employed the Wilcoxon rank-sum test. We calculated the correlation between two groups using the Spearman rank test.For identifying factors that influence prognosis, both univariate and multivariate Cox proportional hazard regression analyses were conducted.

We performed survival analysis using the Kaplan-Meier analysis, along with the log-rank test. All statistical analyses were carried out using R software, version 4.2.1. In this study, a p-value of less than 0.05 was considered statistically significant, with notations *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 indicating increasing levels of significance.

Results

The Expression of *Slit2* in pan-cancer

We conducted an integrated analysis of Slit2 expression across 33 cancer types using TCGA-GTEx and TCGA datasets. Slit2 exhibited differential expression patterns, with some cancers showing elevated expression and others decreased levels (Figure 1a). . This observation largely consistent with the results obtained from the TCGA dataset (Figure 1b). Additionally, we investigated *Slit2* expression in 22 tumor types with paired samples from the TCGA database (Figure 1c).

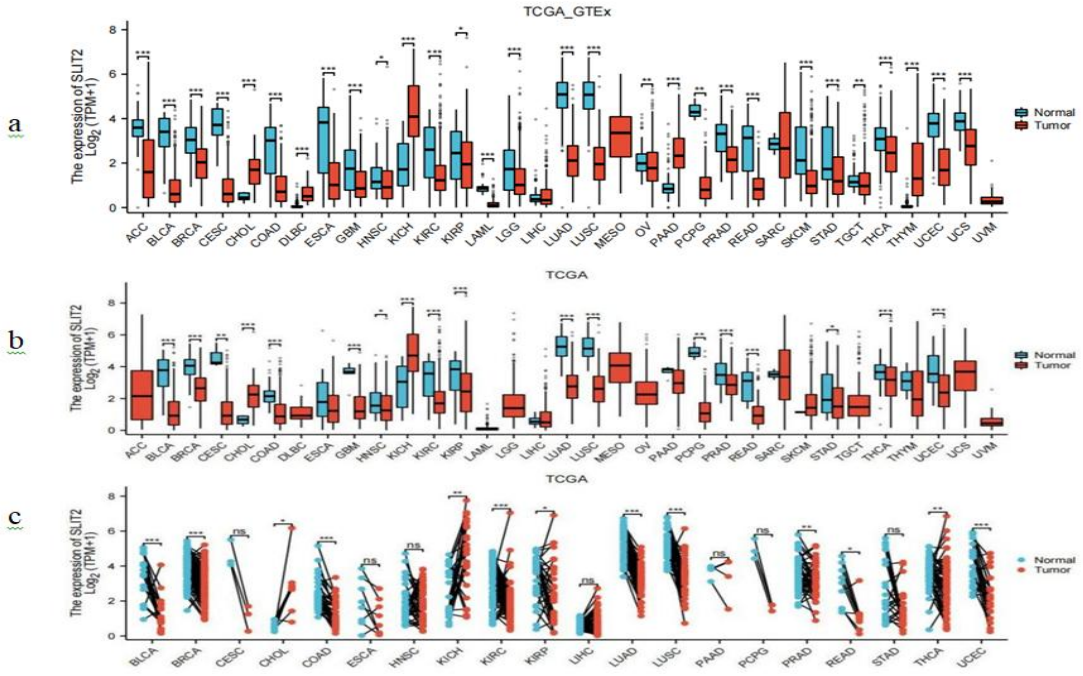
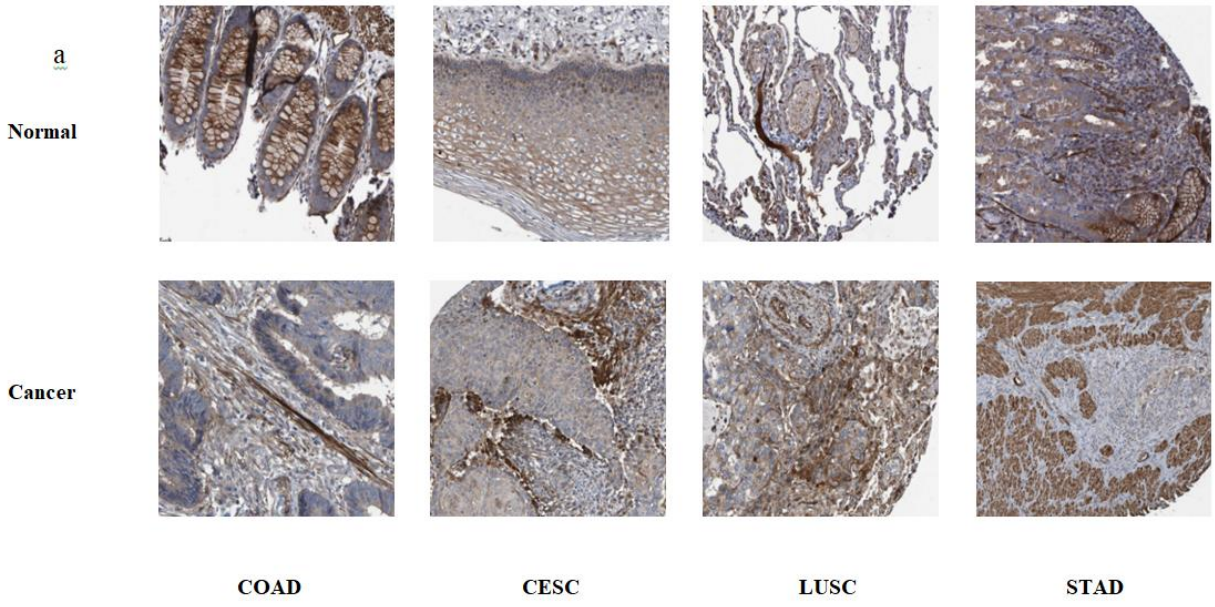


Fig 1 The mRNA expression of *Slit2* in pan-cancer was investigated. (a) The TCGA_GTEx samples

were utilized to analyze the mRNA expression of *Slit2* across 33 tumor types.(b) Investigating the mRNA expression pattern of *Slit2* across 33 tumor types within the TCGA database. (c) The expression of *Slit2* of 20 paired tumor samples from the TCGA database.ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical and endocervical cancers; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma. (ns, p > 0.05; *p < 0.05; **p < 0.01; ***p < 0.001).

We conducted a detailed analysis of *Slit2*'s RNA expression in normal and tumor tissues across multiple human organs, utilizing the Human Protein Atlas (HPA). We selected representative immunohistochemical (IHC) images to illustrate the differential expression in colon, cervix, lung, and stomach tissues (Figure 2a).

And we did some antibodies against slit2 in colon cancer, and it showed that there was significantly more slit2 expression in normal tissues adjacent to cancer than in cancer(Figure 2b).



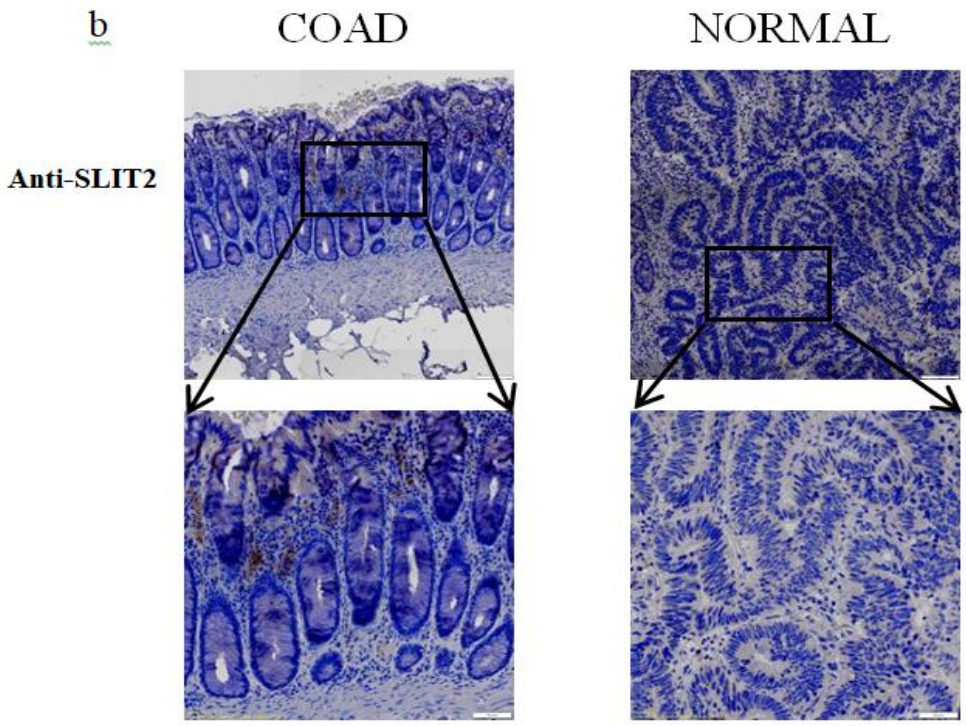


Figure 2 The IHC images depicting the expression of *Slit2* in normal and tumor tissues were obtained from HPA.(a) Representative images of SLIT2 immunohistochemical staining in paracancer non-tumor tissue and paracancer colon cancer tissue. Long scale 100μm, short scale 50 μm.

The association between *Slit2* expression and prognosis in pan-cancer

To evaluate the prognostic value of *Slit2* across various cancers, we conducted a Kaplan-Meier survival analysis to explore how *Slit2* expression correlates with clinical outcomes.Initially, we investigated expression of *Slit2* and its association with overall survival (OS) across 33 types of cancer (Figure 3a). And the result revealed a significant relation of *Slit2* expression with the OS in ACC (Figure 3b), BLCA (Figure 3c),LAML (Figure 3d),OV(Figure 3e) and SARC(Figure 3f).

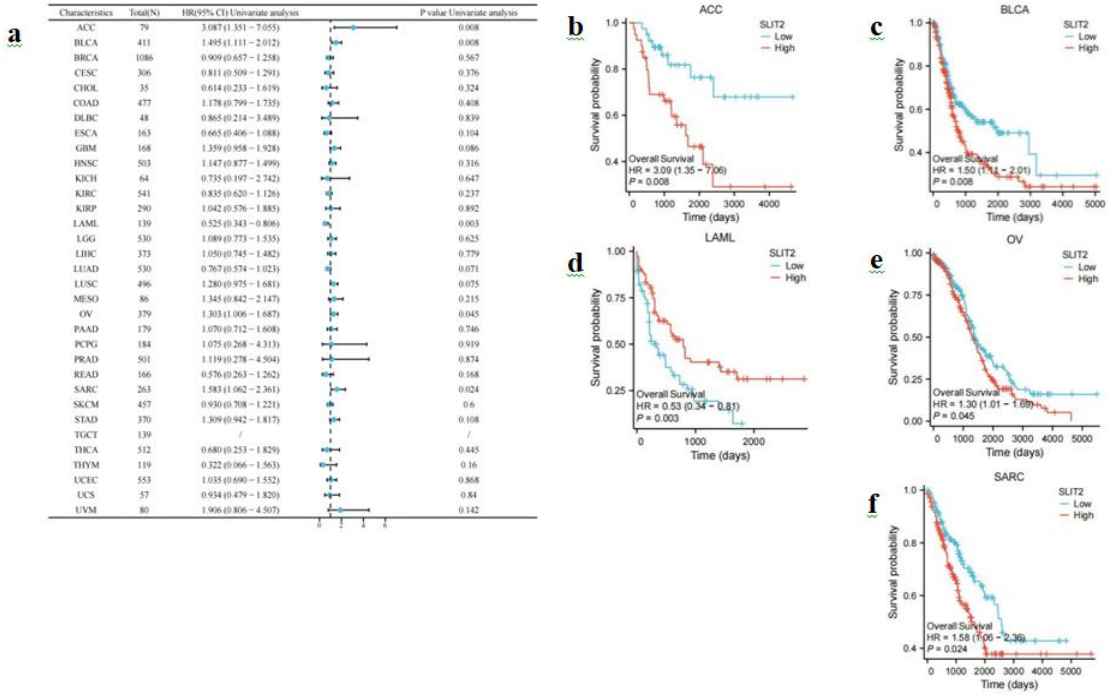


Figure 3 The association between the *Slit2* gene and OS in pan-cancer was investigated. (a) The impact of *Slit2* expression on OS in pan-cancer was demonstrated using a forest plot. (b–f) The effects of *Slit2* expression on OS were examined in ACC, BLCA, LAML, OV, and SARC.

Subsequently, we examined the connection between *Slit2* expression and disease-specific survival (DSS) (Figure 4a). The data revealed a significant relation of *Slit2* expression with the DSS in ACC (Figure 4b) and BLCA (Figure 4c).

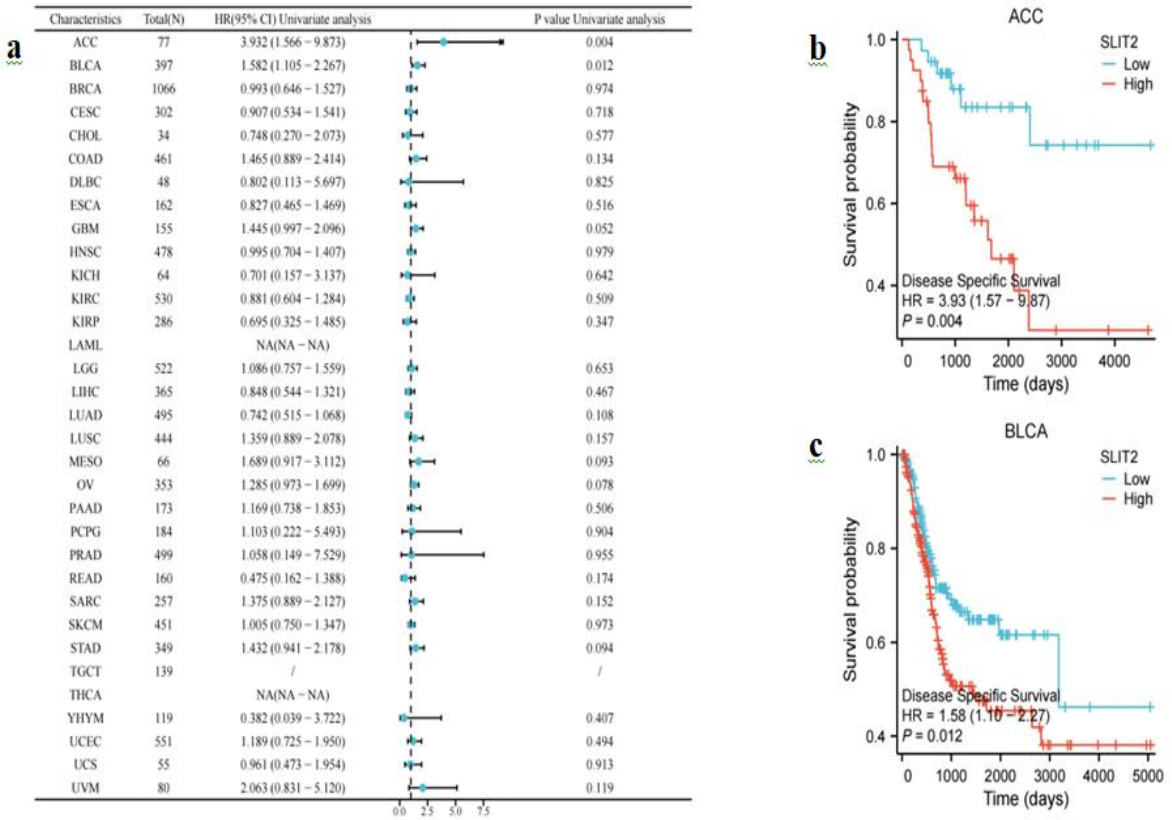


Figure 4 The association between the *Slit2* gene and DSS in pan-cancer was investigated. (a) A forest plot was utilized to demonstrate the impact of *Slit2* expression on DSS in pan-cancer. (b–c) The influence of *Slit2* expression on DSS was separately examined in ACC and BLCA.

In both ACC and BLCA, high *Slit2* expression was linked to poorer DSS outcomes.Finally, we evaluated the relationship between *Slit2* expression and progression-free interval (PFI) (Figure 5a). The results revealed that *Slit2* expression correlates with PFI in adrenocortical carcinoma(ACC) (Figure 5b), BLCA (Figure 5c), COAD (Figure 5d), and lung squamous cell carcinoma(LUSC) (Figure 5e), with higher *Slit2* expression indicating poorer PFI in these cancers.

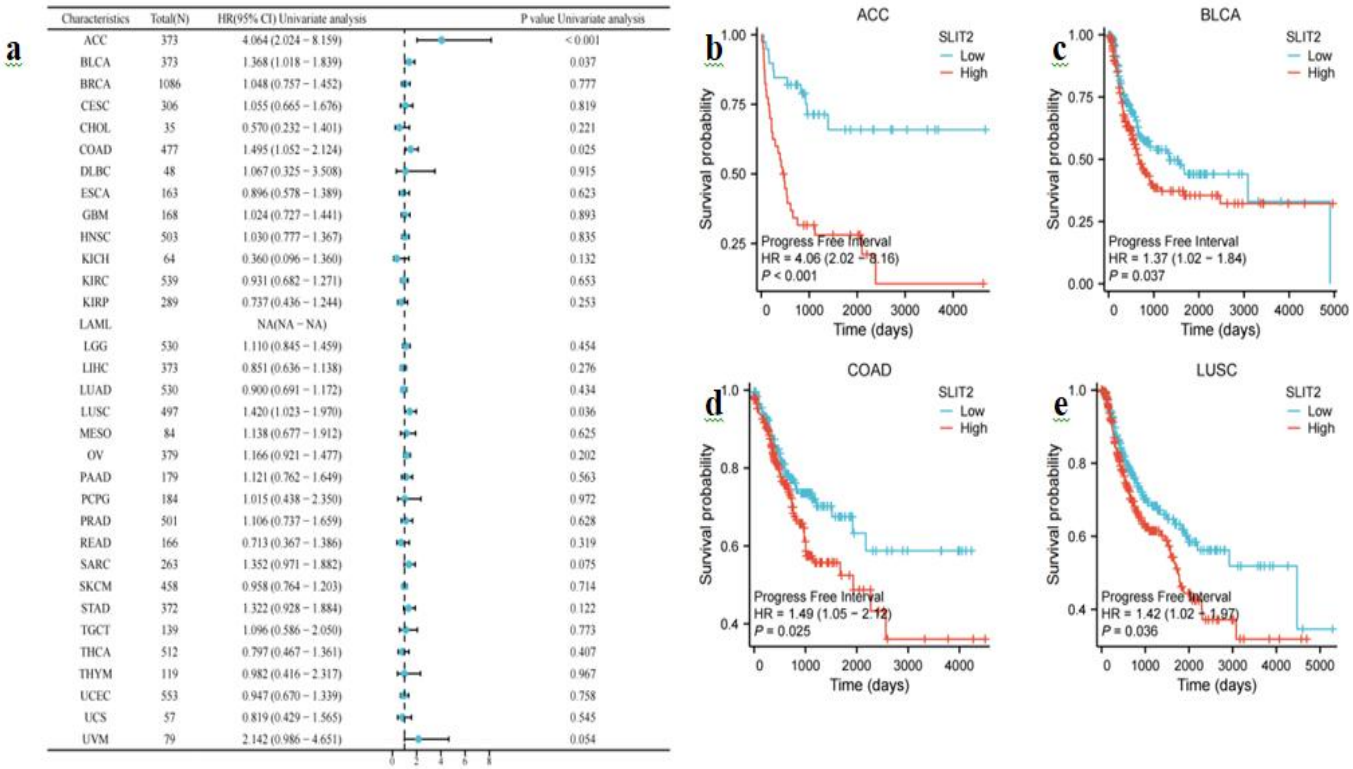


Figure 5 The correlation between *Slit2* gene and PFI in pan-cancer. (a) The effects of *Slit2* expression on PFI in pan-cancer were exhibited through a forest plot. (b–e) Effects of *Slit2* respectively expression on PFI in ACC,BLCA,COAD and LUSC.

Furthermore, the study showcased ROC curves for four tumors associated with *Slit2* expression(Figure 6), illustrating diagnostic potential of *Slit2*.

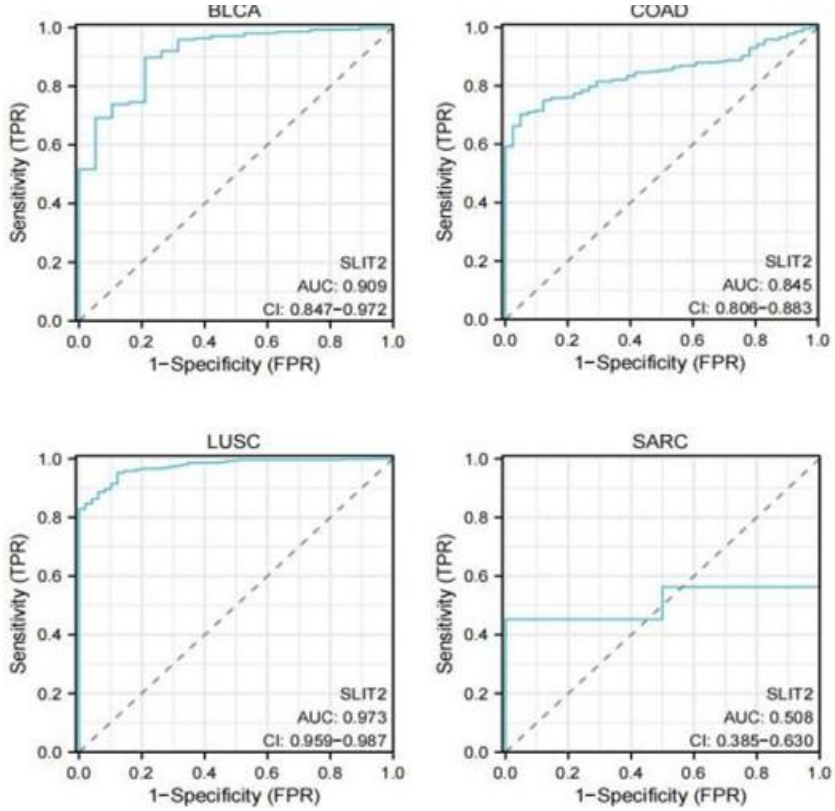


Figure 6 ROC curves of BLCA, COAD, LUSC and SARC in TCGA database.

Correlations Between *Slit2* Expression and Clinical Parameters

In a comprehensive analysis of 33 distinct tumor types within the TCGA database, a significant association between Slit2 expression and patient prognosis was identified in six specific malignancies: Adrenocortical carcinoma (ACC), Bladder urothelial carcinoma (BLCA), Colon adenocarcinoma (COAD), Acute myeloid leukemia (AML), Lung squamous cell carcinoma (LUSC), and Ovarian serous cystadenocarcinoma (OV). This investigation elucidated the correlation between Slit2 expression levels and various clinicopathological parameters in these neoplasms, with a particular emphasis on the pathological staging in ACC and BLCA (Figures 7a-b). Furthermore, the study demonstrated a significant correlation between Slit2 expression and tumor dimensions in BLCA and COAD (Figures 7c-d), as well as its association with lymph node metastasis in these two tumor types (Figures 7e-h).

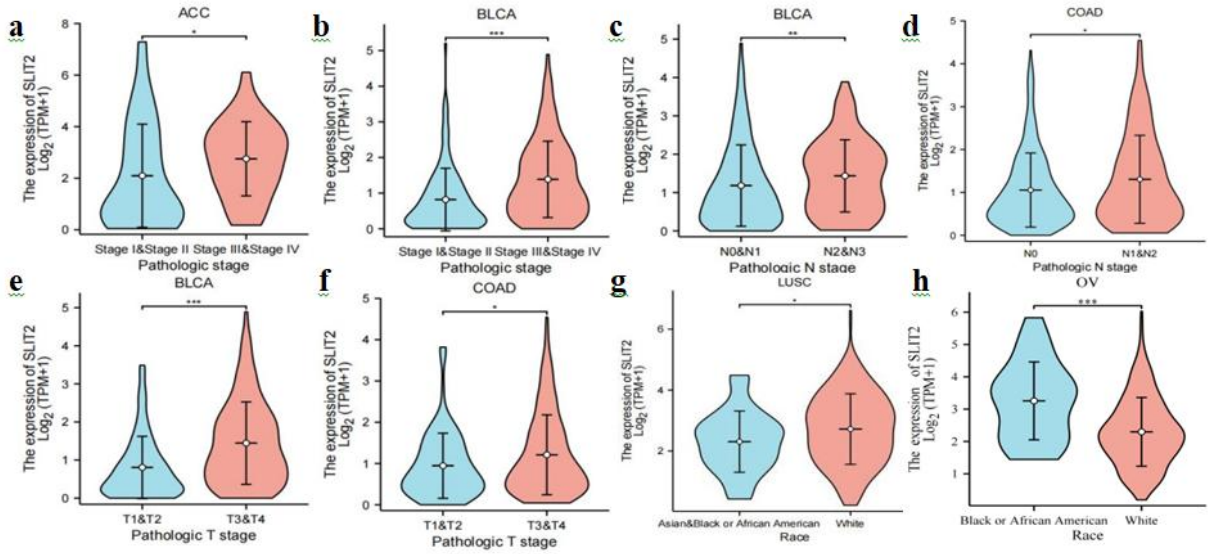


Figure 7 Correlation of *Slit2* expression with clinicopathological parameters. (a–b) *Slit2* expression correlated with the pathologic stage in ACC and BLCA.(c-d) *Slit2* expression correlated with the N stage in BLCA and COAD. (e-f) *Slit2* expression associated with T stage in BLCA and COAD. (g-h) *Slit2* expression associated with race in LUSC and OV. (*p < 0.05; **p < 0.01; ***p < 0.001).(*p < 0.05; **p < 0.01; ***p < 0.001).

Construction and Evaluation of Nomogram Models in Bladder Urothelial Carcinoma and Colon Carcinoma

To delve deeper into the prognostic significance of Slit2 in a spectrum of malignancies, a univariate Cox proportional hazards regression analysis was conducted to evaluate overall survival (OS) in the six tumor types influenced by Slit2 expression, as detailed in Supplementary Tables S1–S7. This analysis served as a preliminary step to identify tumors with significant prognostic associations.Subsequent to the univariate analysis, Bladder urothelial carcinoma (BLCA) and Colon adenocarcinoma (COAD), each with a sample size exceeding 400, were selected for nomogram development. This selection was based on their potential to validate the prognostic values of Slit2, notwithstanding the fact that COAD's association did not achieve statistical significance across all datasets.To rigorously evaluate the predictive accuracy of the nomogram models, calibration curves were employed to assess their performance at 1, 3, and 5-year intervals. The results indicated that Slit2 was a significant prognostic factor and demonstrated a robust predictive capacity for overall survival in both BLCA (Figure 8a) and COAD (Figure 8c). Furthermore, the calibration curves for the 1, 3, and 5-year survival predictions substantiated the high precision of these models in forecasting OS, as evidenced by the close alignment of the observed and predicted survival rates (Figures 8b,d).

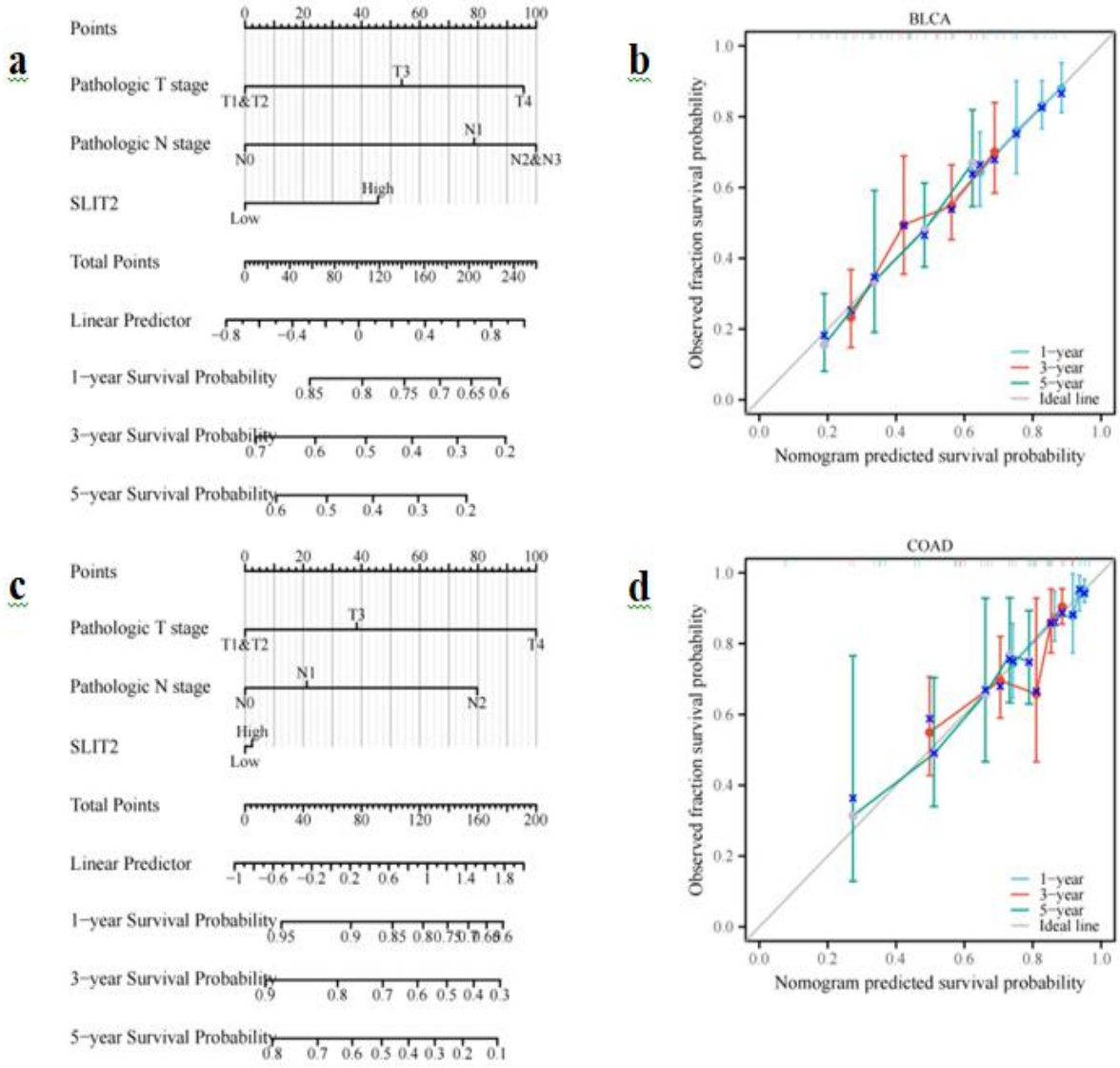


Figure 8 Nomogram models were developed and evaluated for both BLCA and COAD. (a)Development of a nomogram model that includes *Slit2* expression for BLCA. (b)Calibration curves for 1-year, 3-year, and 5-year periods were used to assess the BLCA nomogrammodel.(c)Constructing a nomogram model with *Slit2*1 expression for COAD. (d) Calibration curves for 1-year, 3-year, and 5-year intervals assessed the predictive accuracy of the COAD nomogram model.

Slit2 Expression and Correlation with the Tumor Immune Microenvironment

The immune microenvironment plays a vital role in tumor development and occurrence.To investigate the relationship between *Slit2* and the immune microenvironment in pan-cancer, we analyzed the correlation of *Slit2* expression with immune cells using the GEPIA2 database.Heatmaps showing the correlation of *Slit2* expression with B cells (Figure 9a) , CD4+ T cells (Figure 9b), CD8+ T cells (Figure 9c) macrophages (Figure 9d) and NK cells (Figure 9E)were presented.

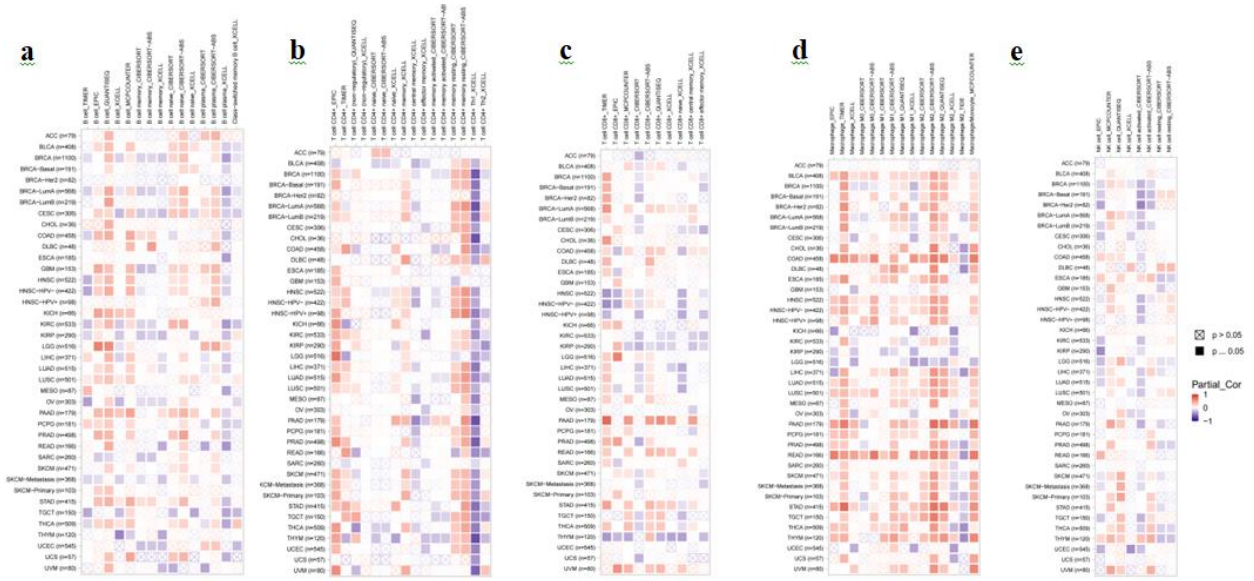


Figure 9 Correlation between *Slit2* expression and immune cell infiltration.(a–e) Heatmaps showed the correlations between *Slit2* expression and B cells, T cell CD4+, T cell CD8+, macrophages, and NK cells in the TIMER2 database.

Functional enrichment analysis and protein-protein interaction analysis of *Slit2*-related genes

To elucidate the biological role of Slit2 in oncogenesis, we utilized the GEPIA2 database to identify the top 100 genes that are co-expressed with Slit2 (as detailed in Supplementary Table S7). Gene Ontology (GO) analysis (Figure 10a) revealed that these Slit2-correlated genes are implicated in a plethora of biological processes, including but not limited to "Rho protein signal transduction," "regulation of Rho protein signal transduction," "fibroblast proliferation," and "regulation of intracellular pH."Moreover, these genes were found to be associated with specific cellular components such as the "proton-transporting V-type ATPase complex," "collagen-containing extracellular matrix," and "endoplasmic reticulum lumen." In terms of molecular functions, these genes are involved in "extracellular matrix structural constituent," "growth factor binding," "collagen binding," and "conferring tensile strength to the extracellular matrix."Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (Figure 10b) suggested that the genes associated with Slit2 may play a role in several critical pathways, including "Calcium signaling," "PI3K-Akt signaling," "Phagosome," "Ras signaling," "Oxidative phosphorylation," and "EGFR tyrosine kinase inhibitor resistance."To further explore the interactions among these Slit2-related genes, a protein-protein interaction (PPI) network was constructed using the STRING website, incorporating the 100 identified genes (Supplementary Figure S1).

This network provides a visual representation of the potential functional associations and interactions between these genes, offering insights into the complex molecular networks in which Slit2 may participate, and highlighting potential therapeutic targets for further investigation..

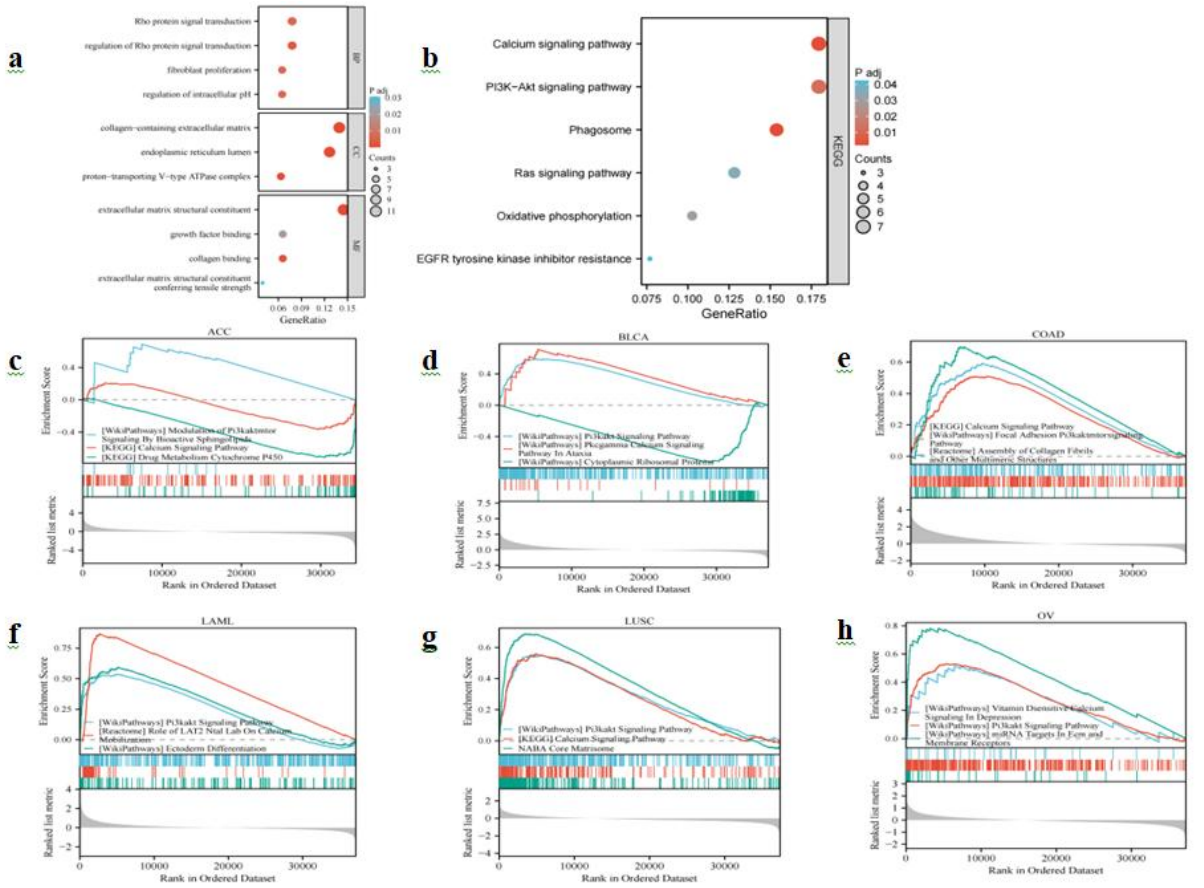


Fig 10 Functional enrichment analysis of *Slit2*-related genes. (a) GO Enrichment Analysis of 100 *Slit2*-Related Genes, Including BP, CC, and MF. (b) KEGG Pathways Analysis of 100 *Slit2*-Related Genes. (c–h) GSEA was performed based on the differential expression analysis conducted in ACC, BLCA, COAD, LAML, LUSC, and OV.

Gene Set Enrichment Analysis

To delineate the functional role of Slit2 in oncogenesis, Gene Set Enrichment Analysis (GSEA) was performed on differentially expressed Slit2 transcripts to elucidate its biological significance in six tumor types where Slit2 expression is prognostic. The tumors under investigation include Adrenocortical Carcinoma (ACC, Figure 10c), Bladder Urothelial Carcinoma (BLCA, Figure 10d), Colon Adenocarcinoma (COAD, Figure 10e), Acute Myeloid Leukemia (LAML, Figure 10f), Lung Squamous Cell Carcinoma (LUSC, Figure 10g), and Ovarian Serous Cystadenocarcinoma (OV, Figure 10h). The GSEA findings underscore a predominant association of Slit2 with the Calcium signaling and PI3K-AKT signaling pathways, highlighting potential therapeutic targets and pathways for further exploration in these malignancies..

Discussion

Tumors pose a serious threat to human health. Cancer is ranked as the second leading cause of death after cardiovascular diseases[13]. Considerable progress has been made in cancer treatment; however, the prognosis for most patients remains poor. Cancer metastasis, dissemination, and relapse are crucial determinants of prognosis. Nevertheless, the underlying mechanisms of malignancy remain unknown[14,15]. Here, we investigate the relationship between Slit2 and pan-cancer for the first time. Recent studies suggest that Slit2 may be associated with some cancers[16–19]. But, there is a dearth of research on Slit2 in pan-cancer. As a consequence, the expression status of Slit2 across various cancers remains unclear.

In this article, we propose conducting pan-cancer expression and prognosis analyses, as well as analyzing immune relationships and gene infiltration in tumors, and we reveal that Slit2 can influence the prognosis of certain tumors and is correlated with various immune cells. Additionally, enrichment analysis indicates that

Slit2 may influence tumor progression through different pathways.

We analyzed the expression differences of Slit2 between normal and tumor tissues from various organs using TCGA_GTEx, TCGA, and paired samples from the TCGA database. Overall, the three datasets generally yielded consistent conclusions that Slit2 had significant differences in expression between cancer tissues and normal tissues. However, there were also some inconsistent or opposite results, likely due to variations in the sample size of the control group. We analyzed the expression and prognosis of bladder uroepithelial cancers related to the Slit2 gene. While some studies support our results, others remain controversial. Consistent with our findings, a previous study showed that Slit2 expression in bladder uroepithelial cancers is lower than that in adjacent samples[20]. Our predicted prognosis also demonstrated that Slit2 has a good ability to predict survival time. The tumor suppressor gene Slit2 has been extensively studied in COAD [21,22]. However, our prognostic results contradict previous findings as we observed that high expression of Slit2 was associated with a poor prognosis. Interestingly, there are also studies supporting our observations, indicating that elevated levels of Slit2 expression are linked to a bad prognosis[23]. Furthermore, Slit2 expression in colorectal cancer increases with the pathological stage, which is consistent with our result[24]. And in LAML, our study found a significant improvement in OS prognosis with high expression of Slit2, which is in line with some studies[25–28]. Based on all results, we speculate that the influence on cancer OS, PFI, and DSS depends on the expression level of Slit2. And our results showed the correlations between Slit2 expression and B cells, T cell CD4+, T cell CD8+, macrophages, and NK cells. This suggests that Slit2 can regulate the immune microenvironment, indicating its potential application in developing targeted drugs for immunotherapy in certain cancers, which could benefit many patients. Our study revealed a significant relationship between Slit2 and the N stage of certain tumors, where tumors with higher Slit2 expression had more lymphatic lesions. Our enrichment analysis items were related to “Calcium signaling pathway”, “PI3K-Akt signaling pathway”, “Phagosome”, “Ras signaling pathway”, “Oxidative phosphorylation”, and “EGFR tyrosine kinase inhibitor resistance”. This suggests that Slit2 may be an important target for prognosis markers in certain unspecified tumors(29).

The following are the advantages of this study. Firstly, we studied the role and mechanism of Slit2 in pan-cancer for the first time, focusing on expression differences, clinical correlations, survival analysis, immune infiltration, and enrichment analysis, thereby deepening our understanding of Slit2. Secondly, based on survival analysis, we chose BLCA and COAD, representative cancers with large sample sizes, to further demonstrate the impact on tumor prognosis, enhancing the reliability of our conclusions. Thirdly, evaluating tumor prognosis using Slit2 combined with immune cells suggested that the function of immune cells might depend on Slit2 expression, further clarifying their relationship to tumor prognosis. Finally, this study suggests that Slit2 may influence tumor development via PI3K-AKT and Calcium signaling pathways, offering directions for future mechanistic research. Mechanistically, it may affect the initiation and development of tumors through PI3K-AKT signaling and Calcium signaling pathways, but the specific roles and mechanisms still need further experimental verification.

Most members of the Slit family have been detected in various tumors in recent years, but the specific role of Slit2 in the progression of pan-cancers remains unclear.Our research suggests that Slit2 may have dual effects on the proliferation of malignant cells, with both beneficial and detrimental outcomes.(30).In various cancers, whether *Slit2* inhibits or promotes cancer development is associated with factors like tumor metastasis(23,31,32).Our pan-cancer data on *Slit2* yielded conclusions both consistent and divergent from previous studies; thus, further research into its signaling pathway expression is required(33,34).While we made every effort to ensure the accuracy and reliability of our research results, certain limitations were unavoidable. For instance, some cancers had a small sample size or the control group was insufficient, leading to inconclusive findings. Therefore, it is imperative to increase the sample size of both groups for more precise conclusions. Additionally, this study primarily focuses on

bioinformatics analysis, and the conclusions drawn must be validated through further in vivo and in vitro experiments. In addition,our study was based on the clinical database,but most of the previous research are vitro experiment,which may cause the different results. Further investigation into the intricate interaction mechanism among genes is necessary to better understand the relationship between Slit2 and other genes.

In conclusion, our systematic analysis offers new insights into Slit2's expression, prognostic value, and relationship with immune cell infiltration across various cancers. These findings underscore the complex role of Slit2 in cancer and highlight the need for further research to validate these observations and explore the mechanisms underlying these relationships.

Data Availability Statement

This study analyzed publicly available datasets, which can be found in the TCGA database and the UCSC database.

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Supplementary Material