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Original Article

Immunological role and prognostic potential of CLEC10A in pan-cancer

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Abstract: Objectives: CLEC10A is expressed in a variety of cells, involved in a variety of biological pathways including immune response, and is closely related to the development of tumor immune response. However, the role of CLEC10A from a pan-cancer perspective has not yet been analyzed, and its role in human cancer prognosis and immunology remains largely unclear. Methods: We studied the expression levels of CLEC10A and investigated its prognostic value in various cancers across distinct datasets including Oncomine, cBioPortal, and TCGA, and conducted immunohistochemical experiments using fresh bladder cancer and breast cancer samples to verify the results. In addition, we also performed GSEA of CLEC10A and explored the relationship between CLEC10A expression and immune infiltration, immune checkpoints, immune activation genes, immunosuppressive genes, chemokines and chemokine receptors. Results: The results showed that the expression level of CLEC10A in most tumors was significantly lower compared with non-cancerous tissue, and the decreased expression was related to poor prognosis and more advanced cancer stages. We also found that the expression of CLEC10A was significantly related to the immunomodulatory interaction between lymph and non-lymphocytes. Furthermore, the expression of CLEC10A was not only significantly correlated with the level of infiltration of CD4+T cells and CD8+T cells, but also closely related to immune checkpoints, immune activation genes, immunosuppressive genes, chemokines, and chemokine receptors. Importantly, our analysis of the relationship between CLEC10A and TMB and MSI also confirmed the speculation that CLEC10A may influence antitumor immunity by regulating the composition and immune mechanisms of the tumor microenvironment. Conclusions: In conclusion, CLEC10A may serve as a new target for tumor immunotherapy and has great potential as a molecular biomarker for predicting pan-cancer prognosis and immune infiltration.

Keywords: CLEC10A, pan-cancer, tumor immunity, tumor microenvironment, prognosis, immune checkpoint inhibitors

Introduction

The World Health Organization statistics stated that cancer is the first or second leading cause of death before the age of 70. Approximately, 19.3 million new cancer cases and nearly 10 million cancer-related deaths occurred worldwide in the year 2020 [1]. Thus, with the global burden of cancer morbidity and mortality increasing rapidly, there is an urgent need for in-depth research to elucidate the mechanisms of cancer development and to identify cancer-related biomarkers for prognosis and treatment. Previously, treatment options were determined based on the location of the tumor, but several studies [2, 3] have demonstrated that although cancers originate in different organs, they do share something in common at the molecular level. The Pan-Cancer Atlas (results based on The Cancer Genome Atlas (TCGA)) contains the data of more than 11,000 tumor patients suffering from 33 of the most prevalent cancer types [4, 5]. Studies of genomic alterations in signaling pathways that control cellular processes, cell death, and cell growth have revealed similarities and differences in these processes across a range of cancers [3], indicating that molecular identification may

help apply specific immunotherapies to several cancers. By integrating major immunogenomic approaches to characterize the immune tumor microenvironment of the 33 cancers analyzed by TCGA, six immune subtypes across multiple tumor types were identified with potential therapeutic and prognostic implications for cancer management, providing further evidence that pan-cancer studies may contribute to the development of combination therapies and individual medical treatment [3].

C-Type Lectin Domain Containing 10A (CLEC10A) is expressed on a number of immune cells including dermal immature peripheral dendritic cells, dendritic cells, and alternatively-activated M2a macrophages [6, 7]. CLEC10A is involved in many biological pathways and processes such as stimulatory C-type lectin receptor signaling pathway, adaptive immune response, endocytosis, innate immune response, and other immune system-related processes [8, 9]. Further, it is also associated with diseases such as Hypotrichosis, Lymphedema, Telangiectasia, and Renal Defect Syndrome. CLEC10A binds to terminal galactose and N-acetylgalactosamine units linked to serine or threonine in a calcium-dependent manner. These sugar moieties are known as Tn-Ag and are expressed in a variety of carcinoma cells. It has been proposed that the expression of Tn structures on tumor cells is accompanied by an increased rate of local recurrence and distant metastasis [10]. CLEC10A can drive Th1, Th17, and CD8+ cytotoxic T lymphocyte (CTL) cellular immune responses by triggering the production of multiple cytokines [11], while tumor cells devise multiple strategies to modulate CLEC10A signaling to suppress effector antitumor immune responses [12, 13].

Immune checkpoint inhibitors (ICIs) therapy for cancer treatment has now been gaining increased attention and has been hailed as the third revolution in cancer therapy. The relationship between CLEC10A and cancer-associated immune response shows great potential in cancer therapy, but the intrinsic mechanism of action of CLEC10A in tumors is not known, and its role in tumor-associated immune responses is also unclear. It has been demonstrated that CLEC10A is associated with ovarian cancer [14] and melanoma [15]; however, there have been no more extensive studies pointing to a potential correlation between CLEC10A and other malignancies.

This is the first comprehensive analysis of CLEC10A expression that studies its association with different types of malignancies using databases such as TCGA, cBioPortal, Oncomine, and TIMER2. CLEC10A expression and its correlation with the prognosis of different types of malignancies was validated using IHC, aiming to explore the potential therapeutic and prognostic value of CLEC10A in pan-cancer studies. Our results provide new insights into the functional role of CLEC10A in pan-cancer, highlighting the potential mechanisms by which CLEC10A affects the tumor microenvironment as well as cancer ICIs therapy.

Methods

Clinical specimens

Paired cancer tissue and normal paracancer tissue samples were collected from 20 patients with bladder cancer and 20 patients with breast cancer from the Cancer Hospital of Guangxi Medical University. All patients were pathologically diagnosed with bladder cancer or breast cancer before tissue collection and had not received chemotherapy or radiotherapy before. We obtained written informed consent from all patients. The study was approved by the Ethics and Anthropology Committee of the Affiliated Tumor Hospital of Guangxi Medical University. All experiments and methods were carried out in accordance with relevant guidelines and regulations.

Data collection

We downloaded relevant data from TCGA [5] and genotypic tissue expression (GTEx) database [16], and RNA expression information and clinical data from the UCSC Xena database [17] (https://xenabrowser.net/datapages/) for more than 1000 tumor samples from 33 human cancers. We also downloaded DNA copy number and methylation data from the cBioPortal database [18] (https://www.cbioportal.org/) and ensured that the acquisition and application methods were consistent with relevant guidelines and regulations. To verify the predictive value of CLEC10A in immunotherapy response, gene expression data and clinical data of IMvigor210 [19], which is an immunotherapy

cohort of uroepithelial carcinoma, were downloaded from the Gene Expression Omnibus (GEO) database.

Immunohistochemical staining

We embedded normal tissue samples next to cancer and tumor tissue samples in formalin. Each tissue was cut to a thickness of 4 mm and fixed on a glass slide. The tissues were left at room temperature for 60 min, dewaxed by immersion in xylene for 10 min, and then immersed in fresh xylene for another 10 min. Subsequently, the endogenous peroxidase activity was blocked by endogenous peroxidase blocking fluid. The sections were then incubated at 4°C overnight with anti-CLEC10A (1:1000 dilution), followed by three consecutive 5-min rinses with PBS buffer solution and another 30-minute incubation with secondary antibodies at 37°C. Finally, the PBS rinse was repeated three more times and the slices were visualized using diaminobenzidine.

Differential expression analysis of CLEC10A

The Oncomine database [20] is currently the world's largest cancer gene chip database and integrated data-mining platform. It has the most complete cancer mutation profile, gene expression data, and related clinical information, and can perform differential expression and co-expression analysis. Through this database, we revealed the differences in CLEC10A mRNA expression levels between tumor tissues of different types of cancer and corresponding normal tissues. SangerBox software was also used to compare the difference between CLEC10A expression in cancer and normal tissues based on TCGA data and the normal sample data from the GTEx database.

Genetic alteration analysis

We used the cBioPortal web (https://www.cbioportal.org/) to query the genetic alteration signatures of CLEC10A. After selecting the "Quick Select" and "TCGA Pan-Cancer Atlas Studies" options, information about alteration frequency, copy number alteration (CNA), and mutation type was described in the "Cancer Types Summary" module of the TCGA database.

Survival analysis

Kaplan-Meier analysis was performed to show the survival of patients in the high and low CLEC10A expression groups and to assess the potential of CLEC10A as a prognostic cancer marker. Survivor, glmnet [21], survivorROC, and survminer were the R packages used in the operations. To explore the correlation between CLEC10A expression and Overall Survival (OS), Disease-free Survival (DFS), Disease-specific Survival (DSS), and Progression-free Survival (PFS) in cancer patients with each cancer type, we performed Cox regression analysis using TCGA in the R environment.

Immune infiltration analysis

The immune infiltration was determined using the TIMER2 database and the CIBERSOFT method. Correlation between CLEC10A expression and cellular abundances was determined using correlation analysis. The stromal score, immune score, and ESTIMATE score for each tumor type sample was calculated using ESTIMATE.

Genome enrichment analysis

We identified the pathways associated with CLEC10A by gene set enrichment analysis (GSEA) using the cluster Profiler R package. Fold changes in mean gene expression between patients with high and low CLEC10A expression were sorted, and the sorted gene list was then represented in the input file. The enriched biological processes were assessed using HALLMARK pathways and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways analyses.

Statistical analysis

Low and high CLEC10A expression groups were defined by transcripts per kilobase of exon model per million mapped reads values. Spearman correlation test was used to explore the association between CLEC10A expression and biomarkers, including microsatellite instability (MSI) and tumor mutation burden (TMB). *P*-values and HRs in survival analysis were calculated using COX regression analysis. *P* values were considered statistically significant at <0.05.

Result

Analysis of CLEC10A expression in pan-cancer

We first evaluated the mRNA expression level of CLEC10A in the pan-cancer data and found

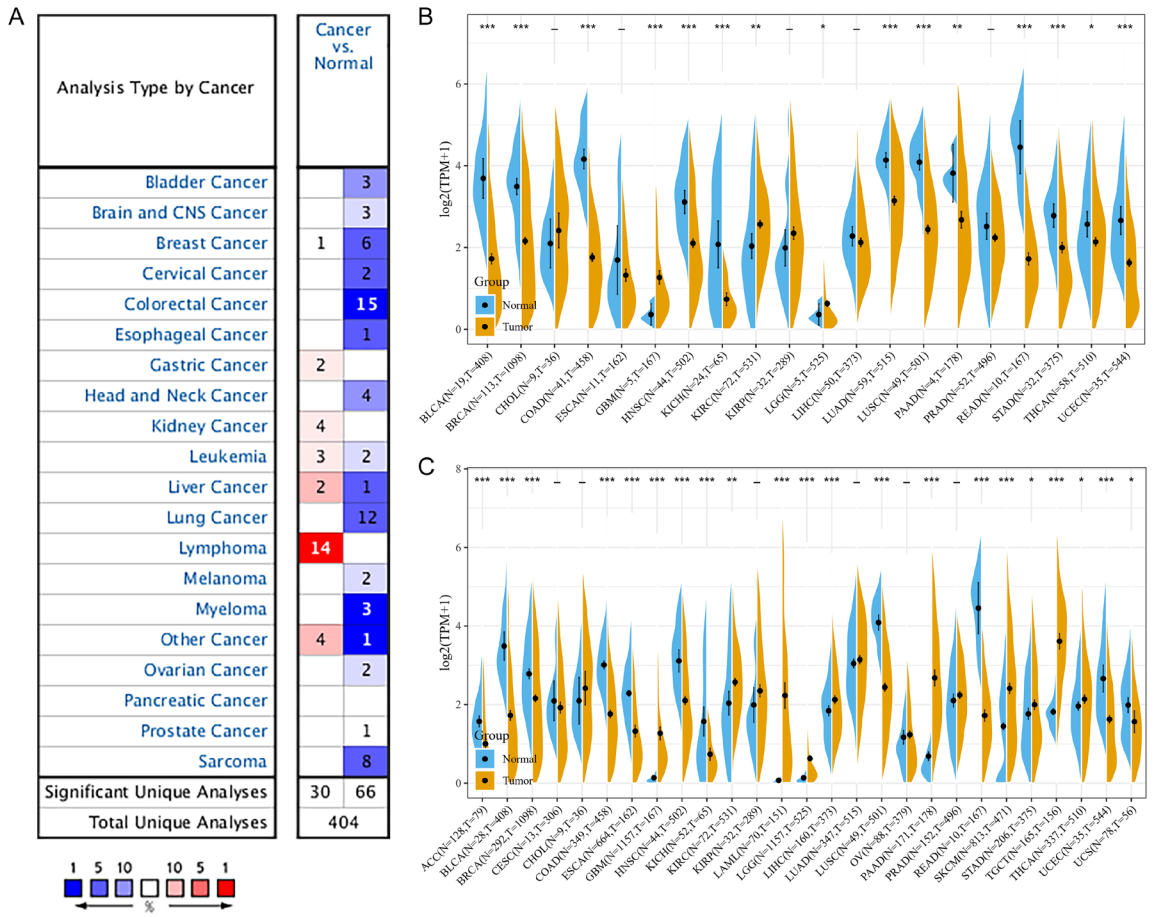

Figure 1. CLEC10A expression levels in human cancers. A. CLEC10A expression in different cancers and paired normal tissues in the Oncomine database. B. CLEC10A expression levels in pan-cancer tissues from TCGA. Tumor tissues are displayed with a yellow spindle and the normal tissues are displayed with a blue spindle (*P<0.05, **P<0.01, and ***P<0.001). C. CLEC10A expression levels in pan-cancer tissues from TCGA and GTEx database. Tumor tissues are displayed with a yellow spindle and the normal tissues are displayed with a blue spindle (*P<0.05, **P<0.01, and ***P<0.001).

that the expression of CLEC10A was significantly different between tumor and normal tissues in human cancers. The Oncomine database showed that compared with the corresponding normal tissues, the mRNA level of CLEC10A was significantly lower in most human cancers, such as bladder cancer, brain and central nervous system cancer, breast cancer, cervical cancer, colorectal cancer, esophageal cancer, head and neck cancer, kidney cancer, liver cancer, lung cancer, ovarian cancer, pancreatic cancer, prostate cancer, leukemia, melanoma, sarcoma, and other cancer types. In contrast, CLEC10A expression was significantly increased in lymphoma (**Figure 1A**). Furthermore, the difference in CLEC10A expression in cancer and adjacent normal tissues was analyzed based on the data from the TCGA database. The results showed that the expressions of CLEC10A in bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), and uterine corpus endometrial carcinoma (UCEC) tissues were significantly lower than those in adjacent normal tissues, but showed a higher level in glioblastoma multiforme (GBM) and kidney renal clear cell carcinoma (KIRC) (**Figure 1B**, all P<0.01). Considering the small number of normal samples in TCGA, we integrated the normal tissue data in the GTEx database and the TCGA tumor tissue

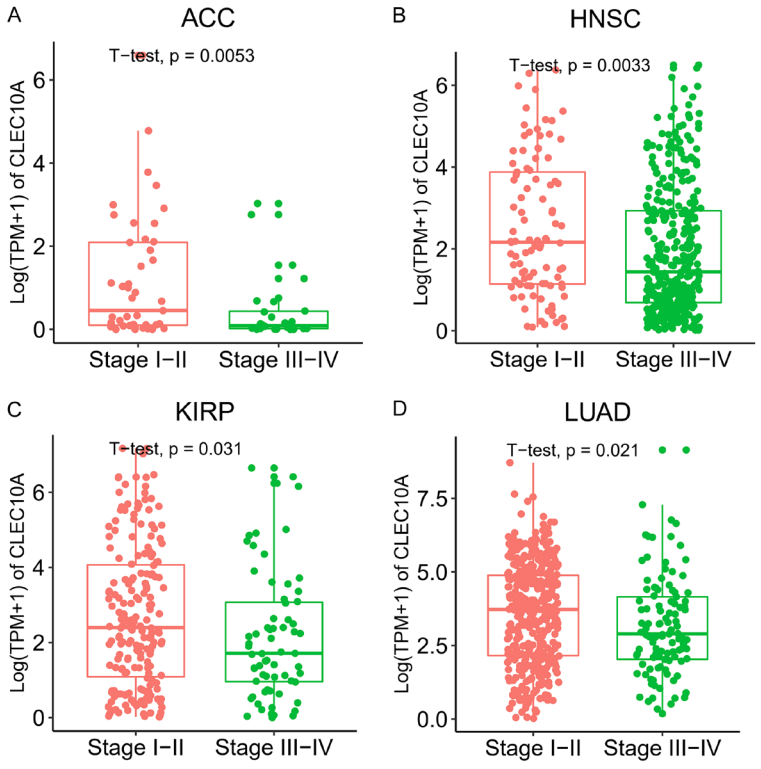

Figure 2. CLEC10A expression in different WHO stages. A-D. Using TCGA data, CLEC10A gene expression was analyzed by the main pathological stage (stage I-II, and stage III-stage IV). Log2 (TPM+1) was used for the log scale. Only tumor types with logarithmic grade P<0.05 are shown here.

data to analyze the expression differences of 27 tumors, and found that CLEC10A was highly expressed in GBM, KIRC, acute myeloid leukemia (LAML), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), skin cutaneous melanoma (SKCM), and testicular germ cell tumors (TGCT) (**Figure 1C**, all P<0.01). The above results indicate that CLEC10A is down-regulated in most tumors, suggesting its protective role in the occurrence and development of tumors.

The relationship between CLEC10A expression and tumor stage

We also used the "Pathological Staging Diagram" module of the World Health Organization to obtain the correlation between CLEC10A expression and cancer pathological staging and found that CLEC10A had low expression in the III-IV phases of ACC, HNSC, kidney renal papillary cell carcinoma (KIRP), and LUAD (**Figure 2A-D**, all P<0.05). Although the expression of CLEC10A in BRCA, COAD, BLCA, cholangiocarcinoma (CHOL), esophageal carcinoma (ESCA), KICH, KIRC, LIHC, LUSC, PAAD, mesothelioma (MESO), READ, SKCM, TGCT, uveal melanoma (UVM), STAD, thyroid carcinoma (THCA), and other tumors in stage III-IV patients exhibited a downward trend, the difference was not statistically significant (Supplementary Figure 1). CLEC10A may be considered a protective factor in tumor staging, as the more advanced stage of cancer was associated with lower expression of CLEC10A.

Genetic changes in CLEC10A expression

To explore the correlation between *CLEC10A* gene mutations and various human cancers, we used cBioPortal tools to study *CLEC10A* mutations in data extracted from TCGA data sets. The highest mutation frequency (more than 4%) was observed in patients with cutaneous melanoma, and no change in gene frequency was found in patients with CHOL, renal chromophobe cell carcinoma, MESO, TGCT, and UVM. At the same time, it should be noted that all patients with uterine carcinosarcoma (UCS) exhibited *CLEC10A* gene amplification with a change frequency of about 2% (**Figure 3A**). The types, loci, and case numbers of *CLEC10A* gene mutations are shown in **Figure 3B**, with the main genetic changes being missense mutations (48 sites). In addition, the copy number was positively correlated with the expression of CLEC10A in KICH, GBM, STAD, PAAD, BRCA, LIHC, and prostate adenocarcinoma (PRAD) (**Figure 3C**, P<0.05).

CLEC10A protein level

We explored CLEC10A expression in-depth with respect to the protein level. The protein level of CLEC10A in human normal tissues such as liver, testis, and skin tissues was higher than that of corresponding cancer tissues, namely liver cancer, testicular cancer, and basal cell carcinoma of the skin tissues, respectively (**Figure 4A**). Its protein level was closely relat-

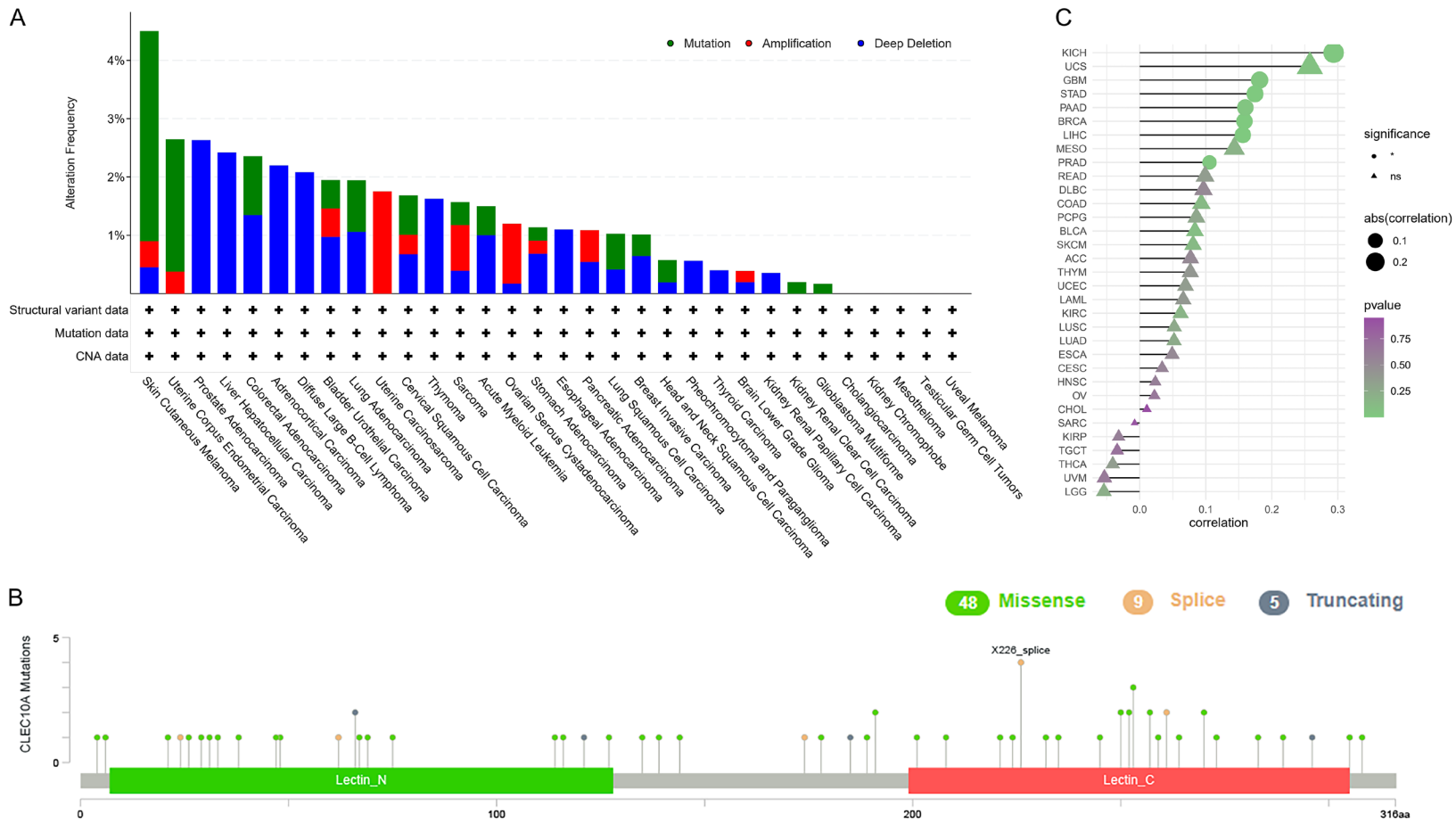

Figure 3. Mutation feature of CLEC10A in different cancers in TCGA. Using the cBioPortal tool, we analyzed the mutation features of CLEC10A for tumors in TCGA. The alteration frequencies with (A) mutation type, (B) mutation site, and (C) copy number change.

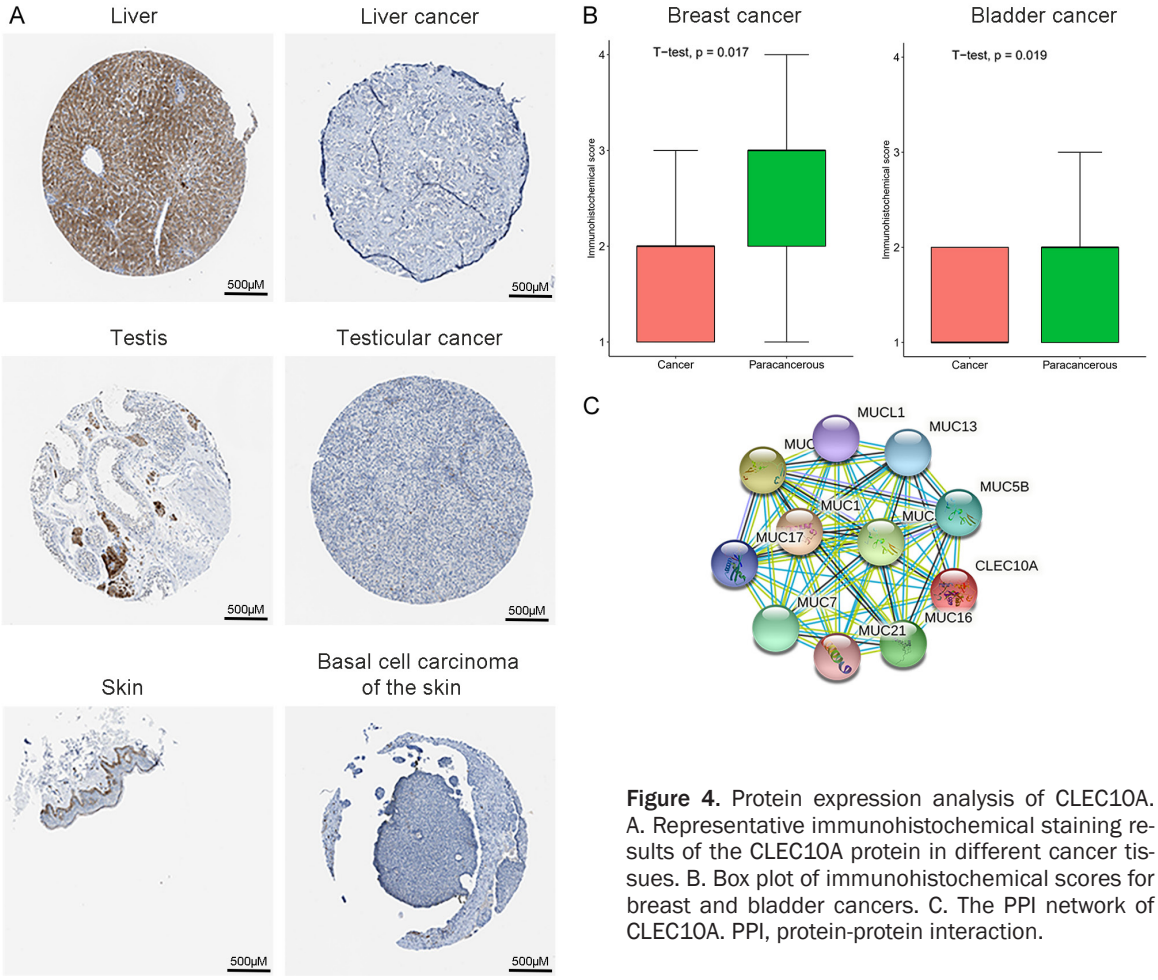

Figure 4. Protein expression analysis of CLEC10A. A. Representative immunohistochemical staining results of the CLEC10A protein in different cancer tissues. B. Box plot of immunohistochemical scores for breast and bladder cancers. C. The PPI network of CLEC10A. PPI, protein-protein interaction.

ed to the mRNA expression level. In addition, we used immunohistochemistry to detect the expression level of CLEC10A in breast cancer and bladder cancer, and the results showed that the expression level of CLEC10A in these cancers decreased significantly (**Figure 4B**, P<0.01). To find the association between CLEC10A and other proteins, we constructed a protein-protein interaction (PPI) network and observed that CLEC10A had a very close relationship with MUC1, MUC4, MUC5AC, MUC16, MUC7, MUC5B, MUC13, MUC17, MUCL1, and MUC21 (**Figure 4C**).

Prognostic significance of CLEC10A

In the present study, we used univariate Cox regression analysis and Kaplan-Meier analysis to explore the impact of CLEC10A on the survival of cancer patients. According to Kaplan-Meier analysis, the high expression of CLEC10A indicated better OS in patients with adrenocortical carcinoma (ACC), BRCA, cervical squamous cell carcinoma, endocervical adenocarcinoma (CESC), COAD, GBM, KICH, HNSC, KIRC, LGG, LUAD, READ, sarcoma (SARC), SKCM, TCGT, and UVM (Supplementary Figure 2, all P<0.05). The high expression of CLEC10A also indicated better PFI in ACC, BRCA, CHOL, BLCA, CESC, COAD, GBM, KICH, ESCA, HNSC, KIRC, KIRP, LIHC, LUAD, MESO, ovarian serous cystadenocarcinoma (OV), READ, SARC, SKCM, STAD, and UCEC (Supplementary Figure 3, all P<0.05). Furthermore, high expression indicated better DSS in ACC, BRCA, CHOL, CESC, COAD, GBM, KICH, HNSC, KIRC, KIRP, LUAD, READ, PRAD, SARC, SKCM, TCGT, UCS, and UVM (Supplementary Figure 4, all P<0.05). From the univariate Cox regression analysis, the OS results showed that the high expression of CLEC10A played a protective role in patients with ACC, BRCA, CESC, COAD, HNSC, KIRC, LUAD, READ, SARC, and SKCM (**Figure 5A**, all P<0.05). PFI results showed that the high

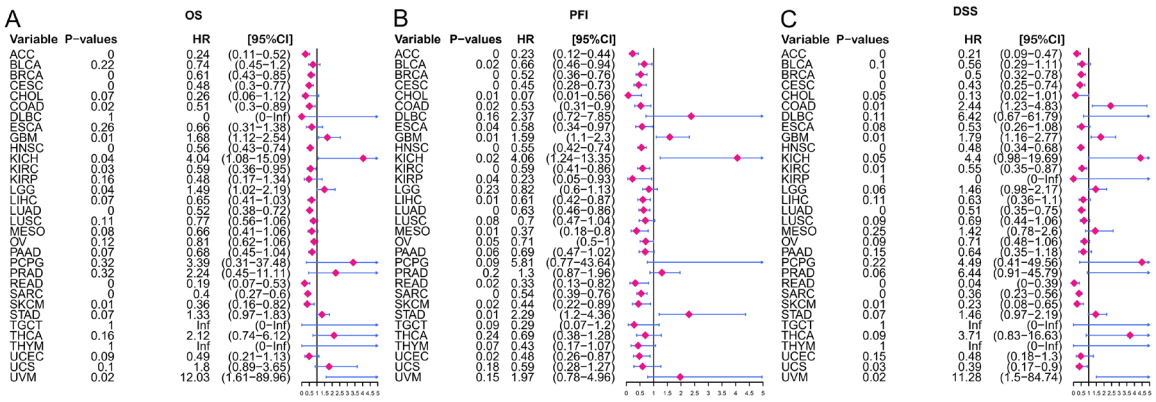

Figure 5. Univariate Cox regression analysis of CLEC10A. A. Forest map showing the univariate cox regression results of CLEC10A for OS in TCGA pan-cancer. B. Forest map showing the univariate cox regression results of CLEC10A for PFI in TCGA pan-cancer. C. Forest map showing the univariate cox regression results of CLEC10A for DSS in TCGA pan-cancer. Red color represents significant results.

expression of CLEC10A played a protective role in patients with ACC, BLCA, BRCA, CESC, CHOL, COAD, ESCA, HNSC, KIRC, KIRP, LIHC, LUAD, MESO, READ, SARC, SKCM, and UCEC, but was a risk factor in patients with GBM, KICH, and STAD (**Figure 5B**, all P<0.05). Finally, the DSS results showed that the high expression of CLEC10A played a protective role in patients with ACC, BRCA, CESC, HNSC, KIRC, KIRP, LUAD, READ, SARC, SKCM, and UCS, but was a risk factor in patients with COAD, GBM, and UVM (**Figure 5C**, all P<0.05). It could be concluded that when the expression of CLEC10A is high, the prognosis of patients is better. Thus, the expression of CLEC10A is closely related to the prognosis of various types of cancer.

GSEA of CLEC10A

To further study the pathways that CLEC10A may regulate, we performed an enrichment analysis of CLEC10A. Interestingly, we found that the high CLEC10A expression group was significantly enriched in cytokine receptor interaction, chemokine signaling pathway, and cell adhesion molecules (CAMs), and the low expression group was significantly enriched in folate biosynthesis, glycosyl phosphate idylinositol (GPI)-anchor biosynthesis, and protein export (**Figure 6A**, **6B**, all P<0.05). At the same time, we used another database for verification and found that the high CLEC10A expression group was enriched in allograft rejection, inflammatory response and complement, whereas the low expression group was enriched in MYC targets v1 and MYC targets v2 (**Figure 6C**, **6D**, all P<0.05). The above results demonstrate that CLEC10A is significantly related to immune-related pathways when it is highly expressed, indicating that CLEC10A may regulate immune-related pathways, which is a potential research direction.

Immune cell infiltration analysis

We used immune cell infiltration databases from two different sources to analyze the correlation between CLEC10A expression and immune cell infiltration. The results of the TIMER2 database showed that there was a positive correlation between the expression of CLEC10A and the level of infiltration of CD8+T cells and CD4+T cells (**Figure 7A**, **7B**, all P<0.05). Furthermore, based on published articles, we also chose the "CIBERSOFT" method to evaluate 22 immune cells. The results showed that the expression of CLEC10A in most tumors was positively correlated with the infiltration of M2 macrophages and CD8+T cells (**Figure 7C**, all P<0.01). These results suggest that CLEC10A may help increase T cell infiltration, which may also explain its possible protective effect in most tumor types.

Correlation between CLEC10A and immune regulation-related genes and chemokines

In addition to studying the above immune-related aspects, we also performed other studies and found that for most tumors, there was a significant positive correlation between CLEC10A expression and stromal score (Supplementary Figure 5), immune score

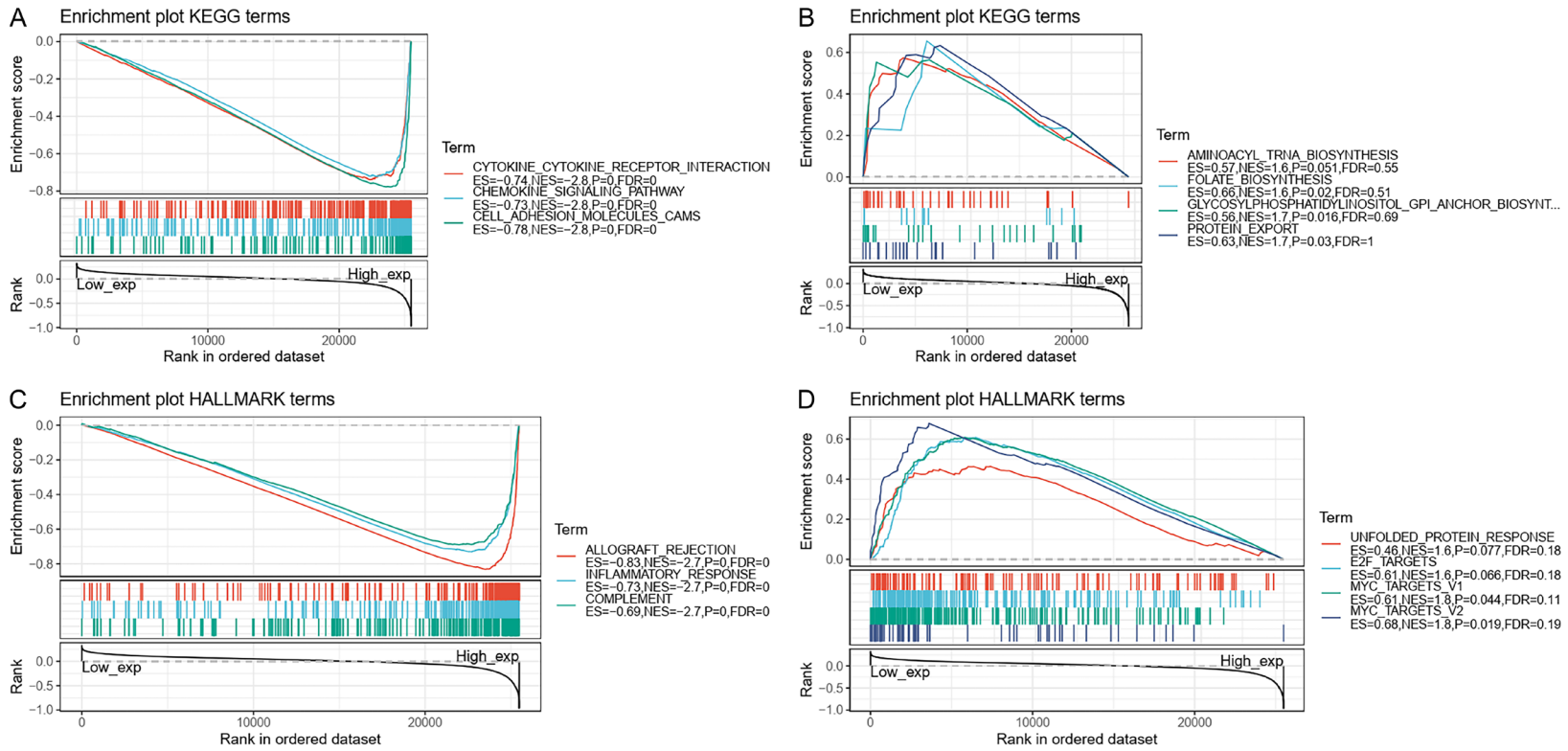

Figure 6. GSEA of high- and low-CLEC10A expression groups. A. Analysis of KEGG pathway in CLEC10A high-expression group. B. Analysis of KEGG pathway in CLEC10A low-expression group. C. Analysis of HALLMARK pathway in CLEC10A high-expression group. D. Analysis of HALLMARK pathway in CLEC10A low-expression group. ES, enrichment score. NES, normalized enrichment score. FDR, false discovery rate. P<0.05 was considered significant.

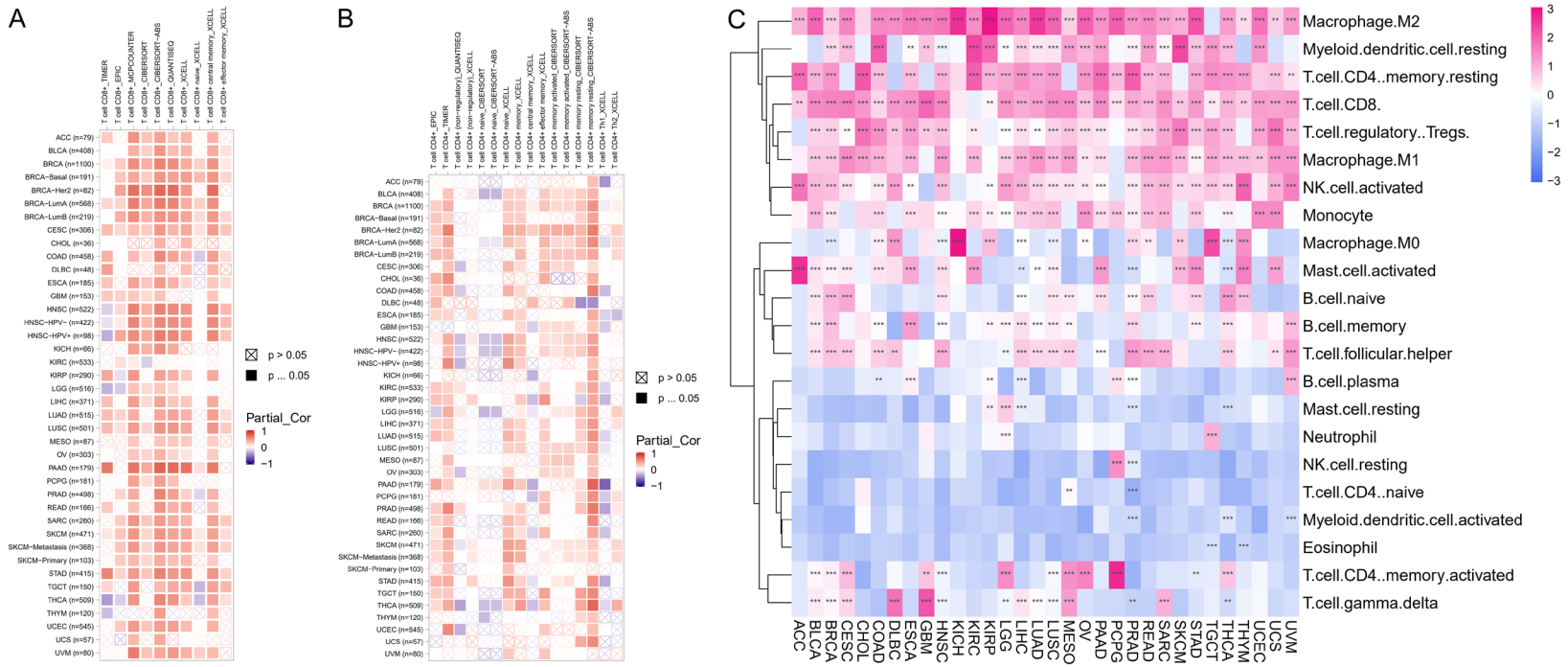

Figure 7. Analysis of the effect of CLEC10A on the immune microenvironment. A. The correlation between CLEC10A and infiltration level of CD8+T cells using the TIMER2 database. B. The correlation between CLEC10A and infiltration level of CD4+T cells using the TIMER2 database. C. The correlation between CLEC10A and infiltration level of indicated immune cells. Red color represents positive correlation, blue color represents negative correlation, and the deeper the color, the stronger the correlation. *P<0.05, **P<0.01, and ***P<0.001.

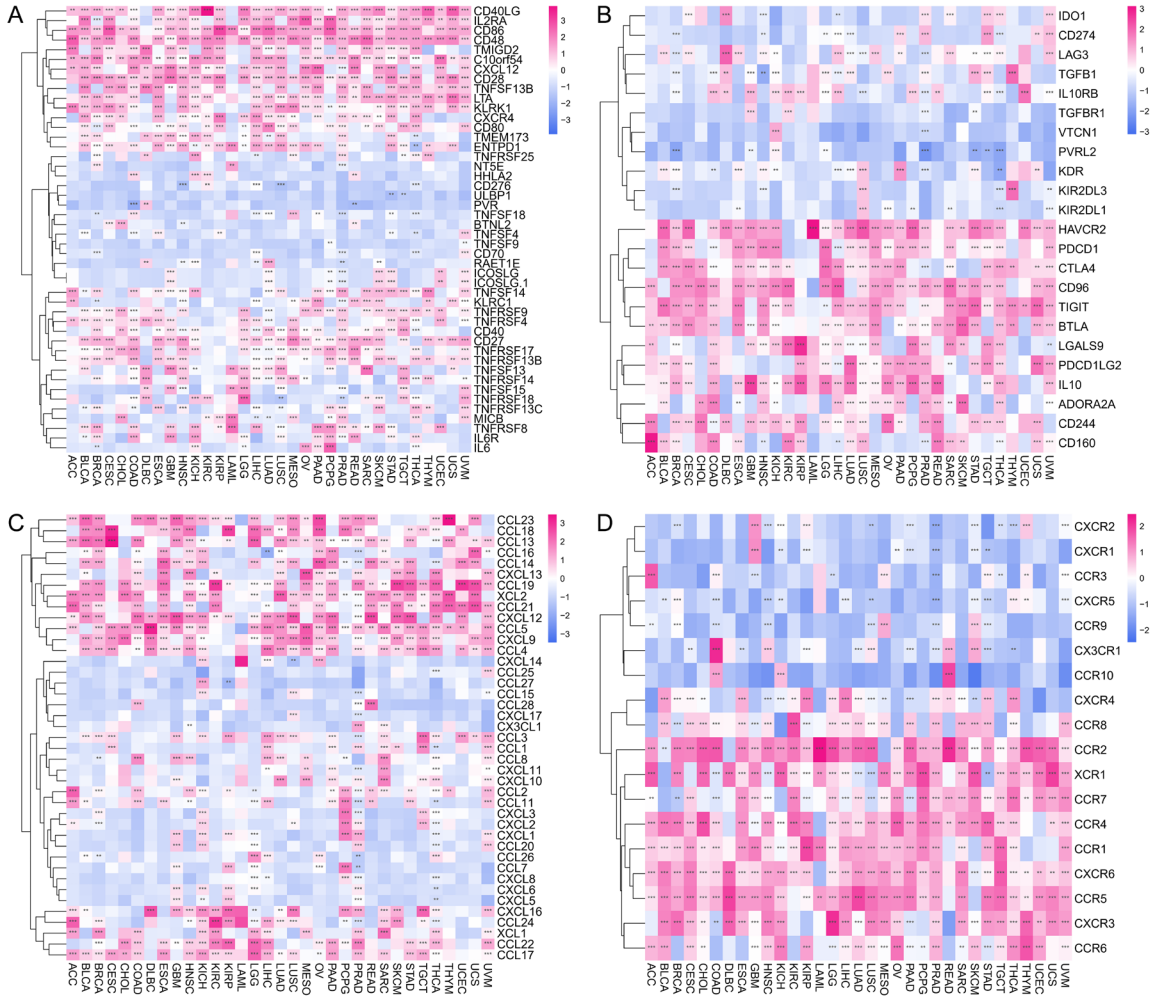

Figure 8. The correlation between CLEC10A and immunoregulation-related genes. A. The heatmap represents the correlation between CLEC10A expression and immune activating genes. B. The heatmap represents the correlation between CLEC10A expression and immunosuppression-related genes. C. The heatmap represents the correlation between CLEC10A expression and chemokine genes. D. The heatmap represents the correlation between CLEC10A expression and chemokine receptor genes. *P<0.05, **P<0.01, and ***P<0.001.

(Supplementary Figure 6), and ESTIMATE score (Supplementary Figure 7). We then explored the relationship between *CLEC10A* and immune activation genes, immunosuppressive genes, chemokines, and chemokine receptors, and concluded that *CLEC10A* expression was positively correlated with that of immune activation genes such as *CD40LG, CD48, CD86, HAVCR2, CD96, TIGIT,* and immunosuppressive genes in TCGA pan-cancer (**Figure 8A**, **8B**, all P<0.01). Furthermore, the expression of *CLEC10A* was positively correlated with chemokines CCL4 and CCL5 and their receptors CCR4 and CCR5 (**Figure 8C**, **8D**, all P<0.01). Combining the two findings, we speculated that CLEC10A may be involved in cell chemotaxis recruitment and immune regulation.

CLEC10A expression is associated with immune checkpoint genes in human cancer

We collected expression data of 47 common immune checkpoint genes, analyzed the relationship between CLEC10A expression and immune checkpoint gene expression, and evaluated the role of CLEC10A as a target in clinical ICIs therapy. The results showed that in most tumors, most of the 47 immune checkpoint genes were closely related to the expression of CLEC10A, and the expression of CLEC10A was positively correlated with these genes (Supplementary Figure 8, all P<0.05). Based on the above findings, high CLEC10A expression may play a significant role in the mediation of immune escape, and the activity of these ICP

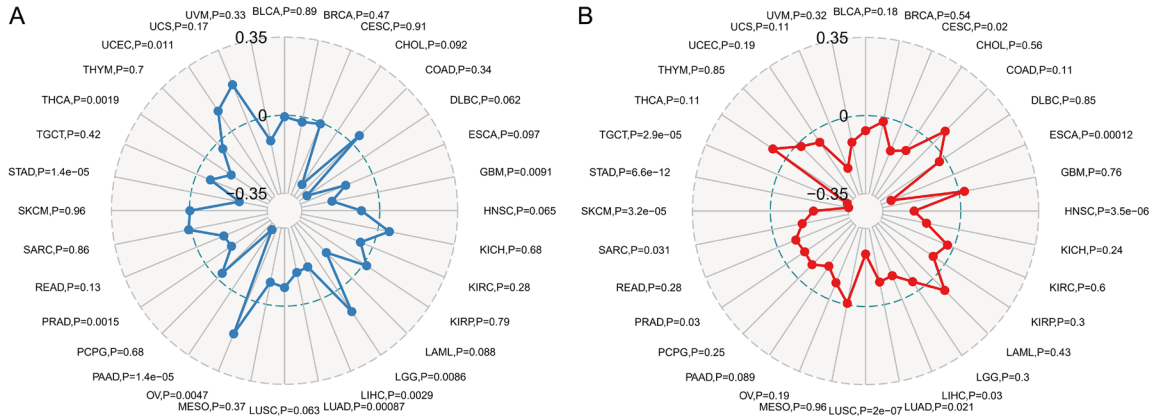

Figure 9. Association between CLEC10A expression and tumor mutation burden (TMB) and microsatellite instability (MSI) in human cancers. A. Association of CLEC10A expression with TMB in human cancers. B. Association of CLEC10A expression with MSI in human cancers. The radar map shows Spearman's correlation coefficient and *p*-value.

genes may be coordinated by CLEC10A in different signal transduction pathways, suggesting that CLEC10A may be a potential pan-cancer biomarker or a new target for ICIs therapy, which could predict ICIs therapy response or achieve promising treatment outcomes.

The relationship between CLEC10A expression and TMB and MSI

TMB and MSI are effective biomarkers of prognosis and indicators of ICIs therapy response in many tumor types. We used the Spearman correlation test to analyze the relationship between CLEC10A expression and TMB and MSI, to explore the immune mechanism of CLEC10A in the tumor microenvironment and its role in immune response. The expression of CLEC10A was positively correlated with TMB in UCEC, GBM, LGG, LIHC, LUAD, OV, PAAD, PRAD, STAD, and THCA, and positively correlated with MSI in CESC, ESCA, HNSC, LIHC, LUAD, LUSC, PRAD, SARC, SKCM, STAD and TGCT (**Figure 9A** and **9B**, P<0.05). The above results further confirm that CLEC10A may affect anti-tumor immunity by regulating the composition and immune mechanism of TME and that CLEC10A could be used to predict the efficacy of tumor ICIs therapy.

Association between CLEC10A expression and prognosis in patients receiving ICIs therapy

To investigate whether CLEC10A has an impact on the prognosis of patients treated with ICIs, we analyzed data from the immunotherapy cohort of uroepithelial carcinoma (IMvigor210). Patients were divided into high and low expression groups based on CLEC10A expression, and we found that patients in the high expression group had significantly longer OS than those in the low expression group (P=0.039, **Figure 10A**). This result suggested that CLEC10A might have influenced the response of patients to ICIs treatment and thus OS. We further explored the potential mechanisms affecting the efficacy of ICIs. We performed GSEA analysis on patients in the response and non-response groups, and the results suggested that the "Antigen processing and presentation" and "Neuroactive ligand-receptor interaction" pathways were significantly enriched in the response group, suggesting that the activation of the "Antigen processing and presentation" and "Neuroactive ligand-receptor interaction" pathways might be beneficial to the efficacy of ICIs (**Figure 10B**). Interestingly, we found that the expression of CLEC10A was significantly and positively correlated with the enrichment scores of the two pathways mentioned above (**Figure 10C**, **10D**). This result suggested that CLEC10A might affect the therapeutic response to ICIs by influencing the above two pathways.

Discussion

In recent years, reports on CLEC10A have increased, and its unique role in tumors is now gaining researchers' attention. A recent report [22] shows that there is a strong relationship between CLEC10A expression and tumor pro-

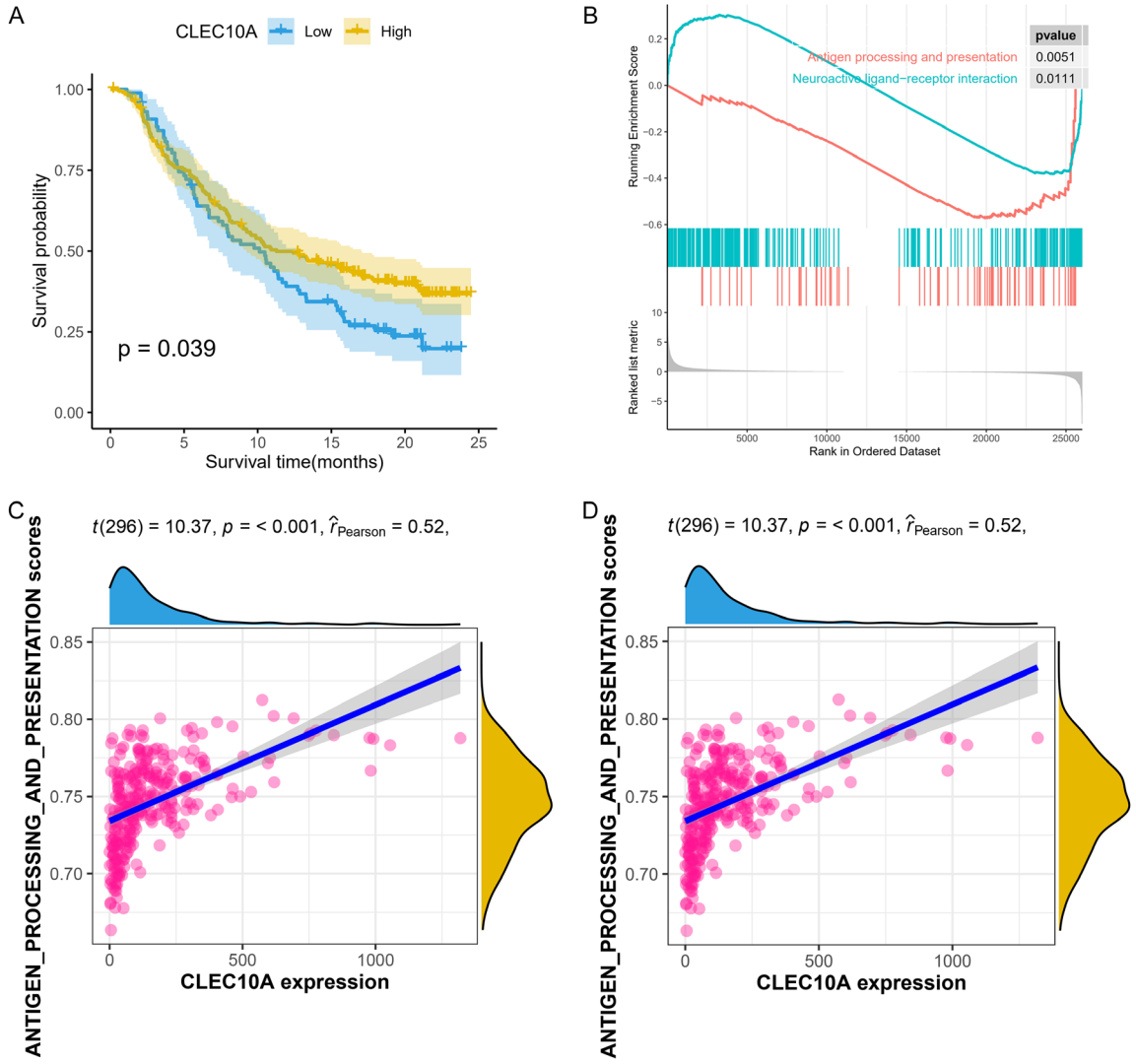

Figure 10. Association between CLEC10A expression and prognosis in patients receiving ICIs therapy. A. Association between CLEC10A expression and OS in patients receiving ICIs therapy. B. GSEA analysis on patients in the response and non-response groups. C. The correlation between CLEC10A expression and the enrichment scores of Antigen processing and presentation pathway. D. The correlation between CLEC10A expression and the enrichment scores of Neuroactive ligand-receptor interaction pathway.

gression. However, the role of CLEC10A has been evaluated only in a number of cancers, such as breast cancer [14], lung cancer [22], and colon cancer [23], while skin melanoma [15] has not yet been evaluated, and there lacks a pan-cancer analysis, resulting in a limited understanding of the role of CLEC10A in human pan-cancer. As mentioned earlier, CLEC10A may act as a double-edged sword in tumor-associated immunity, indicating that it can either prevent the occurrence of cancer or promote tumor development of some types of cancers. However, only a small number of published studies [14] on the relationship between CLEC10A and tumor immunity are available. Considering its potential roles in tumorigenesis and development, we speculated that the study of the internal mechanism of the relationship between CLEC10A and tumor immunity may be clinically significant. It would also be helpful to explore the correlation between the expression of CLEC10A and the prognosis of various human cancers. From this point of view, we carried out a series of related studies on CLEC10A.

We first investigated the pan-cancer expression of CLEC10A and its prognostic value and found that its expression was significantly low-

er in most tumors compared to normal tissues, and a significant increase in CLEC10A expression was observed only in lymphomas. The results of Kaplan-Meier analysis and univariate Cox regression analysis could also verify the good prognostic value of high CLEC10A expression. Therefore, we speculate that the significantly high expression of CLEC10A in most tumors may benefit the survival of cancer patients. Some studies have pointed out that there is a correlation between the expression of CLEC10A and disease progression, and that the different expression levels are closely related to the prognosis of the disease. For example, in colon cancer, CLEC10A is a reliable indicator of the prognosis of patients, and high expression of CLEC10A is associated with a better prognosis [23]. High expression of CLEC10A is associated with a better prognosis even in breast cancer patients [24]. These findings are consistent with our findings, suggesting that CLEC10A played an important in the development of tumors. We thus hypothesized that CLEC10A plays a protective role in tumor development and is a potential pan-cancer prognostic biomarker. In addition, our study found that CLEC10A was expressed at different levels in different tumor stages, suggesting that CLEC10A may play a role in tumorigenesis and development. The low expression of CLEC10A was associated with advanced cancer stages, as CLEC10A expression was lower in stages III-IV of ACC, HNSC, KIRP, and LUAD, suggesting that CLEC10A may inhibit cancer progression. This result also further validates our conclusion that high expression of CLEC10A in pan-cancer is associated with a good prognosis.

By performing GSEA of CLEC10A, we found that it was significantly associated with immune-related pathways, especially lymphoid and non-lymphoid pathways, which play a key role in regulating the response of lymphoid-derived cells such as B cells, T cells and NK cells to self and tumor antigens and to pathogens [25-29]. In addition, there was a positive correlation between CLEC10A expression and the infiltration level of CD8+T cells and CD4+T cells. Meanwhile, we investigated the relationship between CLEC10A and immunoregulatory-related genes and chemokines and found that CLEC10A may be involved in the chemotactic recruitment of antigen-presenting cells and their immune regulation, which may directly affect the prognosis and survival of patients. From these results, we can infer that the relationship between high CLEC10A expression and a good prognosis of cancer patients may involve the infiltration of immune cells. As a member of the CLR, CLEC10A, like other members, was identified to be involved in enhancing the immune response activity of immune cells. CLEC10A recognizes and acts on tumor-associated TN antigens, effectively presenting the antigen to CD4+T cells [30]. In addition, the binding of CLEC10A to tumor-associated antigens carrying α-N-acetylgalactosamine significantly enhances activation of antigen-specific CD8+T cells [31]. Effective tumor eradication requires tumor-specific CD8+T and CD4+T cells. The infiltration level of CD8+T cells and CD4+T cells was higher in patients with high CLEC10A expression, and the number of infiltrating macrophages expressing CLEC10A increased during cancer development. Furthermore, the killing effect of cytotoxic CD8+T cells on tumor cells was enhanced; thus, tumor growth was inhibited by high CLEC10A expression. These results are similar to that of the previous work by Eggink et al. who reduced the number of tumor cells by activating tumor-associated macrophages to express CLEC10A [14]. These results suggest that the increased level of CD8+T cell infiltration in the tumor microenvironment is intrinsic to the better prognosis of patients with high CLEC10A expression.

The tumor microenvironment is an important component of tumor tissue. Although its composition varies by tumor type, the main constituents include immune cells, stromal cells, blood vessels, and extracellular matrix [32-36]. Tumor progression is largely dependent on the interaction between tumor cells and the surrounding microenvironment, and growing evidence reveal the clinicopathological significance and application of the tumor microenvironment in predicting outcome and treatment effectiveness [37-41]. Previous studies [42] have shown that the increase in the number of CD8+T cells in TME is positively correlated with a good prognosis of tumors, which is consistent with our findings. We found that CLEC10A was significantly associated with immune-related pathways through a series of analyses, which predicted that the role of CLEC10A in tumor immunity is closely related to CD8+T cells and

the anti-tumor effects of CD8+T cells in TME are related to antigen presentation and T cell initiation, trafficking, differentiation and function [42]. Cytotoxic CD8+T cells destroy tumor cells once they enter the tumor site, and the cells and factors of TME provide an immunosuppressive environment that inhibits CD8+T cell function [43]. The role of CLEC10A in enhancing the anti-tumor activity of immune cells has attracted much attention and has been suggested as a target for tumor ICIs therapy. These findings may explain the protective role of CLEC10A in most tumor types and help it receive more attention in tumor ICIs therapy in recent years [44-46]. CLEC10A expression was positively correlated with most of the 47 immune checkpoint genes in multiple tumor types. Moreover, we analyzed the relationship between CLEC10A and TMB and MSI using Spearman's correlation test and found that CLEC10A was positively correlated with TMB and MSI in most tumors, which provides a theoretical basis for future combined molecular targeted ICIs therapy. The major targets of tumor ICIs therapy are the immune checkpoint genes, and their involvement in immune checkpoint blockade therapy is an emerging therapeutic approach. The composition of the tumor microenvironment affects the response to immune checkpoint blockade, and in turn immune checkpoint blockade can utilize immune cell infiltration within the tumor to activate an effective anti-tumor immune response [47]. Therefore, we conclude with high confidence that CLEC10A, as a potential new immune checkpoint, has potential immunotherapeutic value in various tumors and may become a new target for tumor ICIs therapy, resulting in therapeutic effects different from those of conventional treatment.

Although our study involves information from various databases and a variety of research methods, our research still has some limitations. First, there exist individual differences between different cancer patients, and all possible differences cannot be covered by our study. Second, considering that our study is a pan-cancer analysis of CLEC10A, not only is it limited by the sample size of each cancer, but there is also no in-depth exploration and summary of individual cancer types. Most importantly, this study is based on bioinformatics, and its development depends to a large extent on public databases, which indicates that further research on the mechanism of CLEC10A and its corresponding clinical application value requires more clinical trials to provide sufficient supporting evidence. Therefore, before clearly understanding and widely accepting the relationship between CLEC10A and cancer ICIs therapy, it is necessary to further analyze and verify our results through experimental work and clinical research.

Conclusions

In summary, we performed the first pan-cancer analysis of CLEC10A in cancer research, clarified the expression level of CLEC10A in a variety of cancers, and revealed its correlation with tumor immune microenvironment and pan-cancer prognosis. We believe that these findings may provide more reference value for the role of CLEC10A in cancer prognosis and related cancer ICIs therapy, and may have a greater impact on clinical application in the future.

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Disclosure of conflict of interest

None.

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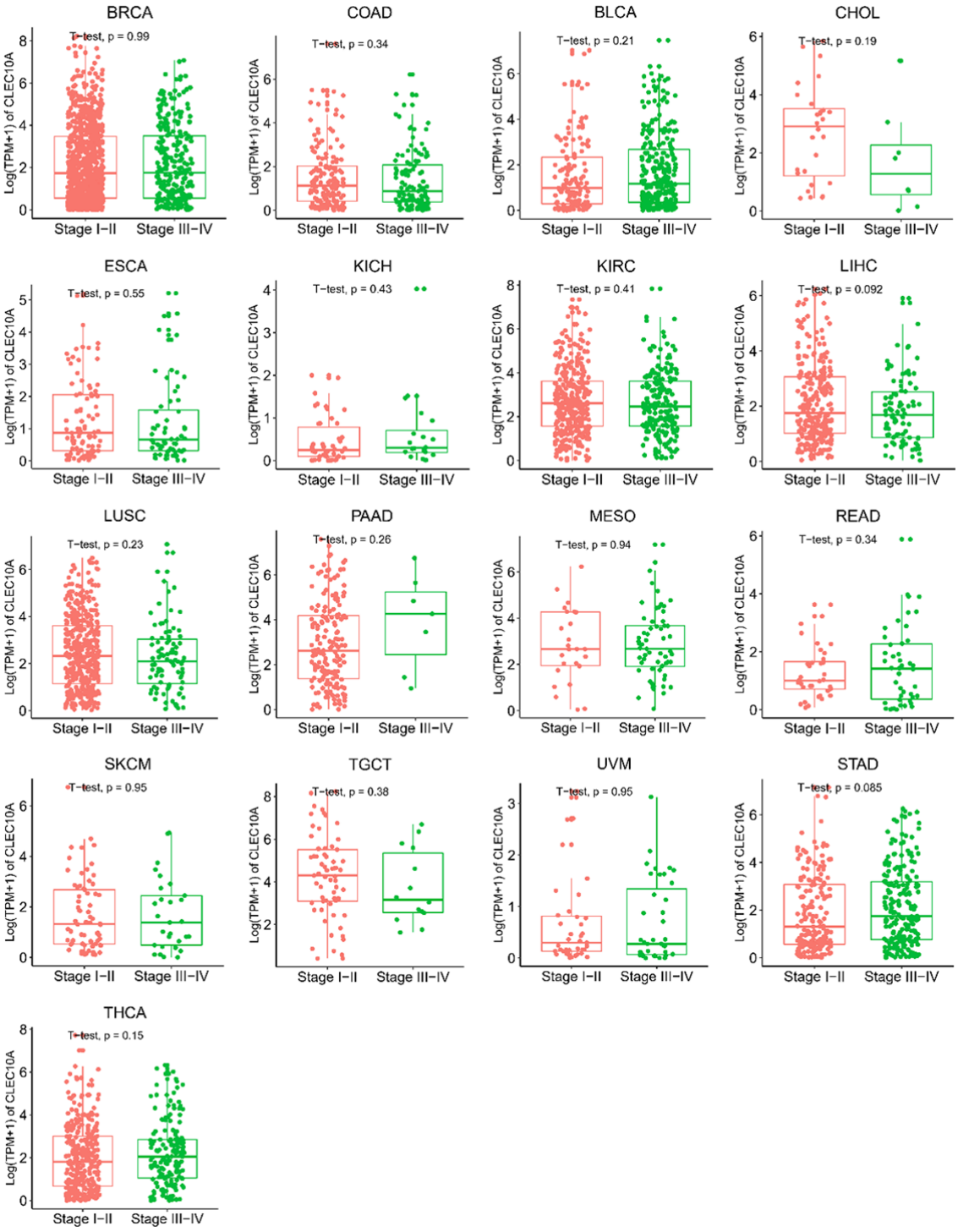
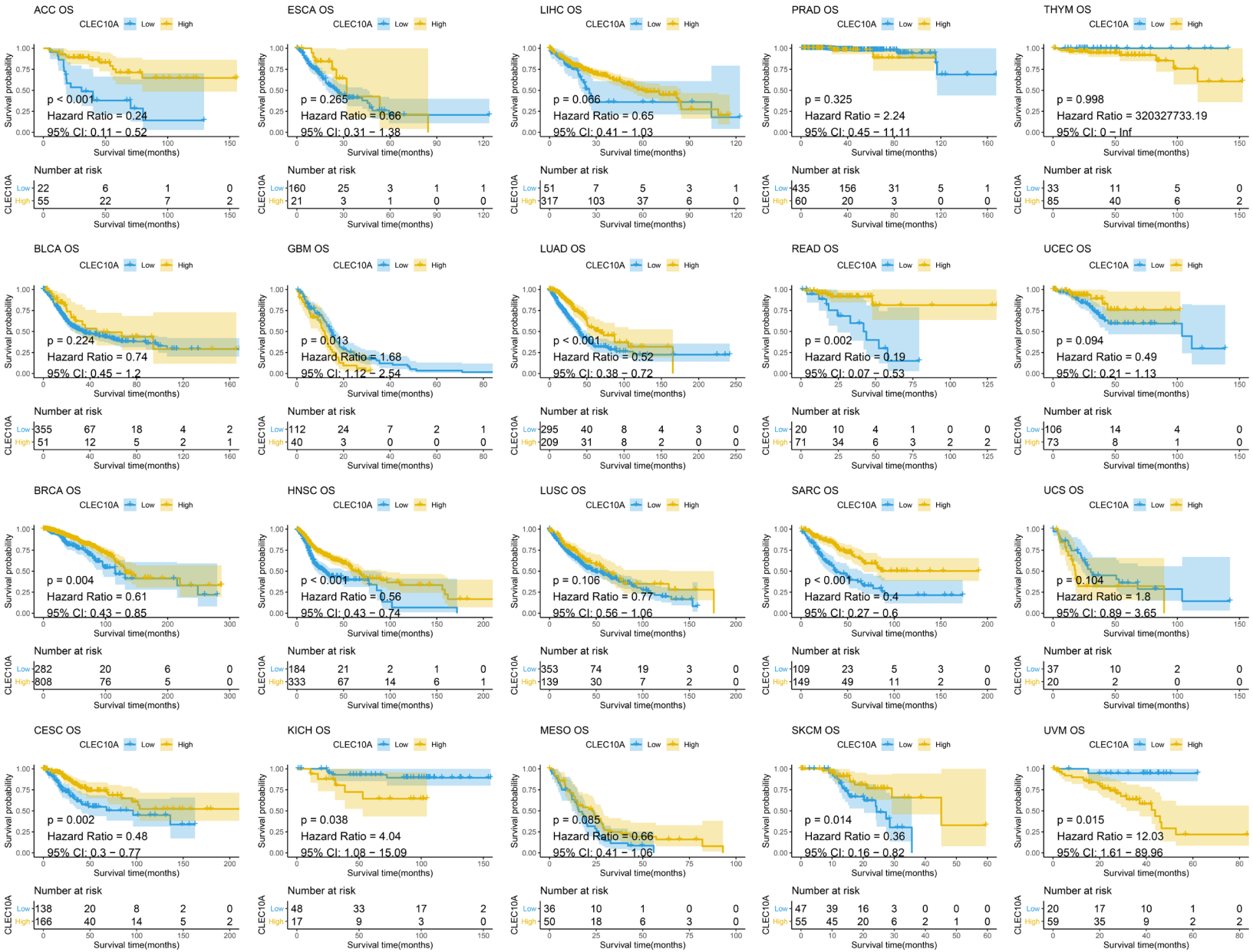
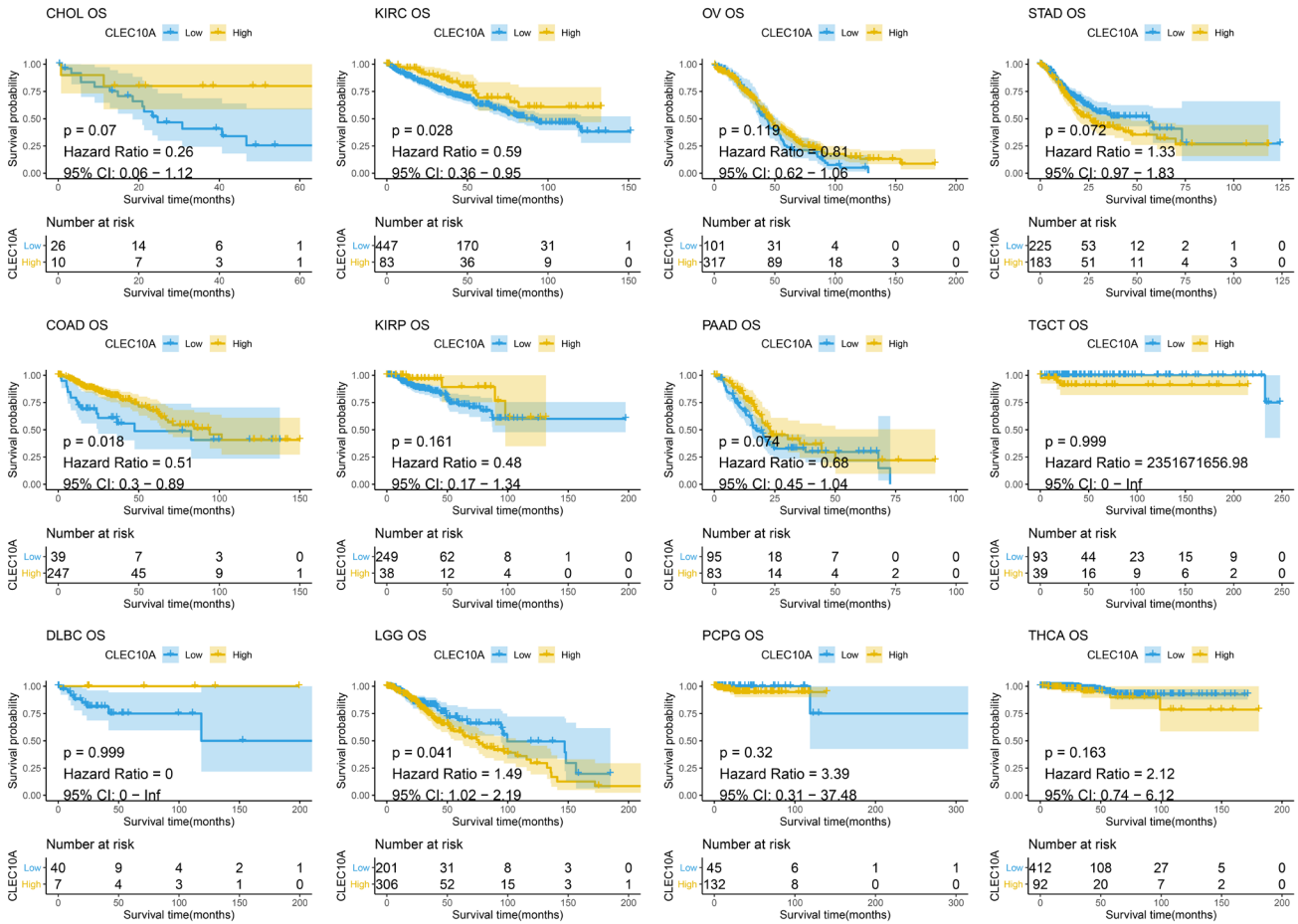


Supplementary Figure 1. CLEC10A expression in different WHO stages. Using TCGA data, CLEC10A gene expression was analyzed by main pathological stage (stage I-II, and stage III-stage IV). Log2 (TPM+1) was used for the log scale. There is no statistical difference here.

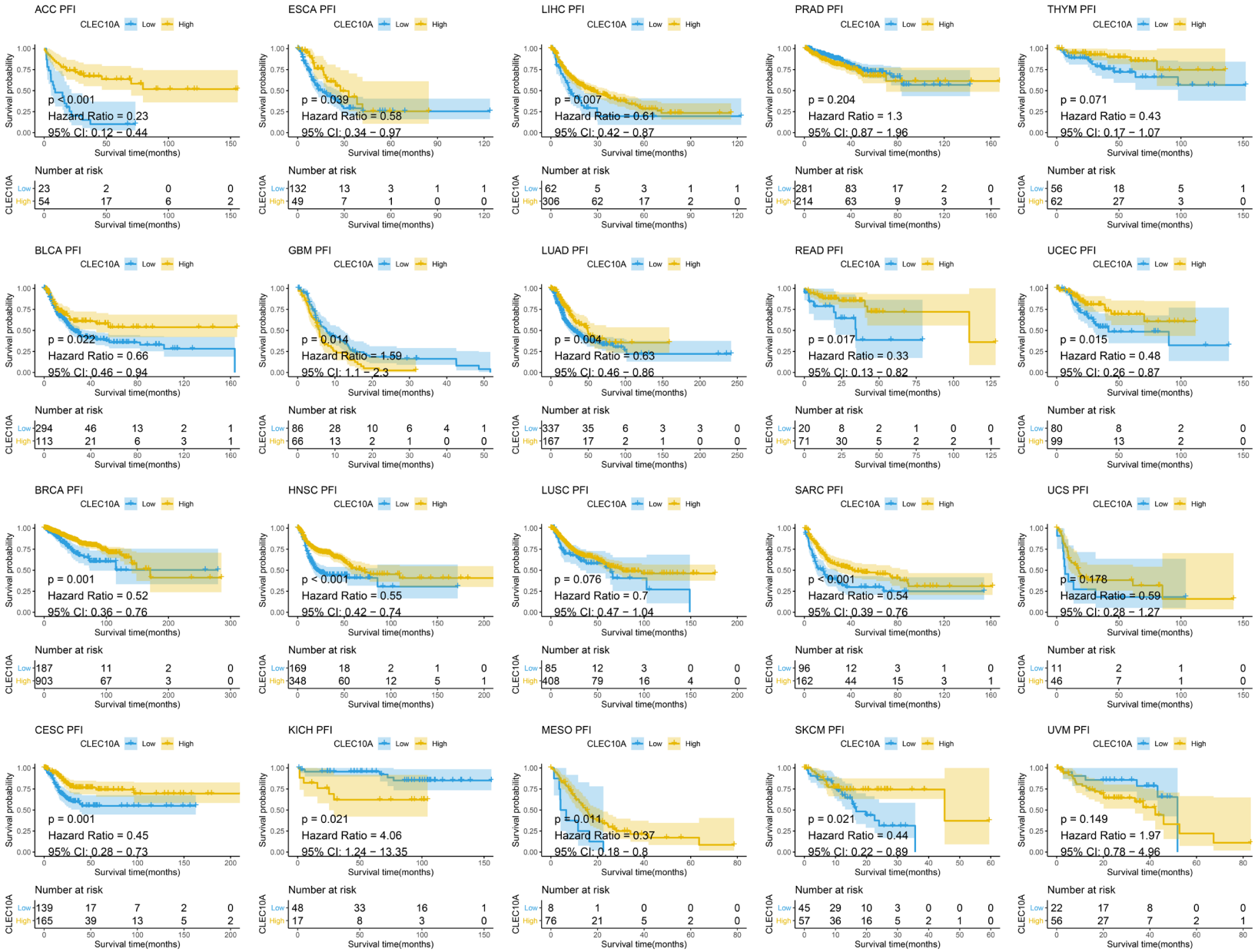
CLEC10A in pan-cancer

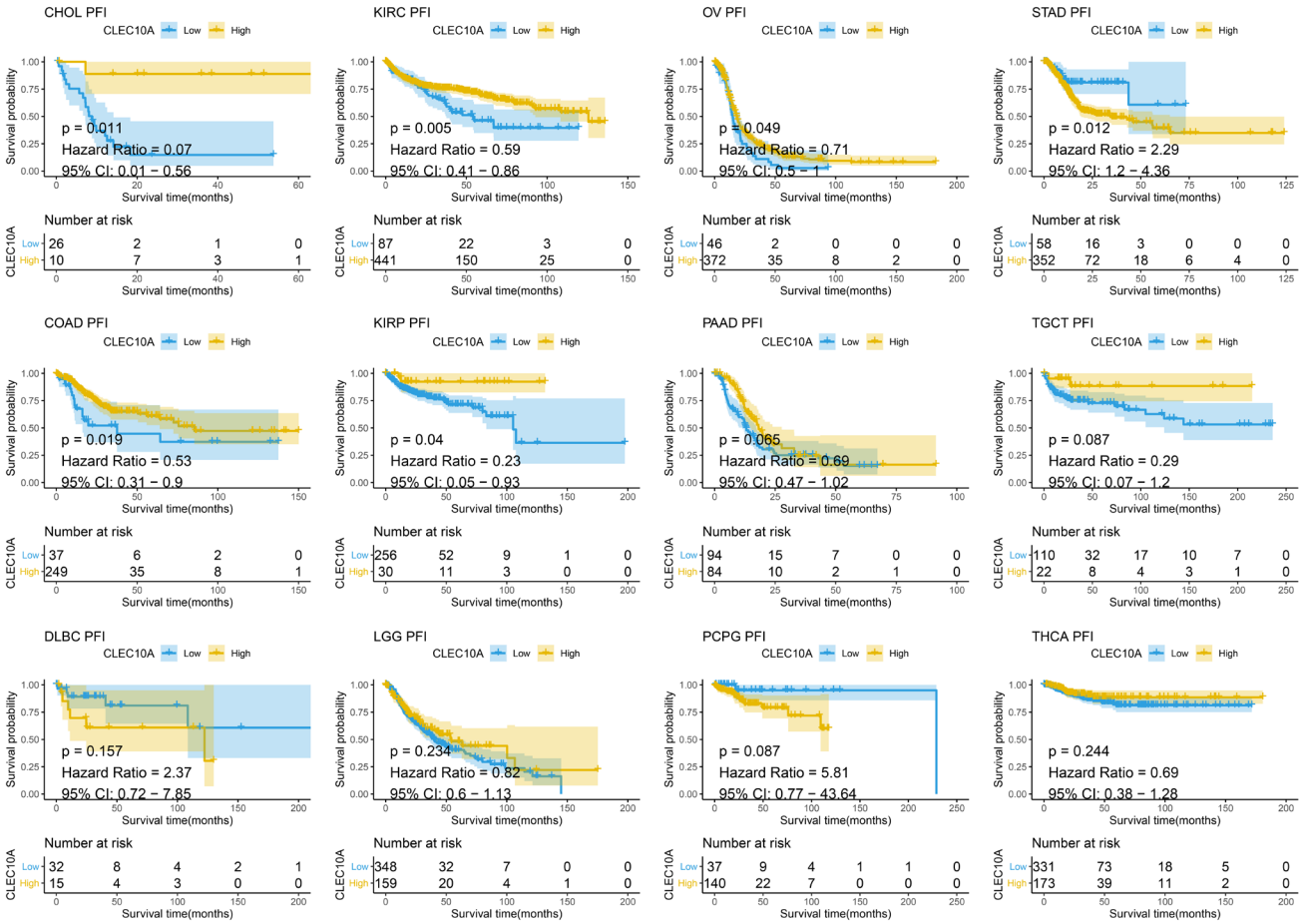




Supplementary Figure 2. Kaplan-Meier survival analysis of CLEC10A in different tumor types for OS.

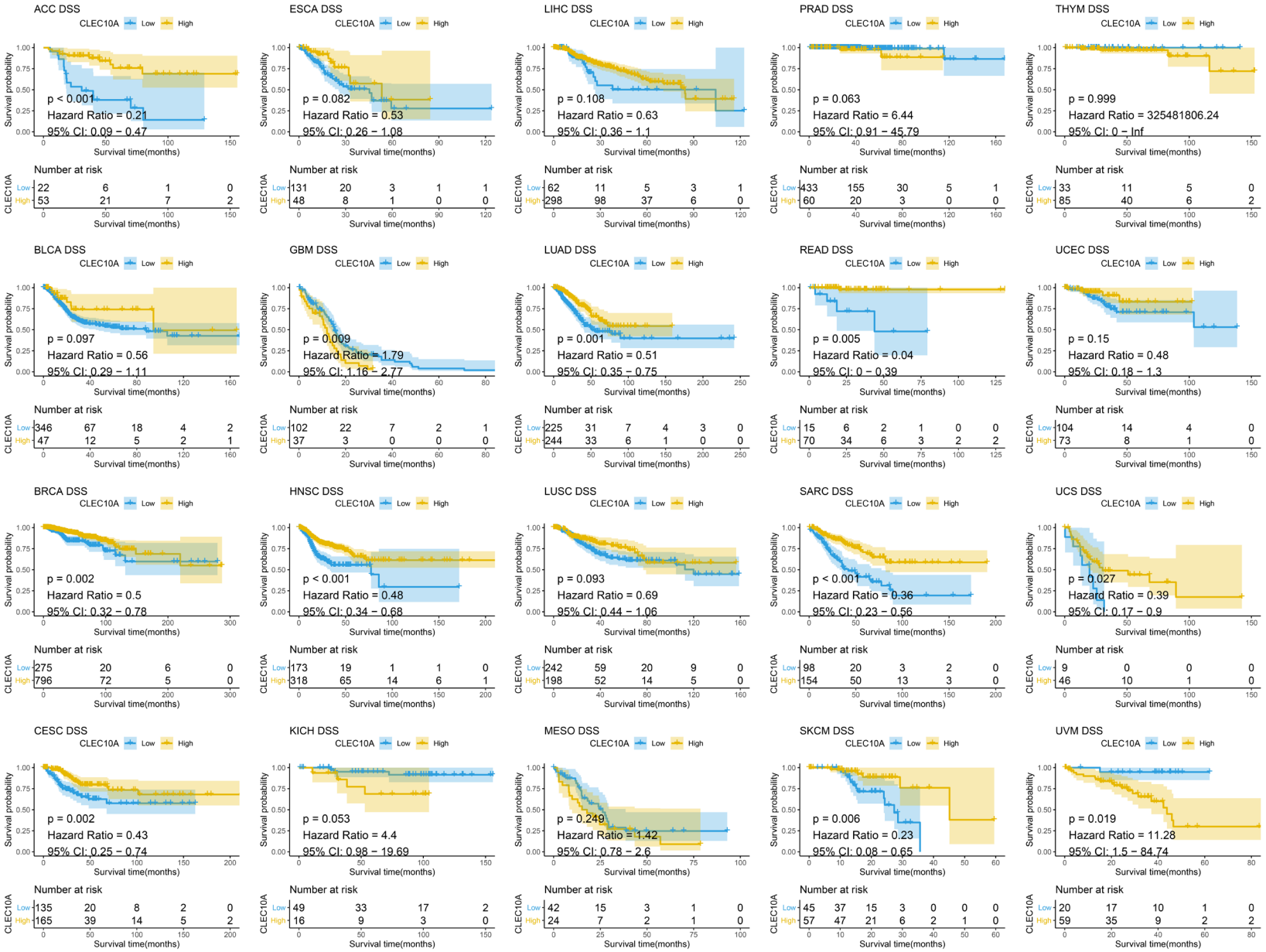
CLEC10A in pan-cancer

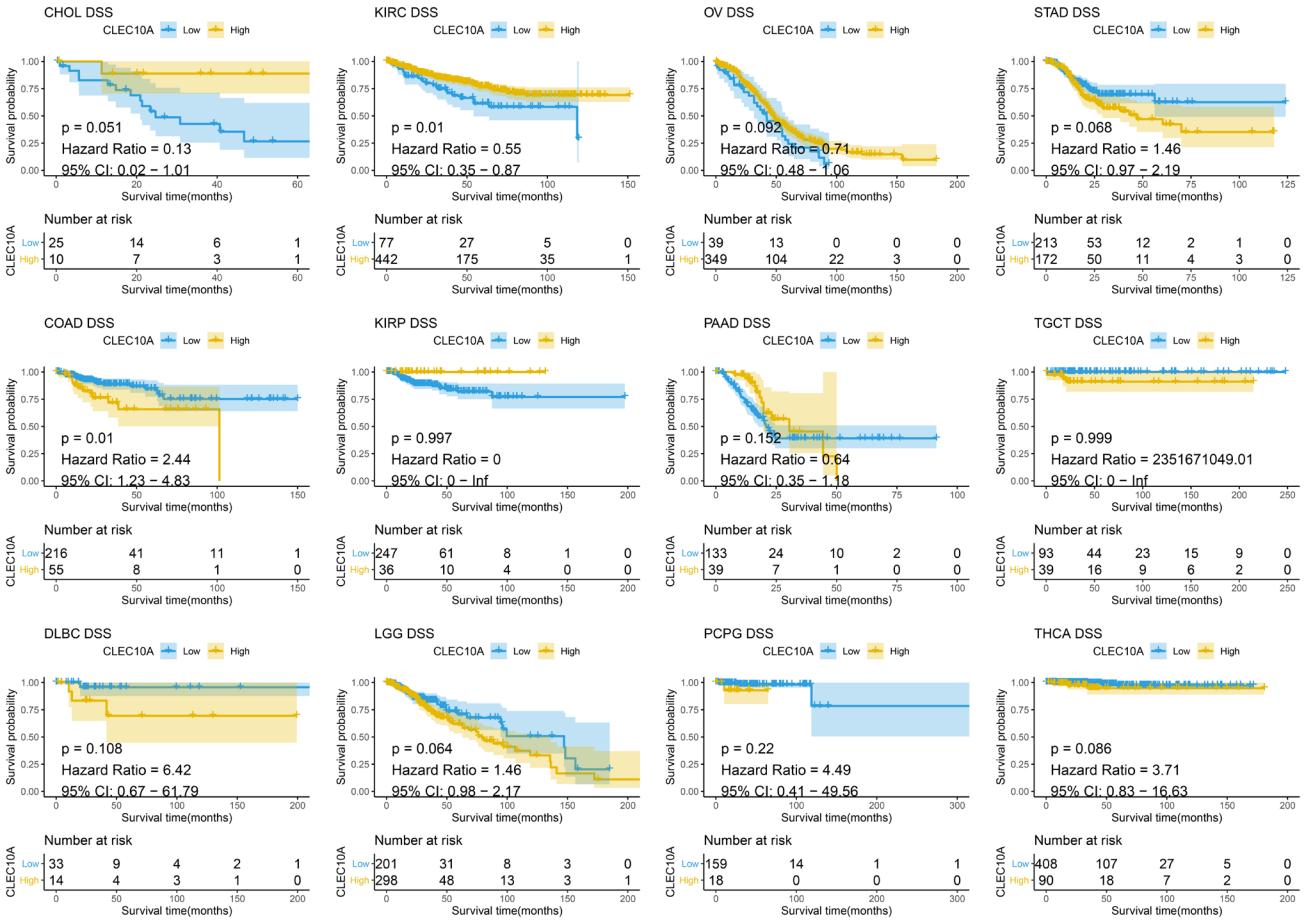




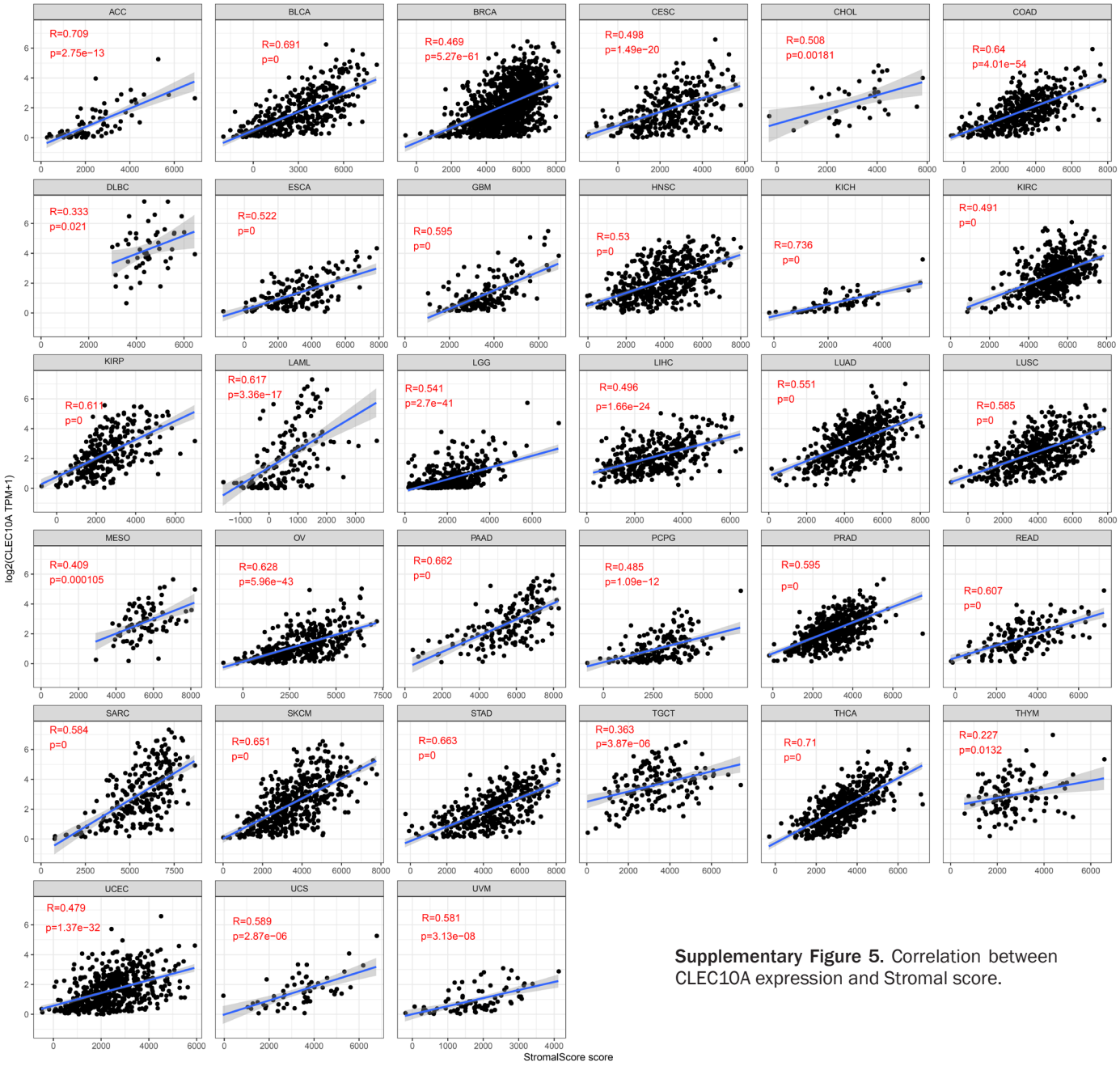
Supplementary Figure 3. Kaplan-Meier survival analysis of CLEC10A in different tumor types for PFI.

CLEC10A in pan-cancer



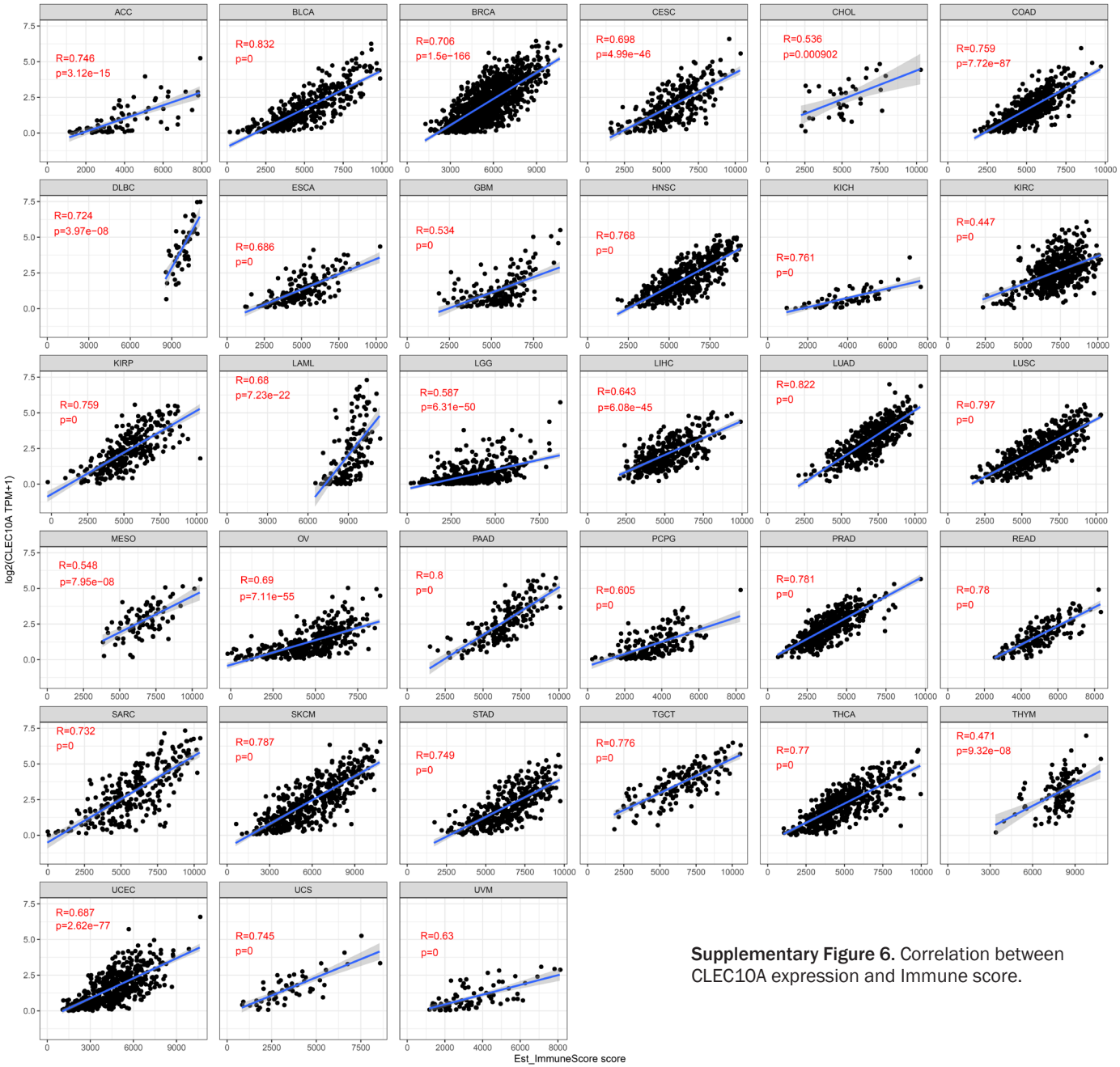


Supplementary Figure 4. Kaplan-Meier survival analysis of CLEC10A in different tumor types for DSS.



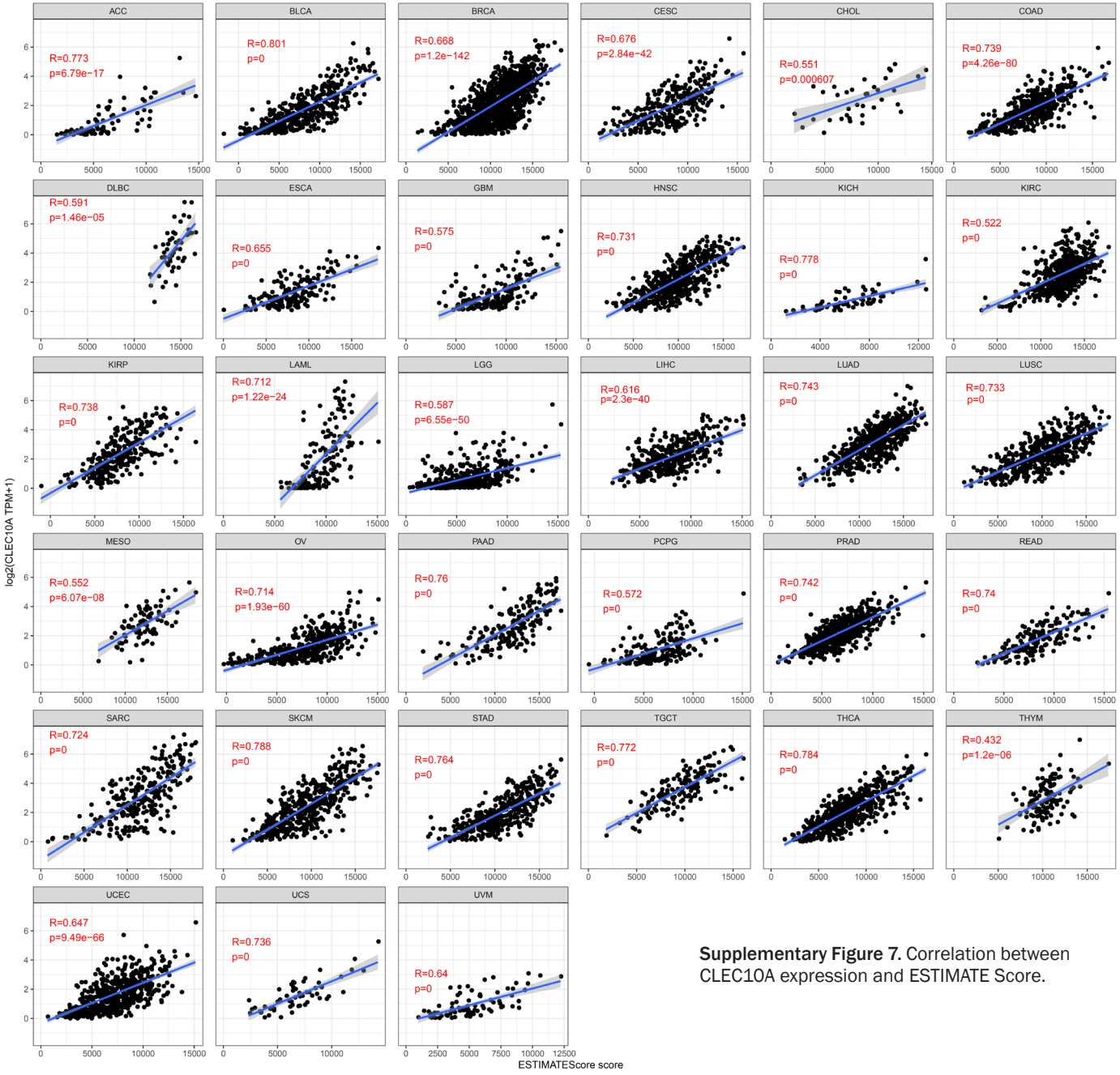
Supplementary Figure 5. Correlation between CLEC10A expression and Stromal score.

CLEC10A in pan-cancer

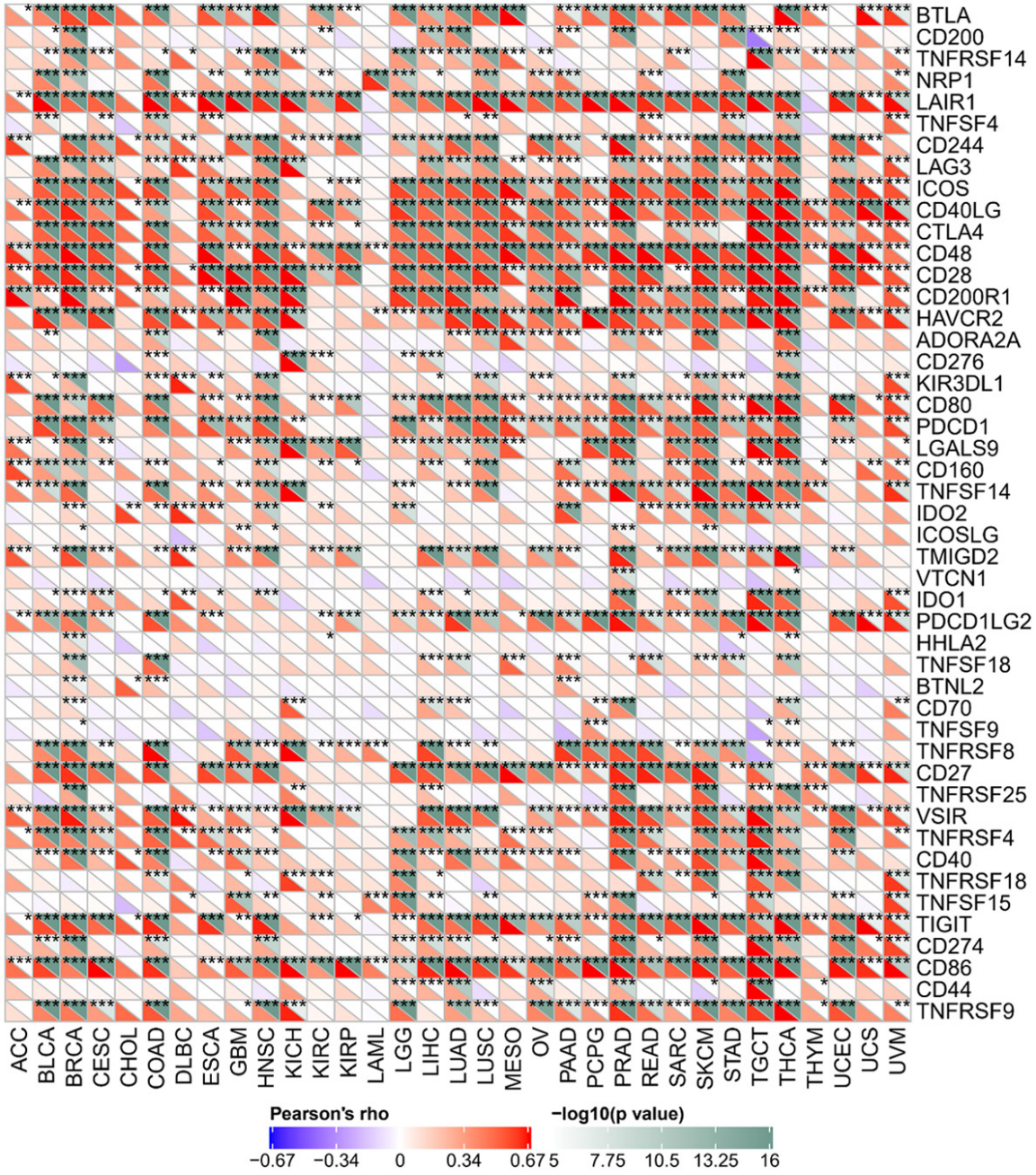


Supplementary Figure 6. Correlation between CLEC10A expression and Immune score.

CLEC10A in pan-cancer



Supplementary Figure 7. Correlation between CLEC10A expression and ESTIMATE Score.



Supplementary Figure 8. Association between CLEC10A expression and pan-cancer immune checkpoint genes. *P<0.05; **P<0.01; ***P<0.001.