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ORIGINAL RESEARCH

The Pan-Cancer Analysis Uncovers the Prognostic and Immunotherapeutic Significance of CD19 as an Immune Marker in Tumor

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Background: The specific cytotoxic effects of anti-CD19 chimeric antigen receptor (CAR) T-cell therapy have led to impressive outcomes in individuals previously treated for B-cell malignancies. However, the specific biological role of CD19(+) target cells, which exert antitumor immunity against some solid tumors, remains to be elucidated.

Methods: We collected information regarding the level of CD19 mRNA and protein expression from various databases including The Cancer Genome Atlas (TCGA), Tumor Immune Estimation Resource (TIMER), Genotype-Tissue Expression (GTEx), and Human Protein Atlas (HPA) for both tumor and normal samples. To evaluate the patient's prognosis according to CD19 expression, a Kaplan-Meier (KM) analysis and univariate Cox regression were performed. Furthermore, using the Estimation of Stromal and Immune Cells in Malignant Tumor Tissues Using the Expression Data (ESTIMATE) algorithm, we estimated the ratio of immune cells infiltrating malignant tumor tissues. Afterward, the GSCALite repository was employed to evaluate the vulnerability of tumors expressing CD19 to drugs used in chemotherapy. To validate the results in clinical samples of certain cancer types, immunohistochemistry was then performed.

Results: Most tumor types exhibited CD19 expression differently, apart from colon adenocarcinoma (COAD). The early diagnostic value of CD19 has been demonstrated in 9 different tumor types, and the overexpression of CD19 has the potential to extend the survival duration of patients. Multiple tumors showed a positive correlation between CD19 expression and tumor mutation burden (TMB), microsatellite instability (MSI), and ESTIMATE score. Furthermore, a direct association was discovered between the expression of CD19 and the infiltration of immune cells, particularly in cases of breast invasive carcinoma (BRCA). Moreover, CD19 is highly sensitive to a variety of chemotherapy drugs.

Conclusion: The study reveals the potential of CD19 as both a predictive biomarker and a target for different cancer immunotherapies.

Keywords: CD19, pan-cancer, prognosis, immune infiltration, biomarker

Introduction

Immunotherapy has gained popularity as a preferred approach for treating cancer due to the achievements of monoclonal antibody-based immune checkpoint blockade and engineered T cells.[1] Stimulatory and inhibitory pathways found in immune checkpoints assist in boosting the immune system's reaction to tumors while maintaining self-tolerance. The main observation is the blocking of pathways such as cytotoxic T lymphocyte-associated molecule-4 (CTLA-4), programmed cell death receptor-1 (PD-1), and programmed cell death ligand-1 (PD-L1).[2] Studies have shown that PD-L1 in breast cancer cells can facilitate the differentiation of CD19(+) B cells and aid in the evasion of immune cells.[3] Chimeric antigen receptor (CAR) -T cells have shown potential as an alternative immunotherapy method because they

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can identify antigens associated with tumors on the outer layer of tumor cells and gene products on the outer layer of healthy cells.[4,5] The primary application of these cells has been for the treatment of blood cancers, including acute lymphoblastic leukemia, chronic lymphocytic leukemia, and multiple myeloma. Although the effectiveness of this treatment has been less noticeable in solid tumors, there have been encouraging results observed in neuroblastoma, non-small cell lung cancer, melanoma, breast cancer, and sarcoma (SARC).[6,7] There is a documented association between the level of CD19 expression in tumors and the effectiveness of engineered T cells in causing cell death.[8] Therapies targeting CD19 include CAR-T cell therapy, monoclonal antibodies, bispecific antibodies, and targeted antibody-drug couplings.[9] Among them, the impressive clinical reactions of resistant B-cell cancers to the transfer of anti-CD19 CAR-engineered T cells have emphasized the crucial function of CD19 protein in the treatment of different tumors.[10] Consequently, it is necessary to investigate the different levels of CD19 expression in all types of cancers and their impact on the immune system.

The B-cell immunoglobulin superfamily's signaling receptor, CD19, is a transmembrane protein involved in regulating B-cell activation in an antigen-dependent manner.[11] CD19 has a high lineage-specific expression across various stages of B-cell maturation.[12] Almost all B-cell lines express this protein, yet its expression is limited in both normal and malignant B-cell lines and is not expressed in multilineage, myeloid, erythroid, or megakaryocytic progenitors, indicating CD19 is a desirable target in B-cell malignancies.[13,14] According to prior studies, B cells have been shown to function as antigen-presenting agents, stimulating T cells to produce tumor-specific antibodies and thereby fulfilling an anti-cancer function.[15] Despite this, B cells can also trigger the immunosuppressive action of macrophages and obstruct the antitumor immunity of T cells. In pancreatic ductal adenocarcinoma, CAR-T cells that specifically target CD19 have demonstrated the ability to eliminate the infiltration of B-cells. A high level of CD19(+) B cells can harm the prognosis of invasive breast carcinoma.[3] CD19(+) B cells are identified as a significant factor in immune evasion from breast cancer due to their high tumor grade, ER-negative status, and expression of Interleukin-10 (IL-10) and PD-L1. Despite this, CD19-positive B cells have been observed to inhibit the aggressiveness of triple-negative breast cancer (TNBC) and the HER2-positive breast cancer subtype, extend patients' lifespans, and reinforce antitumor immunity.[16] Additionally, other studies have demonstrated that CD19(+) B cells prolong the life of those with muscle-invasive bladder cancer.[17] The part of CD19(+) tumor-infiltrating B cells in certain solid tumors is still a matter of contention. Insufficient systematic investigations have been carried out on CD19 across various types of cancers, highlighting the need for more comprehensive studies to ascertain its importance as a marker for tumors and a promising target for immunotherapy.

A study was carried out to compare the levels of CD19 mRNA and protein expression in tumor and normal samples. This study utilized several databases including The Cancer Genome Atlas (TCGA), Tumor Immune Estimation Resource (TIMER), Genotype-Tissue Expression (GTEx), and Human Protein Atlas (HPA). A comprehensive assessment was conducted to examine the correlation between CD19 expression and prognosis, gene mutation, DNA methylation, and immune infiltration. Coexpression analysis of CD19 and immune-related genes was also utilized to investigate the capacity of the CD19 gene in predicting tumor immunity. To identify possible chemotherapy drugs that specifically target CD19, we performed an analysis of drug sensitivity. Immunohistochemistry was then conducted to confirm the varying expression of CD19. The initial findings suggest that CD19 could serve as a predictive indicator for tumor immunotherapy. Additionally, they offer unique perspectives for future investigations on the tumor microenvironment (TME) and potential mechanisms of immunotherapy.

Materials and Methods

Data Collection

We collected clinical and RNA-sequencing data for 33 types of cancer from TCGA (https://portal.gdc.cancer.gov/) and TIMER 2.0 (http://timer.cistrome.org). To supplement the gene expression and clinical data of the normal and tumor groups with partial cancer deletion, the GTEx (https://commonfund.nih.gov/GTEx) database was utilized. The HPA (http://www.proteinatlas.org/) was employed to contrast CD19 protein expression between cancer and normal tissues, while the Gene Expression Profiling Interactive Analysis (GEPIA, http://gepia.cancer-pku.cn/) database was used to

compare the expression of CD19 across different pathological stages of samples from various types of cancer. The copy number of DNA and the frequency of gene mutations in CD19 were acquired from the cBioPortal (https://www.cbioportal.org/) database. We employed R 3.6.3 to integrate raw data from the above database.

Prognostic Analysis

Patients were categorized into high and low expression groups based on the median CD19 expression level. The survival of patients in both groups was compared by KM analysis to assess their overall survival (OS). Survival data was obtained on the 07-20-2019 version of the TCGA database with an estimated 11,400 clinical samples. Additionally, a study was performed to analyze the disease-specific survival (DSS) and progression-free interval (PFI) of the TCGA cohort. DSS could exclude the influence of nontumor death, and PFI could exclude the impact of other crossover and subsequent treatments. To evaluate the risk ratio of CD19 in predicting OS, DSS, and PFI across various types of cancer, a univariate Cox regression analysis was performed. A statistical significance was indicated when the P value was less than 0.05.

Methylation Analysis

We utilized the GSCALite (http://bioinfo.life.hust.edu.cn/GSCA/) system to examine the differences in CD19 promoter methylation between cancerous and healthy tissues. Subsequently, we explored the importance of CD19 methylation in relation to mRNA expression in different types of cancers. By utilizing the UALCAN platform (http://ualcan.path.uab.edu/), it is possible to compare the methylation levels of the CD19 promoter in both cancer and normal groups.

Assessment of TMB and MSI

To explore the role of CD19 in the immune system of the TME, we investigated the correlation between CD19 expression and two crucial biomarkers of the TME, namely TMB and MSI.[18,19] TMB denotes the count of base mutations per million bases in every tumor specimen. MSI is the abbreviation for the occurrence of insertions or deletions of repetitive segments of microsatellites in cancerous cells, causing a modification in the length of microsatellites and ultimately resulting in MSI. Using R 3.6.3, we performed an evaluation to examine the correlation between CD19 expression and biomarkers in the TME.

Gene Set Enrichment Analysis (GSEA)

For the analysis of The Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO), we utilized the GSEA (https://www.gsea-msigdb.org/gsea/index.jsp) database to explore the function and downstream targets of CD19. Statistical significance was indicated when the P value was less than 0.05. The association between CD19 and immune-associated genes was assessed by generating a coexpression heatmap of CD19 and immune-related genes across various types of cancer. Additionally, a coexpression heatmap was generated to compare the relationship between CD19 expression and the expression of genes associated with immune activation, immunosuppression, chemokines, and chemokine receptors. The publicly accessible multi-omics database LinkedOmics (http://www.linkedomics.org/login.php#dataSource) conducts cancer analysis, explores potential target genes, and carries out enrichment analyses.[20] The Pearson correlation coefficient between CD19 and its associated genes was assessed using LinkedOmics. Subsequently, the coexpression maps and volcano plot were generated.

Evaluation of Infiltration of Immune Cells

By utilizing the ESTIMATE score, which combines the matrix and immune elements within the TME, it is possible to assess the quantity of stromal and immune cells present in cancerous tissue. A higher rating indicates a larger quantity of these cells. The overall score represents the total of the matrix and immune scores, indicating the collective proportion of the two elements in the TME. We performed a correlation analysis of gene expression and immune infiltration using the TIMER 2.0 tool (cistrome.shinyapps.io/timer). The database provided is an extensive resource for examining immune infiltration across different forms of cancer.[21,22] The TIMER algorithm facilitated the calculation of the infiltration prevalence of six immune cell categories: B cells, CD4(+) T cells, CD8(+) T cells, neutrophils, macrophages, and dendritic cells (DCs).[23] To illustrate a specific form of cancer, we categorized individuals with tumors into two factions

according to their levels of CD19 expression. This allowed us to assess the enrichment scores of immune cells between the two groups.

Drug Sensitivity Analysis

By conducting a Spearman correlation analysis, the GSCALite database combined the data on drug sensitivity and gene expression profiles from cancer cell lines in GDSC and CTRP. This allowed for the evaluation of the connection between the expression of individual genes in the gene set and the IC_{50} of small molecules/drugs. Drug sensitivity analysis based on CD19 expression was conducted using the GSCALite platform. A positive correlation implies that tumor cells expressing high levels of CD19 are more prone to developing drug resistance, whereas a negative correlation suggests that tumor cells with high CD19 expression are more susceptible to drug effects.

Immunohistochemistry

Immunohistochemical staining was performed on three instances of stomach adenocarcinoma (STAD), three instances of kidney renal clear cell carcinoma (KIRC), and their corresponding adjacent samples at the Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. The main treatment received by those cancer patients was surgical resection. The clinical samples used in this study adhered to the principles outlined in the Declaration of Helsinki. The research was approved by the Ethics Committee of Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. Anti-CD19 antibodies were procured from Abcam's website. After the manufacturer's declaration, immunohistochemical staining was conducted, and then the images were taken with various fields randomly selected under a light microscope.

Statistical Analysis

The expression of the CD19 gene in both the tumor and its corresponding normal tissues was analyzed using Wilcox's test. Cox regression analysis, Kaplan-Meier method and Log Rank test were used to perform survival analysis, and Spearman correlation test to calculate the correlation between CD19 gene expression levels and other variables. Statistical analyses of R versions 3.6.3, and 4.2.1 were conducted with $p < 0.05$ being deemed statistically significant.

Results

The Expression of CD19 Varies in Pan-Cancer Tissues

Examining CD19 mRNA levels in 33 cancer types from the TCGA database, it was discovered that BRCA, diffuse large B-cell lymphoma (DLBC), glioblastoma multiforme (GBM), head and neck squamous carcinoma (HNSC), KIRC, lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), and uterus corpus endometrial cancer (UCEC) exhibited higher CD19 expression compared to normal tissues. In contrast, the levels of CD19 were reduced in COAD, kidney chromophobe (KICH), rectum adenocarcinoma (READ), prostate adenocarcinoma (PRAD), and thyroid carcinoma (THCA) (Figure 1A). According to the TIMER database, the expression of CD19 in DLBC was showing a notable level of expression, consistent with the findings of the TCGA database (Figure 1B).

Due to the limited number of normal samples in the TCGA database, it was necessary to compare normal samples from GTEx with tumor tissues from the TCGA database. CD19 expression was higher in adrenocortical carcinoma (ACC), BRCA, DLBC, esophageal carcinoma (ESCA), GBM, HNSC, LUAD, LUSC, skin cutaneous melanoma (SKCM), STAD, and UCEC when compared to normal samples. CD19 exhibited a noticeable difference, appearing significantly lower compared to the healthy tissues of COAD, KICH, kidney renal papillary cell carcinoma (KIRP), acute myeloid leukemia (LAML), ovarian serous cystadenocarcinoma (OV), PRAD, READ, testicular germ cell tumor (TGCT), THCA, and thymoma (THYM) (Figure 1C). For the paired tumor samples, CD19 expression changed significantly in the eight corresponding normal samples (Figure 1D). The HPA database compared the expression of CD19 in four tumor sections with normal sample sections (Supplementary Figure 1).

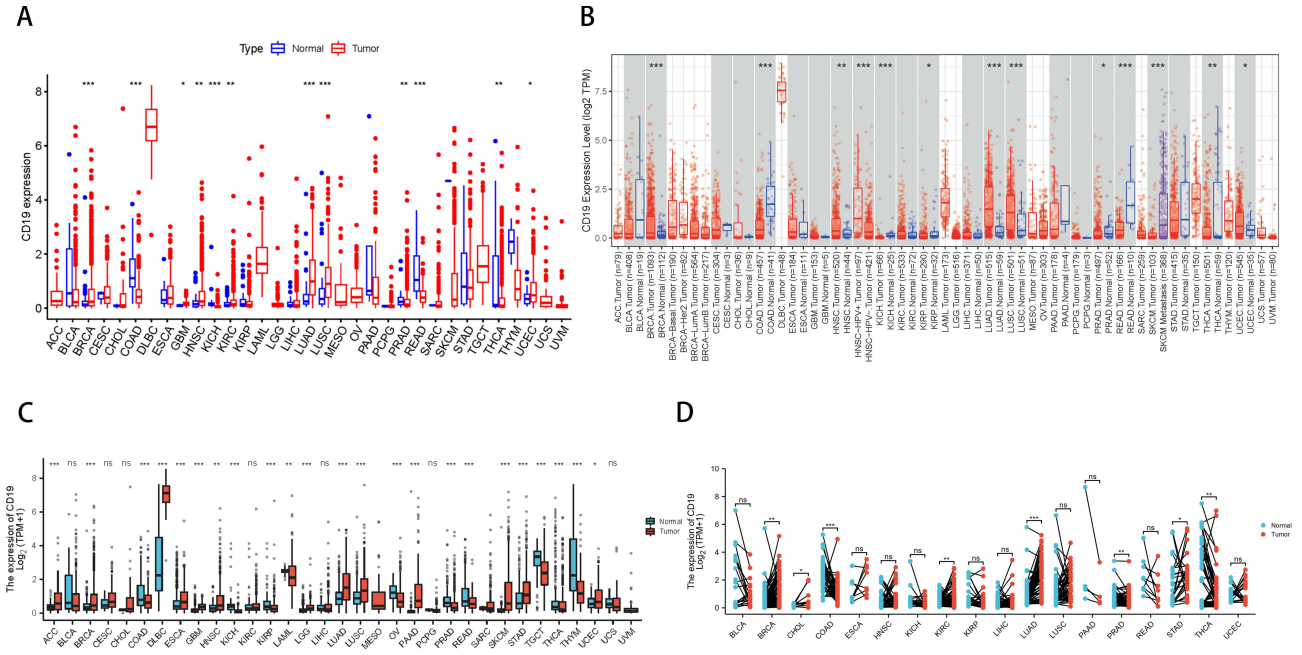

Figure 1 The mRNA expression levels of CD19 in both healthy tissues and cancerous tissues. (**A**) Comparison of CD19 expression differences among 24 types of cancer and adjacent tissues using data from the TCGA database. (**B**) Expression differences in CD19 between tumor and normal tissues in 21 cancers were obtained from the TIMER database. (**C**) The differential expression of CD19 between tumor and normal tissues in 31 cancers was compared in the TCGA and GTEx databases. (**D**) Differential expression of CD19 in tumor and paired normal samples. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ns, not statistically significant.

Advanced Stages of Cancer Show an Inverse Correlation with CD19

CD19 expression in different pathological stages of cancer was assessed using the stage plot feature of the GEPIA database. Figure 2 showed a notable decrease in CD19 expression in advanced THCA, UCS, UCEC, pancreatic adenocarcinoma (PAAD), SARC, OV, and TGCT. Therefore, we suggest that tumor disease progression may be associated with decreased CD19 expression in the advanced stages of many cancers. High CD19 expression is expected to improve the prognosis of tumor patients.

Prognostic Importance of Elevated CD19 Levels in Various Types of Cancer

Survival association analyses, including OS, DSS, and PFI, were used to investigate the correlation between CD19 expression and prognosis in different tumor types. Initially, data from the TCGA database was used to employ Univariate Cox regression analysis to further investigate the connection between CD19 expression and OS in cancer. The findings indicated that CD19 posed a risk element for KIRC (p=0.005), brain lower grade glioma (LGG) (p<0.001), and KIRP (p=0.006). Nonetheless, it acted as a safeguarding element for HNSC (p<0.001), SKCM (p<0.001), LUAD (p=0.002), CESC (p=0.007), UCEC (p=0.01), BRCA (p=0.041), and ESAD (p=0.05) (Figure 3A). According to a KM survival analysis, high CD19 expression was positively associated with high OS in patients with BRCA (p=0.046), GBM (p=0.040), HNSC (p<0.001), LUAD (p=0.010), SKCM (p=0.001) and UCEC (p=0.032) (Figure 3B–G). Nevertheless, a negative outlook in LGG individuals (p=0.031) was linked to elevated CD19 levels (Figure 3H). Median survival with high CD19 expression was 86 days higher in BRCA, 128 days higher in GBM, 91 days higher in HNSC, 7 days higher in LUAD, 561 days higher in SKCM, and 15 days higher in UCEC compared to median survival with low CD19 expression. However, the median survival of CD19 high expression was 5 days lower in LGG compared to CD19 low expression. The results obtained from the DSS were similar to those obtained from the OS. CD19 was identified as a protective role in HNSC (p<0.001), SKCM (p=0.001), LUAD (p=0.006), CESC (p=0.007), UCEC (p=0.007), and OSCC (p=0.045) (Supplementary Figure 2A). The KM analysis demonstrated a significant improvement in the prognosis of cancer patients with high expression of CD19 (Supplementary Figure 2B–J). The Cox regression analysis of PFI revealed that CD19 acted as a protective factor in CESC (p=0.006), BRCA (p=0.007), CHOL (p=0.041), and HNSC

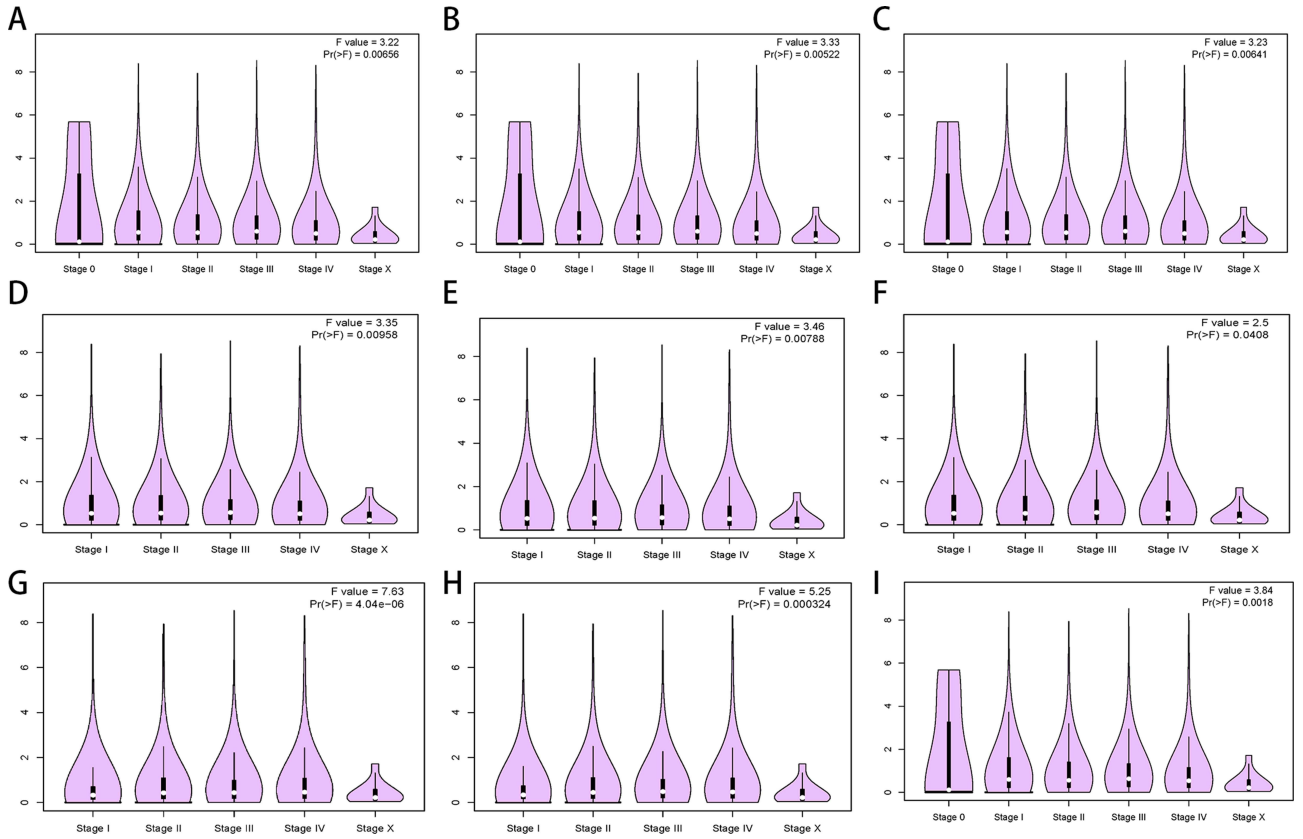

Figure 2 Correlation between CD19 expression and pathological staging in different cancers. (**A**) THCA, (**B**) UCS, (**C**) UCEC, (**D**) PAAD, (**E**) SARC, (**F**) OV, (**G**) LIHC, (**H**) LGG, (**I**) TGCT.

(p<0.001) (Supplementary Figure 3A). LGG patients who had higher levels of CD19 expression experienced a poorer PFI compared to those with lower CD19 expression (Supplementary Figure 3B–G). The results were similar to those of OS. The potential reduction of unfavorable prognosis in most tumors by the high expression of CD19 makes it a promising marker for improving the outlook of various types of cancer.

Correlation Between CD19 Expression and DNA Methylation in Human Cancers

The GSCALite platform was utilized to evaluate the correlation between DNA methylation and mRNA expression of CD19 and immune-related genes (CD28, CD48, CD276, CD80).[24] In all tumors studied (Figure 4A), a negative association was found between the expression of CD19 mRNA and DNA methylation, as well as between DNA methylation and other immunity-related genes. Additionally, significantly reduced CD19 methylation was revealed by the results in HNSC, LIHC, BRCA, LUSC, and LUAD (Figure 4B). We also verified the results using the online tool UALCAN. The UALCAN platform allows for a comparison of the DNA methylation level of CD19 between cancer patients and normal controls. The CD19 promoter methylation level was notably decreased compared to the normal group (Figure 5).

Mutational Characteristics of CD19 Across Cancers

The cBioPortal database facilitates the examination of genetic mutations, structural variants, and copy numbers in cancers. Exploring the genomic alterations in CD19 among patients with various cancers, we employed the cBioPortal database. CD19 gene changes are mainly reflected in copy number amplification and gene mutation. Out of all the individuals, those with UCEC exhibited the most frequent alterations in the CD19 gene, with a remarkable occurrence of nearly 6% (Figure 6A). A total of 112 mutations were found in CD19's tumor samples from the TCGA database, including 94 missense mutations, 13 truncating mutations, and 5 splice mutations. The protein encoded by CD19 had the

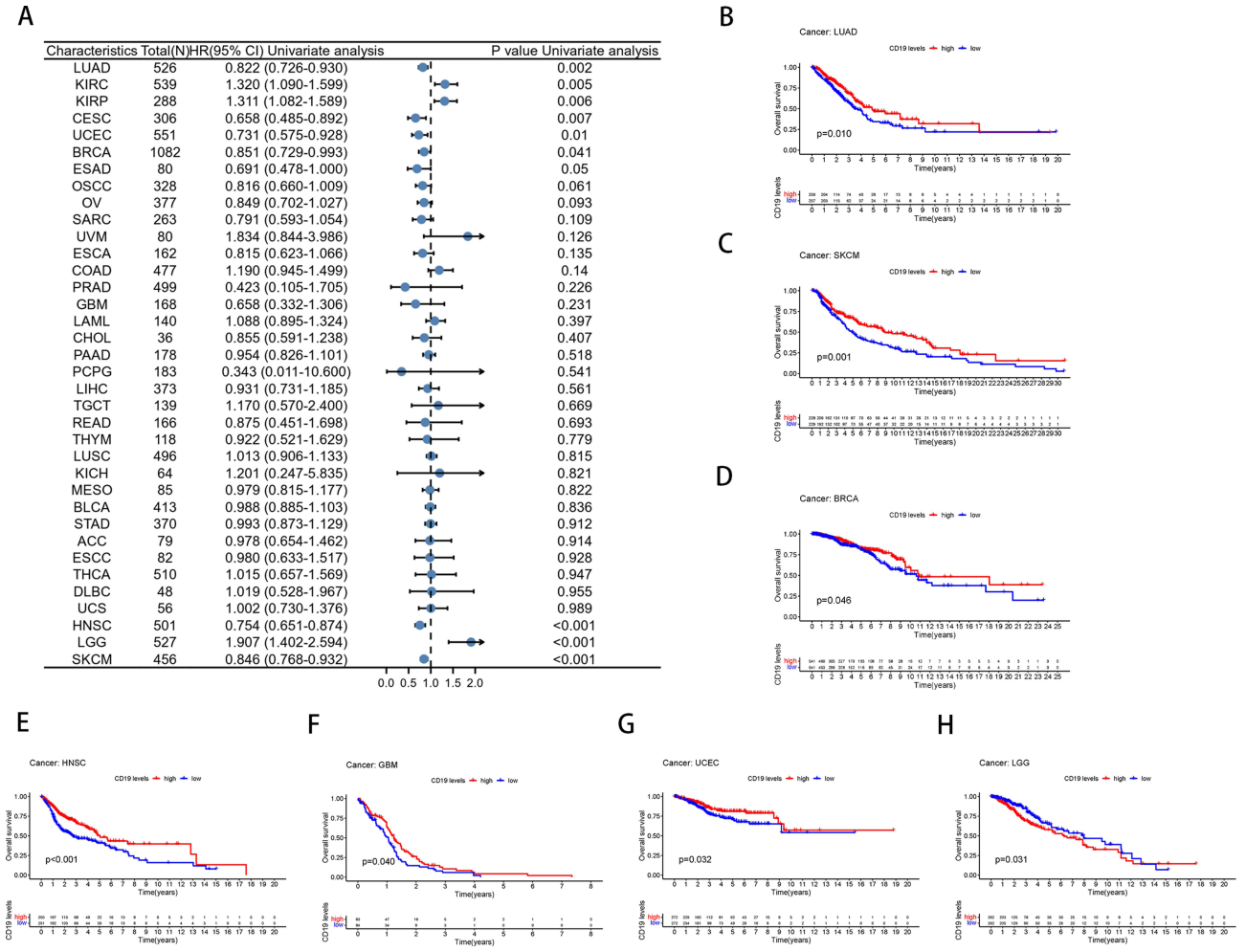

Figure 3 The correlation between the expression of CD19 and the overall survival (OS) of individuals. (**A**) Forest plot displaying the outcomes of the univariate Cox regression analysis of CD19 in pan-cancer samples obtained from the TCGA database. (**B–H**) Kaplan–Meier curve demonstrates the correlation between CD19 expression and OS in samples from various types of cancer.

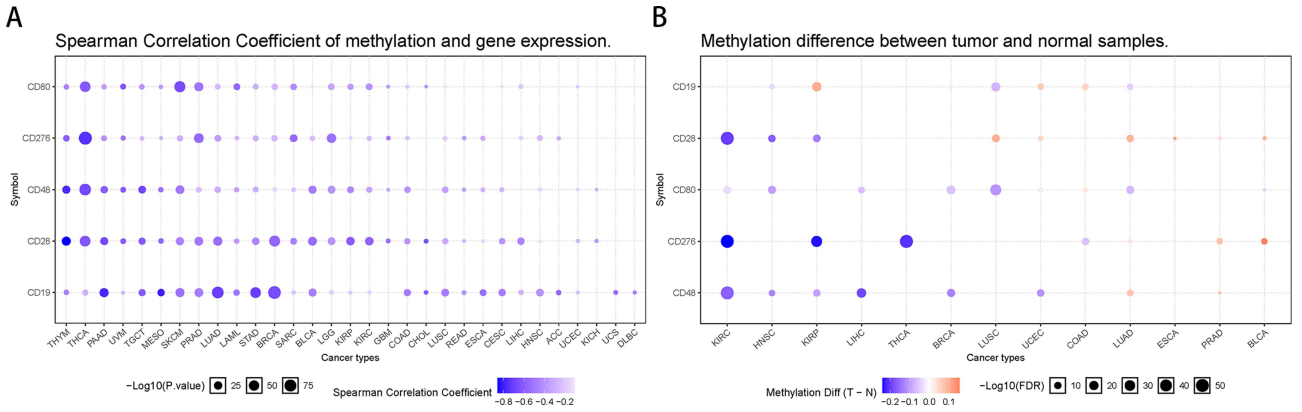

Figure 4 Methylation levels of CD19 in human cancers. (**A**) Relationship between CD19 mRNA expression and DNA methylation across 33 different types of cancer. (**B**) Evaluation of CD19 DNA methylation differences between tumor and normal tissues in 13 different types of cancers using the GSCALite platform.

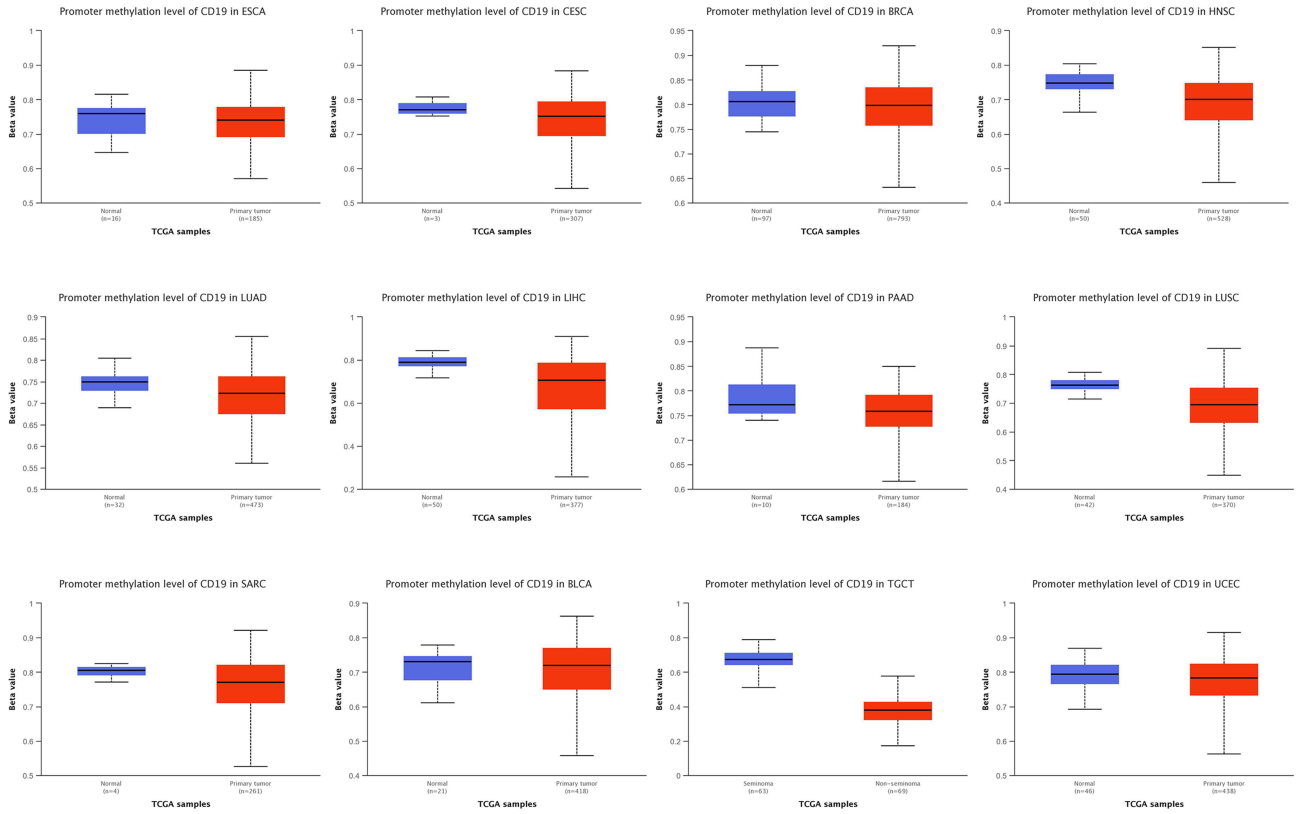

Figure 5 The methylation level of the CD19 promoter was compared between the tumor group and the normal group.

most mutations in residues 99–103, with 27 mutations (Figure 6B). It is conjectured that CD19 could be implicated in tumorigenesis and cancer growth, due to the varying expression and genetic alterations that take place during cancer development.

The effectiveness of tumor immunotherapy can be predicted by TMB and MSI in the TME, which is associated with antitumor immune response.[25] To examine if the CD19 status can serve as a predictor of tumor immunity, we conducted a comparison of the gene expression profiles in tumors exhibiting high and low levels of CD19 expression. In UVM, THCA, TGCT, STAD, PRAD, PAAD, MESO, LUSC, and LIRC (Figure 6C), TMB showed a negative correlation with CD19. A positive correlation between MSI and CD19 expression was observed in THCA, LUAD, and DLBC tumors, while a negative correlation was found in TGCT, STAD, SKCM, and LUSC (Figure 6D). These findings suggest that CD19 may have a potential impact on immunotherapy, potentially influencing the composition and mechanism of the TME.

Enrichment Analysis of CD19-Related Genes

In the assessment of 33 tumor types, we employed GSEA to analyze the pathways associated with CD19. In CESC, PAAD, PCPG, UCEC, and UVM, the correlation between the signaling pathway of the B-cell receptor and CD19 suggests that CD19 might impact the TME by participating in this pathway (Figure 7A–E). Next, we used the LinkedOmics database and OV as an example to verify the genes related to CD19 expression. In the OV dataset, there were 1034 genes (represented by red dots) that exhibited a significant positive correlation with CD19, whereas 166 genes (represented by green dots) showed a significant negative correlation with CD19 (FDR<0.01) (p<0.05) (Figure 7F). Heatmaps illustrate a positive and negative correlation between genes and CD19 expression (Figure 7G and H). CD19 exhibited a positive correlation with the majority of immune genes in OV among the aforementioned factors.

Evaluation of Genes Associated with the Immune System

In order to explore the molecular mechanism underlying the regulation of immunity during tumor formation by CD19, we performed a screening of 47 genes related to the immune system and examined their association with CD19 in

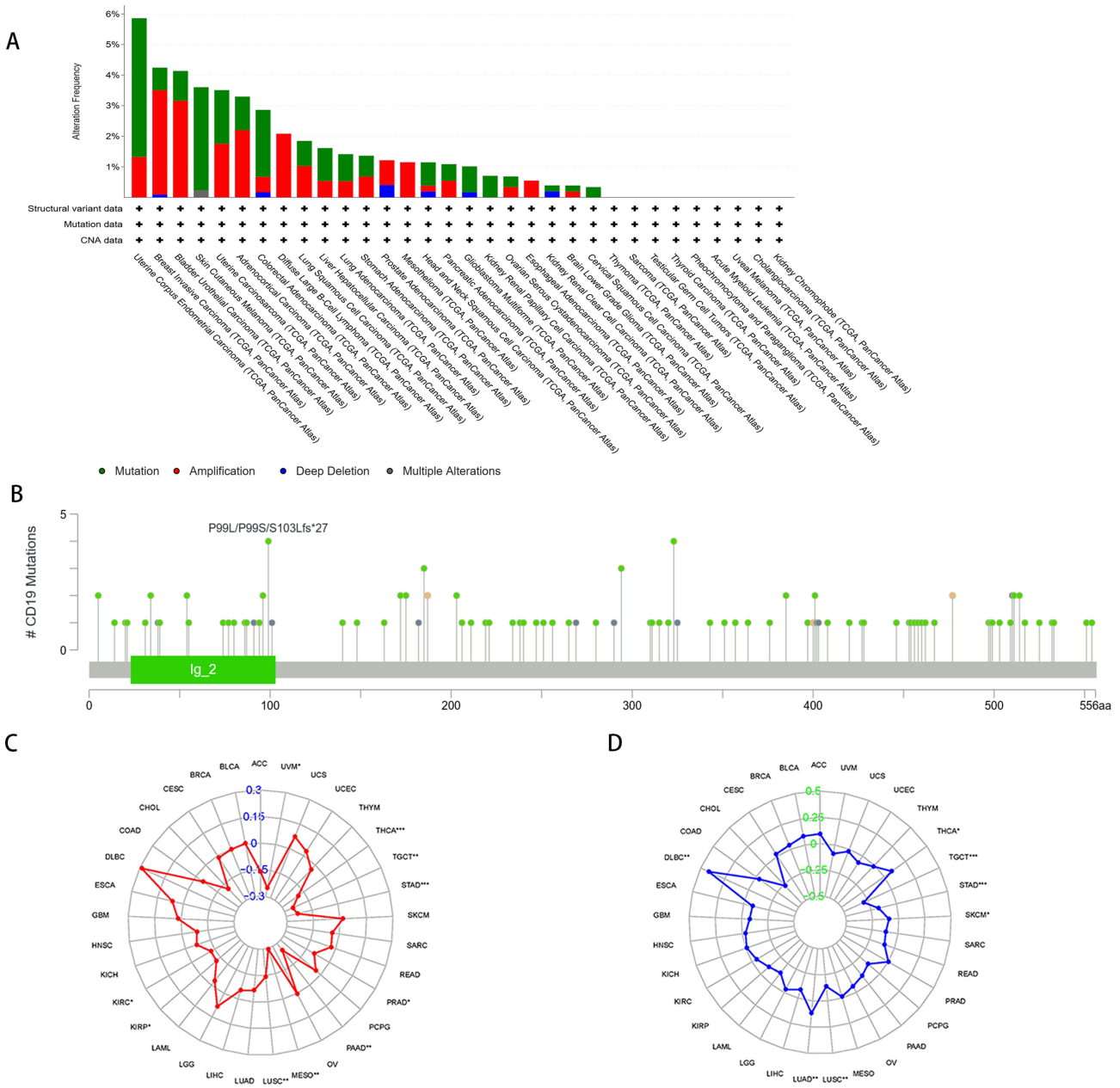

Figure 6 Mutational profiles of CD19 in human cancers. (**A**) Changes in the CD19 gene in multiple tumors according to the cBioPortal tool. (**B**) The mutation sites in CD19 in multiple tumors according to the cBioPortal tool. (**C**) Relationship between CD19 expression and TMB in human cancers. (**D**) Relationship between CD19 expression and MSI in human cancers.

different types of cancer. In various types of cancer, such as BLCA, BRCA, CESC, CHOL, COAD, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LGG, LIHC, LUAD, LUSC, MESO, OV, PAAD, PCPG, PRAD, READ, SARC, SKCM, STAD, TGCT, THCA, THYM, UCEC, and UVM, our analysis discovered a positive relationship between CD19 and the expression of genes related to the immune system (Figure 8A). Hence, CD19, a novel target for anticancer immunotherapy, is anticipated to be utilized in conjunction with immune-related genes to enhance targeted immunotherapy. Tumors can evade the immune system by depleting T-cell populations. To gain further insight into the potential involvement of CD19 in immune evasion, we investigated the correlation between CD19 and immune-stimulating genes (Figure 8B), immune-inhibiting genes (Figure 8C), genes encoding chemokines (Figure 8D), and genes encoding chemokine receptors (Figure 8E) in T-cells that had undergone depletion. The analysis showed a strong connection between the expression of CD19 and the majority of genes that activate or suppress the immune system, including TNFRSF13B, CD27, and TIGIT.

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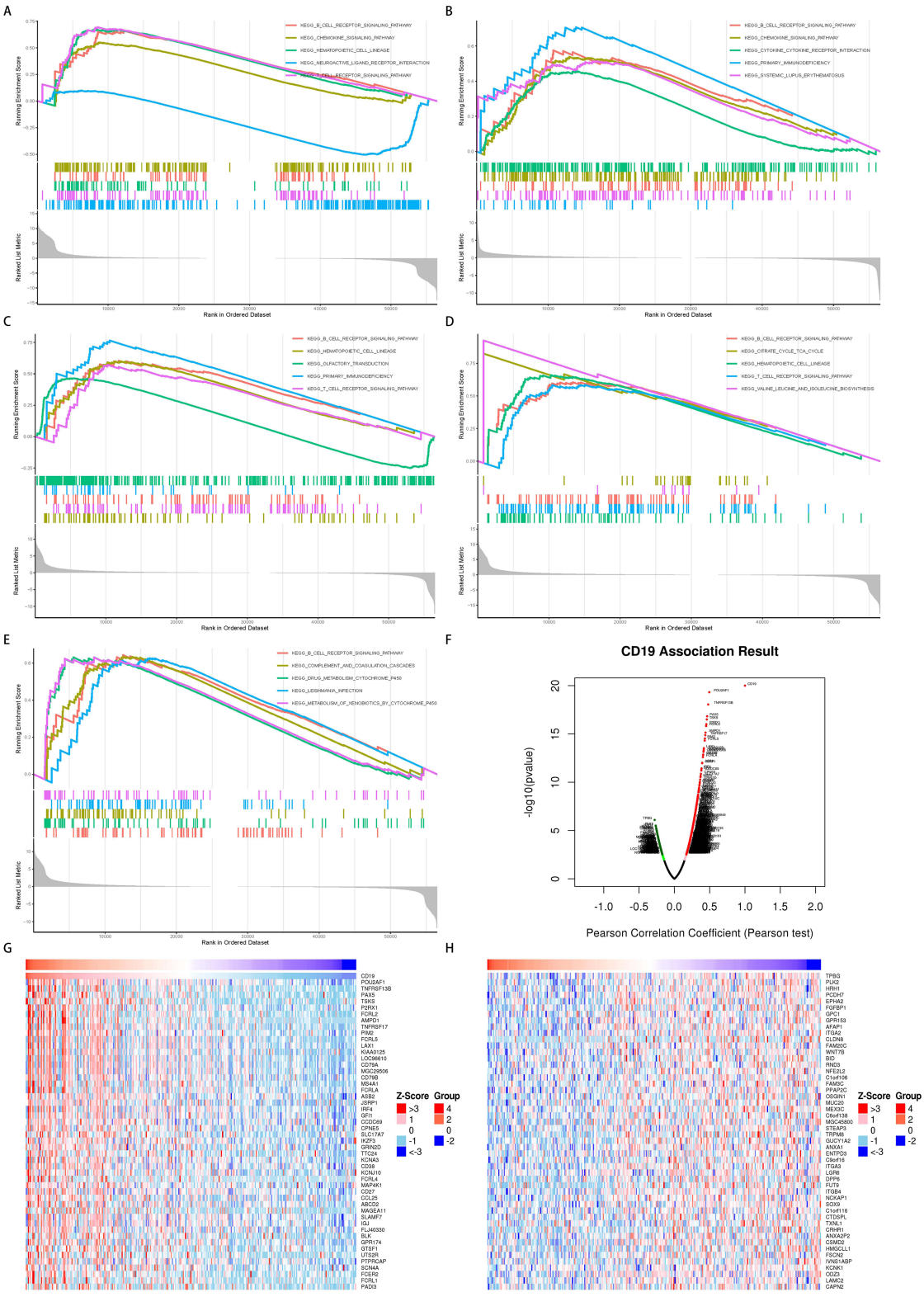

Figure 7 GSEA of CD19. (**A**–**E**) KEGG pathways enriched for CD19 across cancers. (**F**) Volcano plot of immune-related genes that were significantly associated with CD19 expression. (**G**) Coexpression heatmap of immune-related genes that were positively correlated with CD19 expression. (**H**) Coexpression heatmap of immune-related genes that were negatively correlated with CD19 expression.

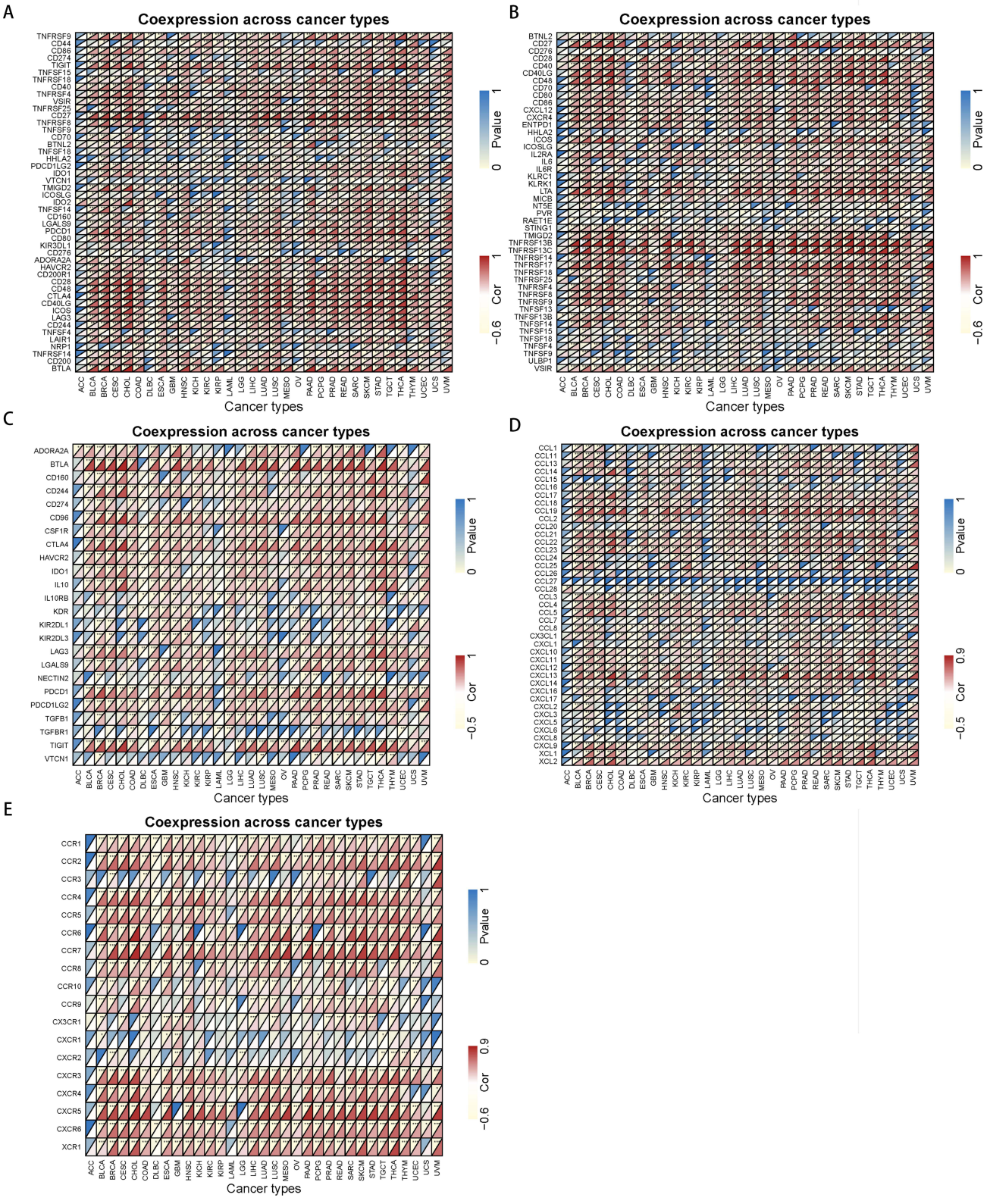

Figure 8 Coexpression analysis of CD19 and immune-related genes. Coexpression of CD19 and (**A**) immune-related genes, (**B**) immune-activating genes, (**C**) immunosuppressor genes, (**D**) chemokine genes, and (**E**) chemokine receptor genes. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. ns, not statistically significant.

An observed positive correlation was found between CD19 and chemokines such as CCL19 and CXCL13, along with chemokine receptors like CXCR5 and CCR7.

TME Analysis

The TME scores, which are crucial for the survival of cancer cells and play a significant role in tumor growth and metastasis, were determined using the ESTIMATE algorithm.[26] The ESTIMATE scores may be perceived as a sign of its purity, while immune and stromal scores can be interpreted as a representation of its immune and stromal components. In most cancers, there was a significant link between CD19 and the infiltration of tumor immune cells as well as the makeup of immune elements in the TME. This association was found to be positively correlated with the ESTIMATE score. However, DLBC exhibited a negative correlation with the ESTIMATE score, as shown in Figure 9.

Correlation Between CD19 Expression and Immune Cell Infiltration

To investigate the correlation between CD19 expression and the infiltration of immune cells, which is closely connected to tumor growth and immunity, an analysis was performed on the TIMER2.0 database.[27] The results demonstrated a favorable association between CD19 expression and the infiltration of CD8(+) T cells, Treg cells, B cells, DCs, macrophages, natural killer (NK) cells, and follicular helper T cells (Figure 10). Conversely, no significant correlation was observed between CD19 expression and the infiltration of other immune cells, including CD4(+) T cells, monocytes, and Th2 cells. By taking BRCA as a case study, we examined how CD19 expression impacts the score of immune cell

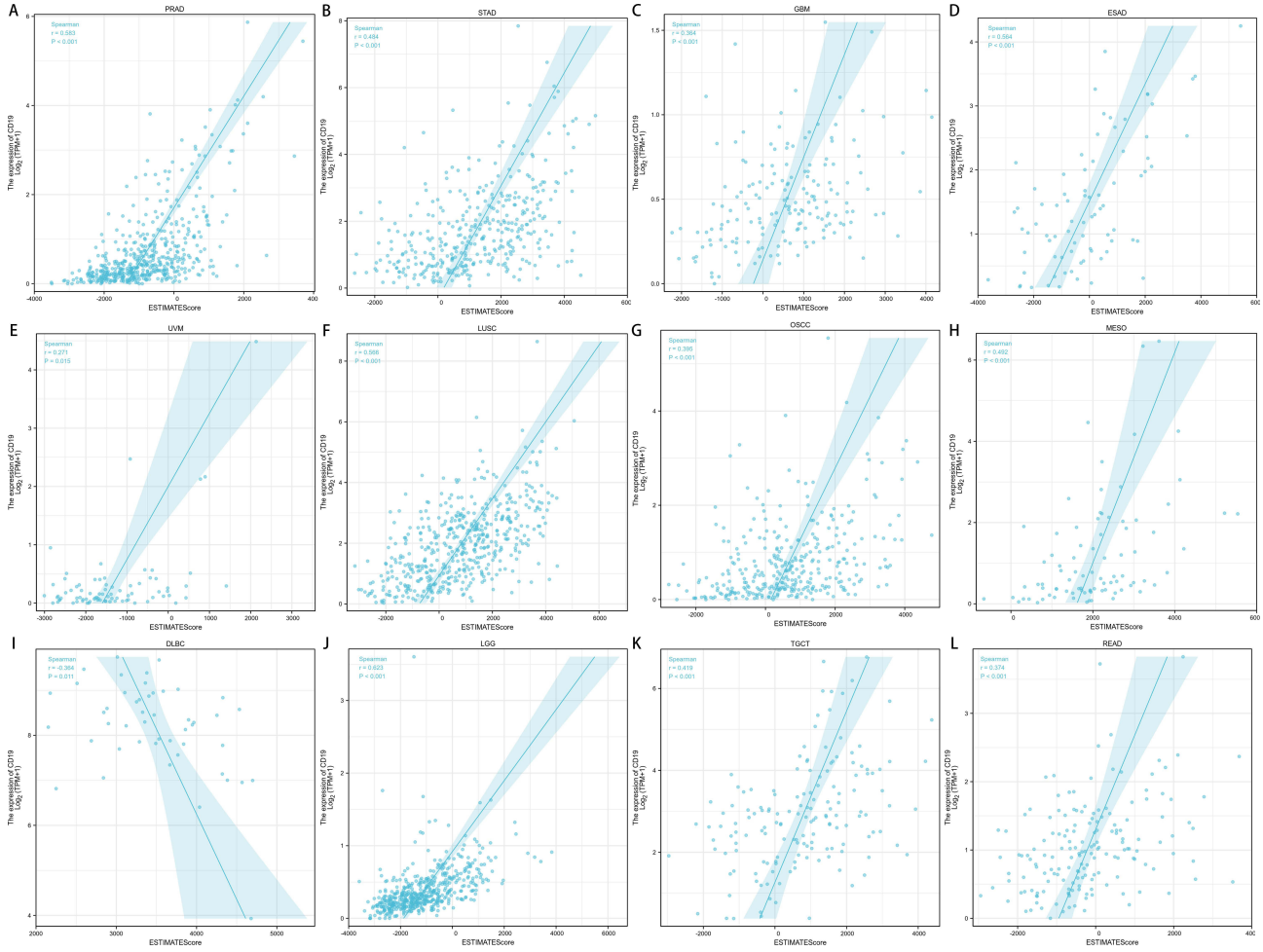

Figure 9 Relationship between CD19 expression in certain cancers and the ESTIMATE score of the TME. (**A**) PRAD, (**B**) STAD, (**C**) GBM, (**D**) ESAD, (**E**) UVM, (**F**) LUSC, (**G**) OSCC, (**H**) MESO, (**I**) DLBC, (**J**) LGG, (**K**) TGCT, (**L**) READ.

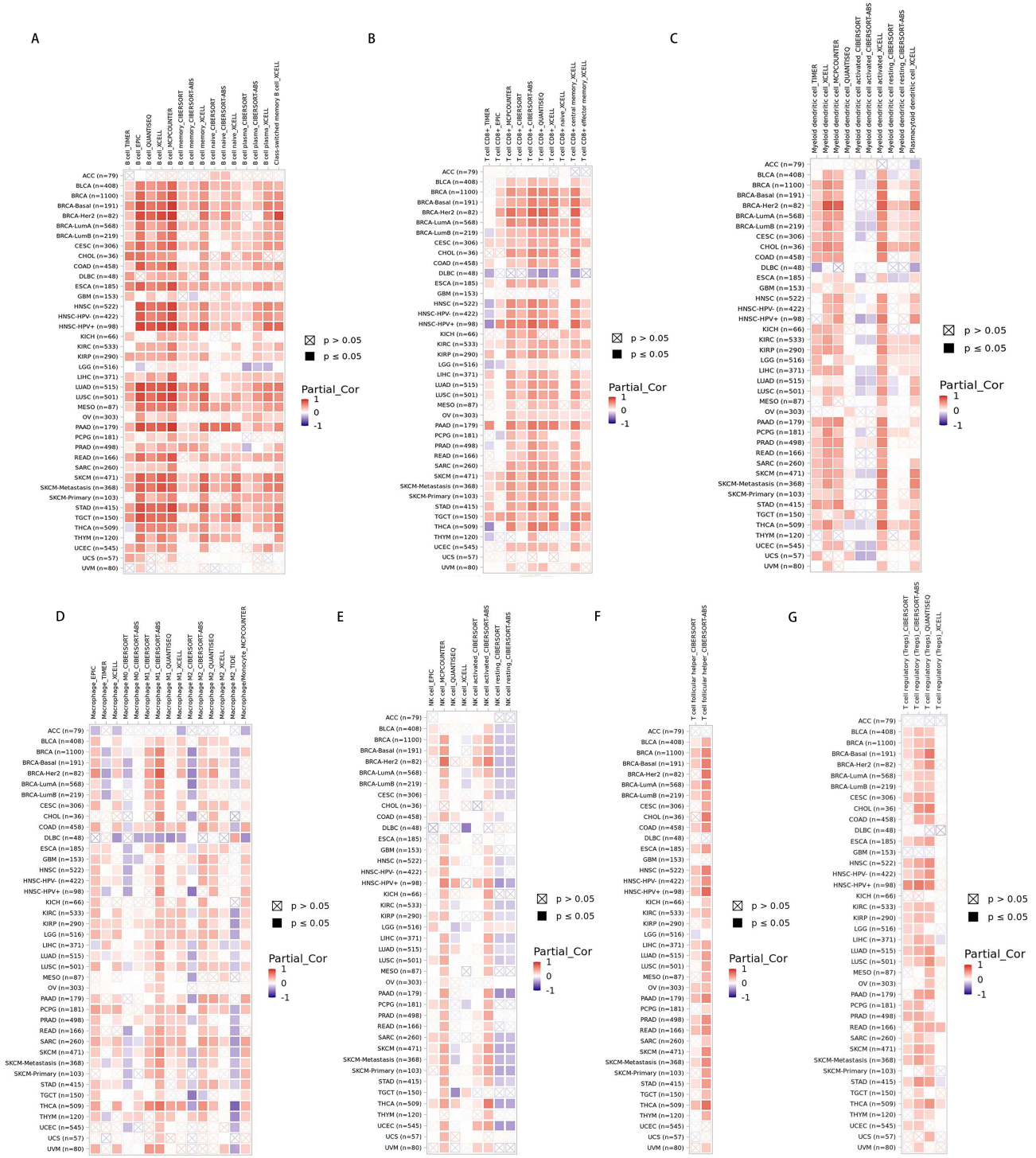

Figure 10 Relationship between CD19 expression and immune cell infiltration in the pan-cancer TME. (**A**) B cells, (**B**) CD8(+) T cells, (**C**) DCs, (**D**) macrophages, (**E**) NK cells, (**F**) follicular helper T cells, and (**G**) Treg cells.

enrichment. We observed a strong association between the expression of CD19 and the presence of diverse immune cells, including CD8(+) T cells, Treg cells, B cells, DCs, macrophages, NK cells, and follicular helper T cells (Supplementary Figure 4). The provided data could potentially elucidate the reasons behind tumor progression inhibition by CD19, indicating a correlation between CD19 and the response to immunotherapy. This discovery offers a fresh perspective on CD19 as a potential biomarker for assessing immunotherapy response.

CD19 and Drug Reactivity

The GSCALite platform was utilized to conduct a correlation analysis between CD19 and drug sensitivity in order to identify potential drugs associated with CD19. The findings indicated a negative correlation between CD19 expression and the antitumor drugs/molecules mithramycin, momelotinib, depsipeptide, TAK-659 (isomer 1), actinomycin D, A-1210477, PF-03758309, A-911, AT-9283, doxorubicin, AT-7519, defactinib, BMS-387032, PF-562271, KW-2449, and MG-132, as illustrated in Figure 11.

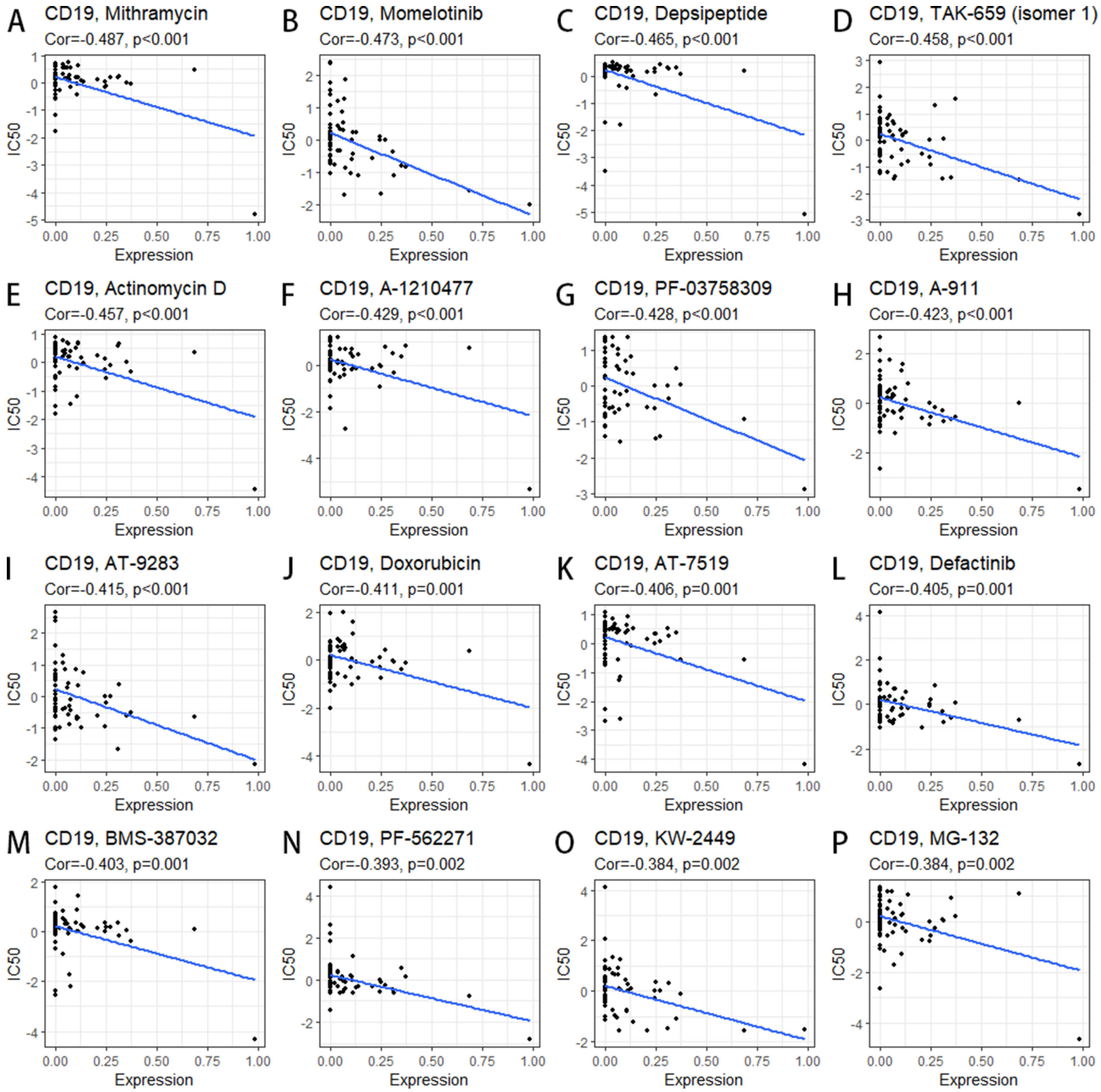

Figure 11 The relationship between CD19 expression and drug response was predicted. (**A**) Mithramycin, (**B**) momelotinib, (**C**)depsipeptide, (**D**) TAK-659 (isomer 1), (**E**) actinomycin D, (**F**) A-1210477, (**G**) PF-03758309, (**H**) A-911, (**I**) AT-9283, (**J**) doxorubicin, (**K**) AT-7519, (**L**) defactinib, (**M**) BMS-387032, (**N**) PF-562271, (**O**) KW-2449, and (**P**) MG-132.

Validation of Some Tumor Expression in Pan-Cancer by immunohistochemistry

The use of immunohistochemistry revealed the presence of CD19 in gastric cancer, renal cancer, and the surrounding tissues adjacent to these cancers. In Figure 12, it was observed that the expression level of CD19 in STAD and KIRC exceeded that in normal tissues.

Discussion

Cancer cells can stimulate inhibitory immune checkpoints to form a TME with immunosuppressive characteristics. The use of CAR-engineered T cells that target CD19 has initiated a fresh era of cancer immunotherapy known as "living drugs".[28] Despite the observation of cytotoxicity in various refractory tumors, such as primary double-hit lymphoma cells,[29] multiple myeloma,[30] and relapsed or refractory B-lineage acute lymphoblastic leukemia,[31] the exact role of CD19 in numerous cancers remains uncertain. This study extensively explored the diverse expression of the CD19 gene in different cancer tissues and its association with pathological progression, cancer prognosis, mutation and DNA methylation, TMB and MSI, immune infiltration, and drug susceptibility. Our research revealed that elevated levels of CD19 expression have the potential to enhance the prognosis of patients with various cancers. Additionally, it can also elevate the extent of immune infiltration in the TME, particularly in cases of BRCA. CD19 gene mutations occur in a variety of tumors. The highest frequency of CD19 gene changes was found in UCEC patients. The CD19 gene expression was hindered by the methylation of DNA in CD19, in many kinds of cancer, where there was a significant decrease in CD19 methylation. In addition, CD19 was strikingly associated with TMB, MSI, and ESTIMATE score in a variety of tumors. CD19 is expressed alongside several immune-related genes, such as TNFRSF13B, CD27, and TIGIT, which serve as indicators of the response to immunotherapy. Tumors with high CD19 expression were sensitive to a variety of chemotherapeutic drugs and molecules, including mithramycin, momelotinib, depsipeptide, TAK-659 (isomer 1), actinomycin D, A-1210477, PF-03758309, A-911, AT-9283, doxorubicin, AT-7519, defactinib, BMS-387032, PF-562271, KW-2449 and MG-132.

Initially, we assessed the CD19 expression, pathological stage, and prognostic importance across various cancers by utilizing TCGA, TIMER, GTEx, HPA, and GEPIA datasets. Initially, the presence of CD19 was detected in various types of cancers and their corresponding healthy tissues, revealing significant differences in gene expression between 23 cancer types and control tissues. The HPA's immunohistochemical findings supported our results, aligning with the CD19 mRNA expression results obtained from the TCGA and GTEx databases. Studies conducted prior have only indicated a correlation between CD19 expression and breast cancer,[3] bladder cancer,[14] acute lymphoblastic leukemia,[31] lymphoma,[29] and myeloma cells.[27] Despite this, there is a lack of comprehensive research on CD19 in many solid tumors.

Improved survival can be achieved by examining the differential expression of CD19 in different pathological stages of cancer, which can serve as early indicators of diseases when cancer is diagnosed early. At later stages, the analysis revealed a reduction in CD19 expression. To gain a deeper understanding of the significance of CD19 in clinical risk stratification, we conducted additional evaluations to examine the association between CD19 and prognosis in various types of cancer. An analysis of KM OS indicated that CD19 acted as a safeguarding element for BRCA, GBM, HNSC, LUAD, SKCM, and UCEC while posing a threat to LGG. To evaluate the association between CD19 and DSS and PFI in cancer patients, a univariate Cox regression analysis was performed. This analysis was conducted because OS, which includes non-cancer-related deaths, does not provide an accurate reflection of the impact of treatment on tumor growth response, migration, and invasion.[32] DSS and PFI analyses further validated that CD19 improves the prognosis of most tumor patients. Therefore, we can infer that CD19 benefits most tumors.

Epigenetic carcinogenesis is fundamentally characterized by aberrant DNA methylation.[33] This study suggests a correlation between the extent of DNA methylation and the suppressive impact of CD19 on tumors. It provides evidence that CD19, along with other genes associated with the immune system, can hinder DNA methylation in different types of tumors. To obtain a thorough understanding of the molecular characteristics of the CD19 gene, which is associated with drug resistance in most tumor cells,[34] the cBioPortal database was utilized. For instance, a research study found that many individuals who had received CART-19 immunotherapy for B-cell acute lymphoblastic leukemia

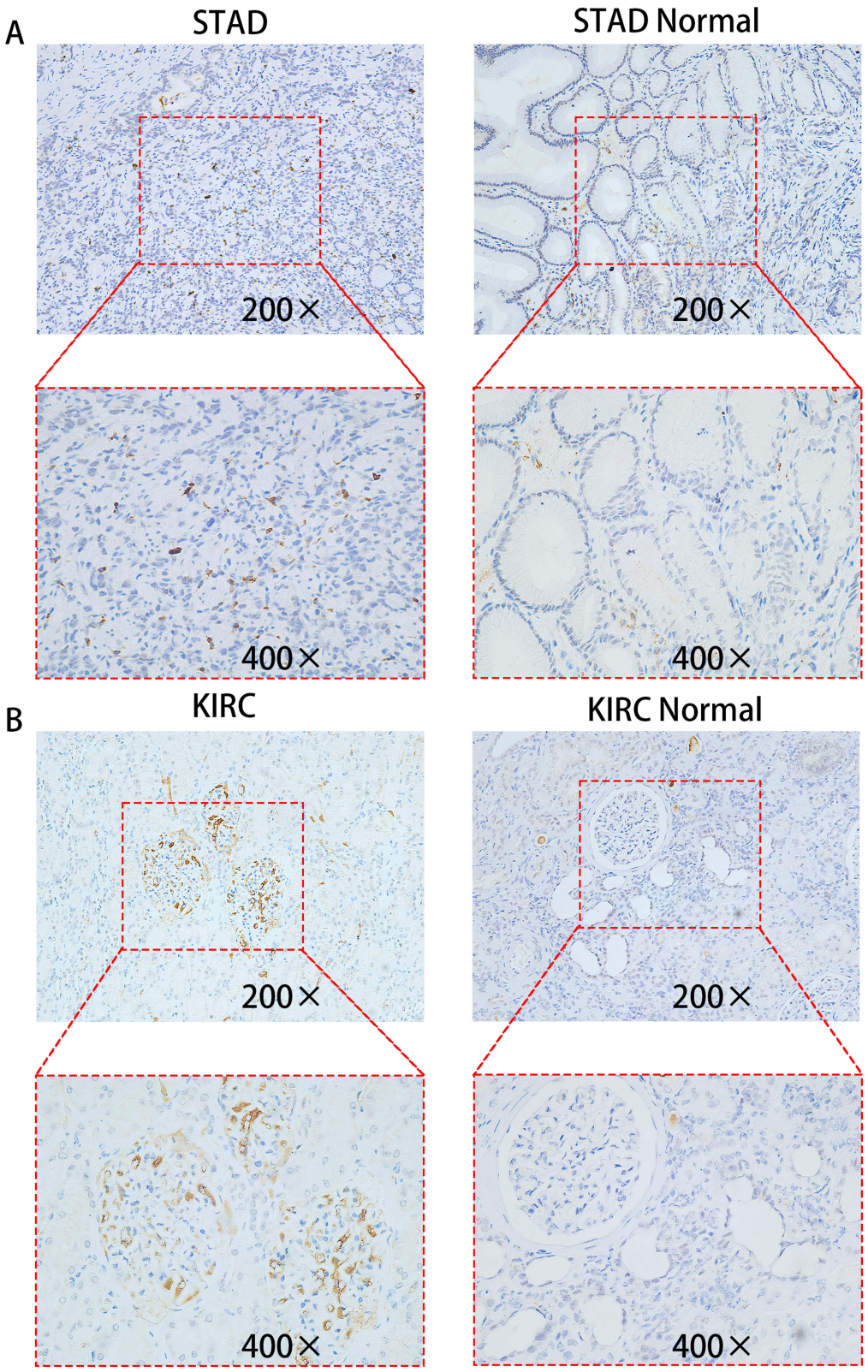

Figure 12 Immunohistochemistry of CD19 in STAD, KIRC, and normal tissues. (**A**) CD19 expression was higher in STAD than that in normal gastric tissues. (**B**) CD19 expression in KIRC was higher than that in normal renal tissue.

(B-ALL) experienced a relapse because they lacked the cognate CD19 epitope.[35] UCEC exhibited the most elevated rate of CD19 gene alteration when compared to other tumors. Rarely was splice mutation seen, whereas amplification was the most frequent alteration. A foundation for further genetic exploration of genes related to CD19 is established by this discovery. Furthermore, we discovered a significant inverse association between CD19 and TMB in the majority of tumors, whereas both positive and negative associations were observed with MSI. These results indicate that CD19 has differential immunomodulatory effects in different cancers. A powerful correlation between CD19 and MSI and TMB implies a powerful bond between CD19 and the TME.

The GSEA uncovered a significant correlation between CD19 and immune-related pathways, specifically highlighting the B-cell receptor signaling pathway. According to reports, CD19 plays a significant role in the development and activation of B-cells. It has been identified as the docking and recruitment site for various kinases and signaling components in the B-cell signaling pathway, along with its involvement in B-cell receptor (BCR) signaling.[36] CD19 activation enhances signaling pathways induced by the B-cell antigen receptor, which are crucial for the proliferation of the B-cell population.[37] Furthermore, we acquired 47 genes associated with the immune system, which encompassed the lymphocyte activation molecule known as CD70. CD70 plays a crucial role in the immune response of T cells, significantly contributing to their successful proliferation. Widely employed in the surveillance of B cells, CD70 is anticipated to become a target for immunotherapy in B-cell malignancies.[24,38] We discovered that CD19 and its related genes, when examined through a coexpression network, could collaborate to control the immune reaction and antigen presentation in tumor immunotherapy. The potential for cancer-targeted immunotherapy is immense due to the amalgamation of CD19 and genes associated with immunity.

The primary immune cells that function as antitumor responders are CD8 cytotoxic T cells, which are activated when their T-cell receptor (TCR) recognizes tumor antigenic peptides on tumor cells.[39,40] Nevertheless, tumor-associated macrophages and Treg cells are responsible for the formation of an immunosuppressive TME, thus enabling tumor immune escape. It is thought that Treg cells, which sustain immune balance, are the primary impediment to antitumor immunity.[41] During our investigation, we discovered a strong connection between the presence of CD19 and the infiltration of CD8(+) T cells, Treg cells, B cells, DCs, macrophages, NK cells, and follicular helper T cells that assist in the development of follicles. This suggests that CD19 can have various effects on tumors by influencing the extent of immune cell infiltration into the tumor. By investigating the correlation between the expression level of CD19 and the sensitivity to drugs, we put forward potential therapeutic medications that specifically aim at CD19, and confirmed its significance as a promising target for cancer treatment.

Conclusion

In conclusion, CD19 is differentially expressed in different cancers. CD19 prolongs the survival of cancer patients, elucidating the prognostic significance of CD19 in pan-cancer samples. CD19 is strongly associated with TMB, MSI and TME. In addition, there is a significant correlation between CD19 and immune-related pathways, and CD19 can synergize with other immune-related genes to control immune responses and antigen presentation in tumor immunotherapy, emphasizing the importance of CD19 as a predictor of tumor response to immunotherapy. The development of drugs targeting CD19 may have a killing effect on a variety of tumors. To summarize, our findings offer fresh theoretical backing for the development of anti-cancer medications that focus on CD19 in the TME.

Abbreviations

CAR, chimeric antigen receptor; TCGA, The Cancer Genome Atlas; TIMER, Tumor Immune Estimation Resource; HPA, Human Protein Atlas; KM, Kaplan-Meier; ESTIMATE, Estimation of Stromal and Immune Cells in Malignant Tumor Tissues Using the Expression Data; COAD, colon adenocarcinoma; TMB, tumor mutation burden; MSI, microsatellite instability; BRCA, breast invasive carcinoma; CTLA-4, cytotoxic T lymphocyte-associated molecule-4; PD-1, programmed cell death receptor-1; PD-L1, programmed cell death ligand-1; SARC, sarcoma; IL-10, Interleukin-10; TME, tumor microenvironment; TNBC, triple-negative breast cancer; GTEx, Genotype-Tissue Expression; GEPIA, Gene Expression Profiling Interactive Analysis; OS, overall survival; DSS, disease-specific survival; PFI, progression-free interval; GSEA, Gene set enrichment analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, gene

ontology; STAD, stomach adenocarcinoma; KIRC, kidney renal clear cell carcinoma; DLBC, diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; UCEC, uterus corpus endometrial cancer; KICH, kidney chromophobe; READ, rectum adenocarcinoma; PRAD, prostate adenocarcinoma; THCA, thyroid carcinoma; ACC, adrenocortical carcinoma; ESCA, esophageal carcinoma; SKCM, skin cutaneous melanoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; OV, ovarian serous cystadenocarcinoma; TGCT, testicular germ cell tumor; THYM, thymoma; BLCA, bladder carcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, cholangiocarcinoma; LIHC, liver hepatocellular carcinoma; PCPG, pheochromocytomas and paragangliomas; UCS, uterine carcinosarcoma; PAAD, pancreatic adenocarcinoma; LGG, brain lower grade glioma; DCs, dendritic cells; NK, natural killer; B-ALL, cell acute lymphoblastic leukemia; BCR, B-cell receptor; TCR, T-cell receptor.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are publicly available in the TCGA (https://portal.gdc.cancer.gov/), TIMER 2.0 (http://timer.cistrome.org), GTEx (https://commonfund.nih.gov/GTEx), HPA (http://www.proteinatlas.org/), GEPIA (http://gepia.cancer-pku.cn/) and cBioPortal (https://www.cbioportal.org/) database. Further inquiries can be directed to the corresponding authors.

Ethics Approval and Informed Consent

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of the Shanghai Sixth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. Informed consent was obtained from all subjects involved in the study.

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This paper has been uploaded to Research Square as a preprint: https://www.researchsquare.com/article/rs-3212266/v1.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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