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ORIGINAL RESEARCH

Interleukin-6 as a Pan-Cancer Prognostic Inflammatory Biomarker: A Population-Based Study and Comprehensive Bioinformatics Analysis

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Purpose: Interleukin-6 (IL-6) is a central factor linking inflammation to cancer. This study aimed to provide a comprehensive assessment of the prognostic value of IL-6 and its immunotherapeutic features using a population-based pan-cancer analysis and comprehensive bioinformatic analysis.

Patients and Methods: In the cohort study, 540 patients were included to explore the prognostic value of serum IL-6 levels in cancer. The differential expression of IL-6 and its association with survival and immune cell infiltration were investigated using the TCGA database. The SangerBox database was used to analyze the correlation between IL-6 expression and immune checkpoint (ICP), tumor mutation burden (TMB), and microsatellite instability (MSI) in cancer. Genomic changes in the IL-6 levels were studied using the c-BioPortal database. The IL-6 co-expression network was analyzed using the LinkedOmics database.

Results: Serum IL-6 is an independent prognostic factor for cancer, especially gastrointestinal cancers. Compared to other serum inflammatory markers, serum IL-6 is an optimal biomarker for cancer prognosis. A comprehensive bioinformatics analysis showed higher IL-6 expression in human cancers than in the paired normal tissues. The IL-6 expression is closely associated with prognosis, ICP, TMB, and MSI. In addition, it is also strongly correlated with tumor-infiltrating cells. IL-6 levels are significantly associated with the prognosis of stomach adenocarcinoma (STAD). The IL-6 co-expression network in STAD is mainly involved in regulating inflammatory pathways and cell communication.

Conclusion: IL-6 is a potential prognostic and immune biomarker of cancer. Compared to other clinical inflammatory biomarkers, IL-6 demonstrates superior prognostic efficacy.

Keywords: Interleukin-6, Prognostic marker, Bioinformatic analysis, STAD

Introduction

Cancer is one of the leading causes of shortened lifespan in humans. In recent decades, the incidence and mortality rates of malignancy have been on a continuous upward trend.[1,2] It is estimated that by 2023, the United States have approximately 1.958 million new cases of cancer, with an average of 5370 new cases per day. The number of cancer-related deaths is expected to reach 610,000, with an average of 1670 deaths per day.[3] In China, the latest data show approximately 4.064 million new cases of cancer and 2.4 million cancer-related deaths. Cancer poses a serious threat to public health, accounting for 24.09% of all deaths.[4] Therefore, there is an urgent need to identify effective prognostic indicators to guide cancer treatment to reduce the mortality rates.

Systemic inflammation plays a crucial role in the progression of cancer.[5,6] Serum biomarkers are among the simplest, most effective, and most widely used means of prognostic assessment in cancer patients. Numerous serum inflammatory markers

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have proven to be robust prognostic biomarkers.[7–9] Interleukin-6 (IL-6) is an essential inflammatory marker that plays a key role in immune responses, inflammatory reactions, acute-phase reactions, and physiological and pathological processes such as cell proliferation, differentiation, and apoptosis.[10–12] Elevated levels of IL-6 are closely associated with the occurrence, development, and prognosis of various cancers.[13,14] Ma et al found that serum IL-6 was correlated with clinical disease stage, lymph node metastasis, as well as with the expression of ER and HER2 antigens.[15] Ma et al showed that High serum IL-6 level is associated with adverse prognosis in cervical cancer and could be a prognosis indicator for cervical cancer.[16] IL-6 activates multiple signaling pathways, such as JAK/STAT, PI3K/Akt, and MAPK, promoting tumor cell proliferation and metastasis.[17,18] It also inhibits apoptosis by inhibiting caspase-8 activity, activating the anti-apoptotic factor Bcl-2 and other pathways, rendering tumor cells resistant to chemotherapy or radiotherapy.[19–21] Elevated serum IL-6 levels are associated with increased stromal IL-6 levels in colorectal cancer. High expression of IL-6 in tumor-infiltrating immune cells is also linked to the accumulation of immunosuppressive cells within the tumor microenvironment (TME).[22]

Although IL-6 has been widely studied, its clinical applications have not been extensively evaluated compared to C-reactive protein (CRP). Therefore, more evidence is needed to validate the value of serum IL-6 as a prognostic indicator in patients with cancer. This study aims to explore the relationship between serum IL-6 levels and overall survival (OS) in patients with cancer. We also compare the serum IL-6 levels with known common prognostic markers to provide scientifically reliable evidence for its use as a predictive marker in patients with cancer. In addition, we have comprehensively analyzed the role of IL-6 in human cancer prognosis and immunology by investigating the potential association between IL-6 expression and immune subtypes in the tumor microenvironment, molecular subtypes in different types of cancer, immune infiltration degree, tumor mutation burden (TMB), microsatellite instability (MSI), and various immune-related effects. Furthermore, we explore the IL-6 pathways and co-expressing genes in stomach adenocarcinoma (STAD). Through these evaluations, we aim to explore the value of IL-6 in human cancer prognosis and provide further insights into new antitumor strategies.

Materials and Methods

Cohort Data

In this study, patients were sourced from the Investigation on Nutrition Status and the Clinical Outcome of Common Cancers (INSCOC) database (registered at chictr.org.cn, ChiCTR1800020329).[23,24] The included patients (a) had confirmed tumors through pathological examination, (b) had not undergone initial treatment to prevent any interference from treatment-related factors, and (c) had comprehensive clinical pathology data, including detailed medical history, imaging examination results, and laboratory test results. Patients under 18 years of age were excluded because their physiological and pathological characteristics may differ from those of adult patients, requiring separate consideration and study. Additionally, patients with severe complications or multiple types of tumors were also excluded because their disease conditions were complex and could interfere with the study results. All patients provided written consent. This study was approved by the ethics committees of all participating institutions. Approved public trial registries: http://www.chictr.org.cn/ showp roj. aspx? proj= 31813 (ChiCTR1800020329). All data were analyzed anonymously by removing any identification information, and the principles of the Declaration of Helsinki were followed.

Within the first 48 hours after admission, trained researchers (medical personnel who underwent three weeks of training on patient enrollment) conducted face-to-face interviews and physical examinations to collect the following information from the patients: age, sex, smoking and drinking status, comorbidities (hypertension, diabetes, and coronary heart disease), family history of cancer, Nutritional Risk Screening 2002 version (NRS2002) score, Patient-Generated Subjective Global Assessment (PG-SGA) score, and Karnofsky Performance Status (KPS). Tumor pathological information (TNM stage), anticancer treatments (surgery, radiotherapy, and chemotherapy), and hospitalization-related information (duration and expenses) were collected. Serum laboratory tests included IL-6, CRP, white blood cell count, neutrophils, lymphocytes, platelets, red blood cell count, hemoglobin, and albumin. All serum tests were conducted upon admission. The method for determining IL-6 concentrations is as follows: Peripheral blood samples are collected from patients upon admission and then centrifuged at 2000 g for 10 minutes to separate the serum. The serum is

subsequently analyzed using a high-sensitivity electrochemiluminescence assay with the MQ60 Auto fully automated chemiluminescence immunoassay analyzer, enabling precise measurement of IL-6 levels (pg/mL).

During the study, trained researchers collected clinical outcome information from participants by phone every year from enrollment to death or the last follow-up. The primary outcome indicator was OS, defined as the time interval (in months) from the date of diagnosis to death or last follow-up.

TCGA and Gene Expression Omnibus Database Analysis

Gene expression and clinical data were obtained from cBioPortal (http://gepia2.cancer-pku.cn/#analysis) and The Cancer Genome Atlas (TCGA) (https://tcga-data.nci.nih.gov/), covering 10,688 samples from 33 different types of cancer. The Gene Expression Profiling Interactive Analysis 2 (GEPIA2) database was used to analyze the expression levels of IL-6 in pan-cancer tissues. COX regression analysis was used to examine the correlation between the IL-6 expression and survival for each cancer type. For the eight cancer types with significant changes in expression levels (KIRC, LGG, KIRP, STAD, SARC, CESC, UVM, and PAAD), Kaplan-Meier curves were generated using the "survival" and "survminer" packages, and the cumulative survival rates were compared using the Log rank test.

Analysis of Tumor IMmune Estimation Resource (TIMER) Database

TIMER (https://cistrome.shinyapps.io/timer/) is a comprehensive resource for analyzing interactions between tumors and the immune system. We used this database to explore the correlation between IL-6 copy number variations (CNV) and the degree of infiltration of six types of tumor-infiltrating immune cells (TIICs), including B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and dendritic cells, and to validate the relationship between IL-6 and immune checkpoints (ICPs) using Spearman correlation statistical methods.

Furthermore, we explored the relationship between IL-6 expression and ESTIMATE score, tumor mutation burden (TMB), and microsatellite instability (MSI) in the tumor microenvironment (TME) through the SangerBox website (http://sangerbox.com/Tool). TMB and MSI are critical biological markers of TME. Estimated Stromal and Immune Cells in Malignant Tumor Organizations Using Expression Data (ESTIMATE) is an algorithm designed to predict TME purity and includes matrix, immune, and estimate scores. We also explored the correlation between IL-6 expression and the immune and molecular subtypes for different cancer types using the TISIDB database (http://cis.hku.hk/TISIDB/index.php).

LinkedOmics Analysis

The LinkedOmics database (http://www.linkedomics.org) was used for the gene enrichment analyses. The LinkFinder module of the LinkedOmics database was used to explore the correlation between differentially expressed genes (DEGs) and IL-6 in the STAD cohort, and the results were visualized using a heatmap. The LinkInterpreter module of the LinkedOmics database was used to identify the pathways and networks of the DEGs. The data from LinkFinder were further analyzed through signature and ranking, and gene set enrichment analysis (GSEA) was applied to elucidate relevant information, such as Gene Ontology (GO) term analysis (including biological processes, cellular components, and molecular functions) and KEGG pathway analysis.

Protein Interaction Network

We analyzed the genes co-expressing with IL6 in the STAD dataset using the cBioPortal database. Significantly, the co-expressors were selected based on screening criteria (p-value < 0.05, Spearman correlation > 0.8), and the STRING database was used to construct a protein-protein interaction (PPI) network. Finally, Cytoscape software was used for visualization, and the two most significant modules were selected. These analytical methods provide insights into the molecular mechanisms of IL-6 in tumor development and support its application as a therapeutic target.

Statistical Analysis

For continuous data, we used the mean (± standard deviation) or median (interquartile range) and performed a Kolmogorov–Smirnov test for normality. Data with normal and non-normal distribution were compared using the

Student's *t*-test and Mann–Whitney *U*-test, respectively. Categorical data are presented as numbers (percentages) and were compared using the chi-square test. Based on optimal stratification and log-rank statistics and considering prognosis, we determined the optimal cutoff value for continuous IL-6. Subsequently, we assessed the survival differences between the high and low IL-6 groups using the Kaplan-Meier method. The relationship between IL-6 levels and survival was analyzed using the Cox proportional hazards model after adjusting for potential confounding factors. A sensitivity analysis was conducted by excluding deaths that occurred within the first three months of follow-up to assess the robustness of the relationship between IL-6 and patient survival. The relationship between IL-6 and the mortality rate was visualized using restricted cubic spline (RCS) analysis based on the Cox proportional hazards model to measure the hazard ratio of continuous IL-6. Finally, we compared the prognostic potential of IL-6 with that of other inflammatory markers by calculating the concordance index (C-index).

In the comprehensive bioinformatics analysis, Mann–Whitney *U*-test were used to analyze the differences between two groups. Kaplan-Meier curves and Log rank tests were used to compare the overall survival (OS) among the different groups. The value of IL-6 as a prognostic indicator was evaluated using Cox proportional hazards. Pearson correlation tests were conducted to determine the associations with subtypes, clinicopathological features, risk scores, immune checkpoint expression, and immune infiltration levels. Statistical significance was determined using a threshold of $p < 0.05$. All statistical analyses were performed using an online bioinformatics database and the R software (version 4.0.4).

Results

Baseline Characteristics

This study included 540 patients (354 males and 186 females) with cancer, with an average age of 62.87 (±12.08) years. Of them, 377 patients (69.8%) had stage III–IV disease, 191 (35.4%) had lung cancer, 148 (27.4%) had gastrointestinal cancer, and 201 (37.2%) had other cancers (brain and other nervous system cancer). At the final follow-up, 188 patients (34.8%) had died. The cutoff value of IL-6 in patients with cancer was determined as 3.47 (Figure S1). The low IL-6 group consisted of 149 patients, while the high IL-6 group included 391 patients. Compared with the low IL-6 group, patients in the high IL-6 group had higher stages of cancer, were more likely to have lung cancer, had higher mortality rates, and incurred higher hospitalization costs. In addition, high IL-6 levels were significantly associated with a high white blood cell count, high neutrophil count, low lymphocyte count, low red blood cell count, low hemoglobin level, low albumin level, and high CRP level (Table S1).

Comparison of IL−6 and Other Systemic Inflammation Biomarkers

Previous studies have shown that systemic inflammation is characterized by the upregulation of inflammatory parameters, including neutrophils, platelets, and C-reactive protein, as well as the downregulation of anti-inflammatory parameters, including lymphocytes and albumin. We summarized 17 combinations of pro-inflammatory and anti-inflammatory parameters (Table S2). Using the C-index, we compared levels of IL-6 with those of other systemic inflammatory biomarkers for the prognosis of patients with cancer. IL-6 emerged as the best predictive biomarker, with the highest C-index of 0.625 (0.583, 0.667). Compared with IL-6, the prognostic efficacy of other systemic inflammatory biomarkers was negative (Table 1). Furthermore, most inflammatory markers provided clinical benefits in terms of pathological staging, with IL-6 increasing the clinical benefit by 5.9% (Table S3). Based on the Pearson correlation analysis, IL-6 had a correlation coefficient of 0.190 ($p<0.001$) with CRP, 0.182 ($p<0.001$) with CAR, 0.130 ($p<0.001$) with IBI, 0.172 ($p<0.001$) with NC, 0.211 ($p<0.001$) with PC, 0.093 ($p<0.05$) with GPS, and 0.102 ($p<0.05$) with mGPS (Figure S2).

Kaplan-Meier Curves Based on IL-6 Levels

During the follow-up period, the number of deaths in the high IL-6 group was 161, while in the low IL-6 group, it was 27. Compared with the low IL-6 group, patients in the high IL-6 group had significantly poorer survival (81.9% vs 58.8%, $p<0.001$) (Figure 1A). In the TNM I–II subgroup, the survival rate in the high IL-6 group was significantly lower than that in the low IL-6 group (91.3% vs 80.9%, $p=0.006$) (Figure 1B). In the TNM stage III–IV subgroup, IL-6 significantly stratified patient prognosis (73.8% vs 51.9%, $p=0.001$) (Figure 1C). In the tumor type subgroup, although high IL-6 levels were

Table 1 Comparative Analysis of the Discrimination of Systemic Inflammation Biomarkers for All-Cause Mortality in Cancer

Discrimination Ability		C-statistic		
	Difference	p value	Difference	p value
IL-6	0.625(0.583,0.667)	<0.001	Ref	
CRP	0.612(0.567,0.657)	<0.001	−0.013(−0.061, 0.035)	0.601
CAR	0.617(0.573,0.662)	<0.001	−0.008(−0.056, 0.039)	0.737
CALLY	0.614(0.569,0.660)	<0.001	−0.010(−0.055, 0.034)	0.658
LCR	0.611(0.565,0.656)	<0.001	−0.014(−0.059, 0.031)	0.552
IBI	0.609(0.563,0.655)	<0.001	−0.016(−0.065, 0.029)	0.508
NC	0.608(0.562,0.654)	<0.001	−0.018(−0.061, 0.030)	0.442
PC	0.599(0.555,0.643)	<0.001	−0.026(−0.077, 0.024)	0.308
GPS	0.585(0.545,0.625)	<0.001	−0.041(−0.085, −0.001)	0.048
mGPS	0.578(0.540,0.616)	<0.001	−0.048(−0.092, −0.008)	0.025
NAR	0.577(0.531,0.622)	<0.001	−0.050(−0.102, 0.008)	0.074
LCS	0.571(0.531,0.610)	<0.001	−0.056(−0.099, −0.019)	0.007
NLR	0.557(0.510,0.605)	0.018	−0.069(−0.127, −0.008)	0.024
SII	0.544(0.497,0.591)	0.064	−0.082(−0.146, −0.006)	0.022
NP	0.544(0.498,0.591)	0.062	−0.083(−0.145, −0.005)	0.021
PAR	0.533(0.488,0.578)	0.154	−0.093(−0.150, −0.027)	0.003
LA	0.530(0.484,0.576)	0.199	−0.095(−0.153, −0.036)	0.001
PLR	0.514(0.468,0.559)	0.558	−0.139(−0.228, −0.096)	<0.001

Abbreviations: IL-6, Interleukin-6; CRP, C-reactive protein; CAR, C-reactive protein-to-albumin ratio; CALLY, C-reactive protein-albumin-lymphocyte index; LCR, Lymphocyte-to-C-reactive protein ratio; IBI, Inflammatory burden index; NC, Neutrophil-C-reactive protein score; PC, Platelet-C-reactive protein score; GPS, Glasgow Prognostic Score; mGPS, Modified Glasgow Prognostic Score; NAR, Neutrophil-to-albumin ratio; LCS, Lymphocyte C-reactive protein score; NLR, Neutrophil-to-Lymphocyte ratio; SII, Systemic-Immune-Inflammation Index; NP, Neutrophil-Platelet score; PAR, Platelet-to-albumin ratio; LA, Lymphocyte-Albumin score; PLR, Platelet-to-Lymphocyte ratio.

associated with poor prognosis in lung cancer, it did not reach statistical significance (65.1% vs 44.6%, p=0.130) (Figure 1D). For gastrointestinal cancers, compared to the low IL-6 group, patients in the high IL-6 group had significantly lower survival rates (89.7% vs 64.4%, p<0.001) (Figure 1E). For other cancers, patients in the high IL-6 group had a relatively poor prognosis, but the difference was not significant (87.5% vs 69.3%, p=0.058) (Figure 1F).

Association of IL-6 with Survival

In model A (unadjusted), RCS demonstrated a consistent nonlinear positive association between IL-6 levels and overall mortality (p for nonlinearity <0.001, p for mortality <0.001) (Figure 2A). After adjusting for age, sex, BMI, tumor stage, and tumor type in model B, the positive nonlinear association between increased IL-6 levels and overall mortality remained significant (p for nonlinearity = 0.003, p for mortality = 0.001) (Figure 2B). In the final model (adjusted for age, sex, tumor type, tumor stage, BMI, surgery, radiotherapy, chemotherapy, smoking, drinking, hypertension, diabetes, and family history), a positive nonlinear association between IL-6 levels and overall mortality persisted (p for nonlinearity = 0.031, p for mortality = 0.028) (Figure 2C). In the multivariable-adjusted Cox regression analysis, as the IL-6 levels increased by a standard deviation, the risk of adverse prognosis increased by 121.9% (hazard ratio = 2.219, 95% confidence interval = 1.144–4.305, p-value = 0.018). The high IL-6 group (≥3.47) had twice the risk of adverse prognosis compared to the low IL-6 group (<3.47) (hazard ratio = 2.001, 95% confidence interval = 1.269–3.157, p-value = 0.003). Furthermore, we tested the trend in IL-6 levels and found that the risk of adverse prognosis increased by 51.0%, 110.0%, and 93.3% in the Q2, Q3, and Q4 groups, respectively, compared to the Q1 group (Table 2). In the sensitivity analysis, we excluded patients who died within three months, and the results demonstrated the robustness of our findings, as high IL-6 levels remained an independent risk factor for tumor patient prognosis (Table S4). Additionally, multivariate forest plots showed that high IL-6 levels remained an independent risk factor for poor prognosis in the majority of subgroups (Figure S3).

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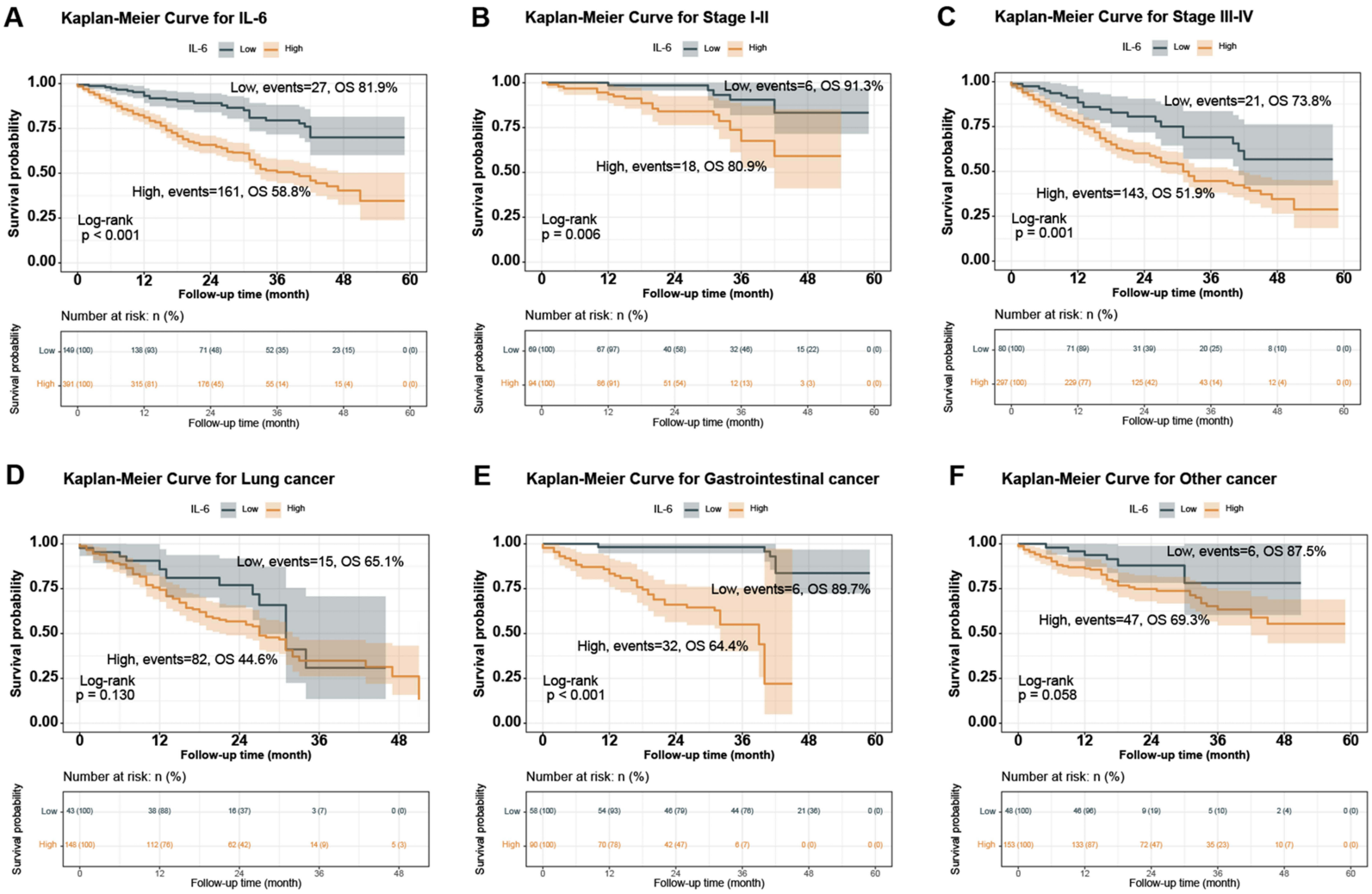

Figure 1 Kaplan-Meier curve of serum IL-6 in patients with cancer.
Notes: (A), All patients; (**B**), Stage I–II; (**C**), Stage III–IV; (**D**), Lung cancer; (**E**), Gastrointestinal cancer; (**F**), Other cancer.

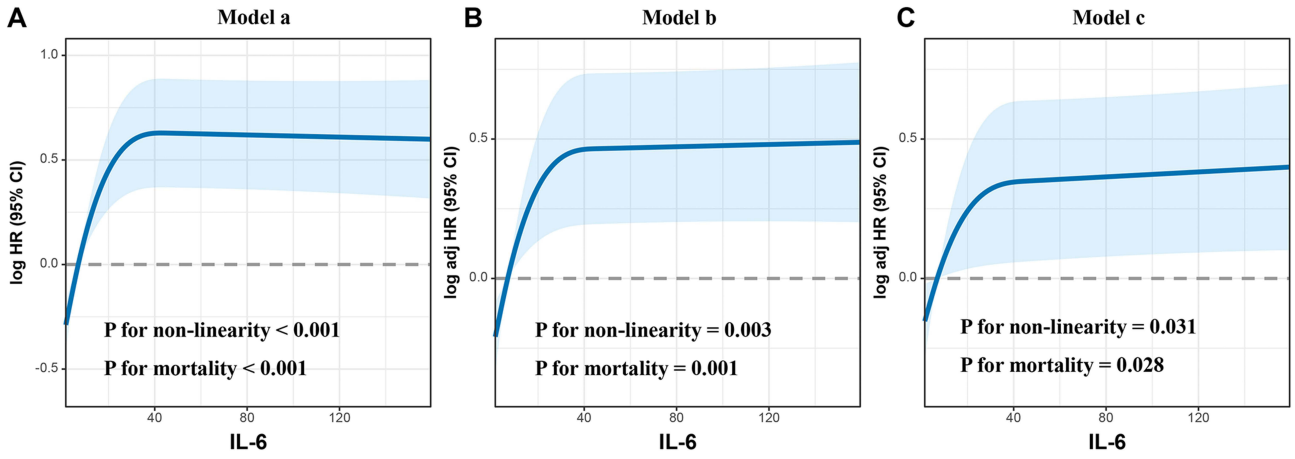

Figure 2 Association Between serum IL-6 and All-Cause Mortality Using a Restricted Cubic Spline Regression Model.
Notes: Model (**A**) No adjusted. Model (**B**) Adjusted for age, gender, BMI, tumor stage, and tumor type. Model (**C**) Adjusted for age, gender, tumor type, tumor stage, BMI, surgery, radiotherapy, chemotherapy, smoking, drinking, hypertension, diabetes, coronary heart disease, and family history.

Correlation Between IL-6 Gene Expression Level and OS

Analysis of the gene expression data and clinical data of pan-cancer patients downloaded from the TCGA database showed an association between high IL-6 expression and poor prognosis (Figure 3A). Higher disease stage was associated with higher IL-6 expression, with the highest expression observed in Stage IV patients (Figure 3B) indicating a possible correlation between elevated IL-6 levels and disease progression. COX regression analysis showed that IL-6 was significantly associated with the prognosis of patients with KIRC, LGG, KRIP, STAD, SARC, CESC, UVM, HNSC, and PAAD (Figure 3C). Kaplan-Meier curves demonstrated that high IL-6 expression was associated with poor prognosis in KIRC, LGG, STAD, KIRP, HNSC, CESC, and UVM, whereas low IL-6 expression correlated with shorter survival in SARC (Figure 3D–K).

Further exploration of the distribution of the IL-6 gene in pan-cancer patients revealed high expression DLBCL, ESCA, GBM, PAAD, and TGCT, whereas it was downregulated in BLCA, BRCA, KICH, LAML, LUAD, LUSC, and THCA (Figure 4A). Based on the different levels of IL-6 gene expression, tumors were divided into high IL-6 tumors (LUSC, HNSC, LUAD, TGCT, STAD, ESCA), moderate IL-6 tumors (BLCA, GBM, READ, PAAD, COAD, KIRC, SARC, CESC, DLBC, and PRAD), and mild IL-6 tumors (BRCA, THCA, SKCM, UCEC, OV, UCS, PCPG, CHOL, KIRP, LGG, KICH, THYM, ACC, LIHC, and LAML) (Figure 4B). In the tumor types where IL-6 was found to be statistically significant in the COX regression analysis, only LGG, KIRP, SARC, STAD, CESC, and PAAD exhibited

Table 2 Association Between Serum IL-6 and Overall Survival of Patients with Cancer

IL-6	Model a	p value	Model b	p value	Model c	p value
Continuous (per SD)	1.488 (0.879,2.517)	0.139	1.96 (1.089,3.527)	0.025	2.219 (1.144,4.305)	0.018
Cutoff value		<0.001		<0.001		0.003
C1 (<3.47)	ref		ref		ref	
C2 (≥3.47)	2.896 (1.92,4.37)		2.271 (1.496,3.448)		2.001 (1.269,3.157)	
Quartiles						
Q1 (<3.09)	ref		ref		ref	
Q2 (3.09–6.88)	1.939 (1.187,3.168)	0.008	1.729 (1.050,2.848)	0.031	1.510 (0.878,2.595)	0.136
Q3 (6.88–20.28)	3.234 (2.033,5.143)	<0.001	2.447 (1.528,3.919)	<0.001	2.100 (1.269,3.475)	0.004
Q4 (≥20.28)	2.979 (1.870,4.746)	<0.001	2.333 (1.458,3.733)	<0.001	1.933 (1.164,3.21)	0.011
p for trend		<0.001		<0.001		0.007

Notes: Model a: No adjusted. Model b: Adjusted for age, gender, BMI, tumor stage, and tumor type. Model c: Adjusted for age, gender, tumor type, tumor stage, BMI, surgery, radiotherapy, chemotherapy, smoking, drinking, hypertension, diabetes, coronary heart disease, and family history.

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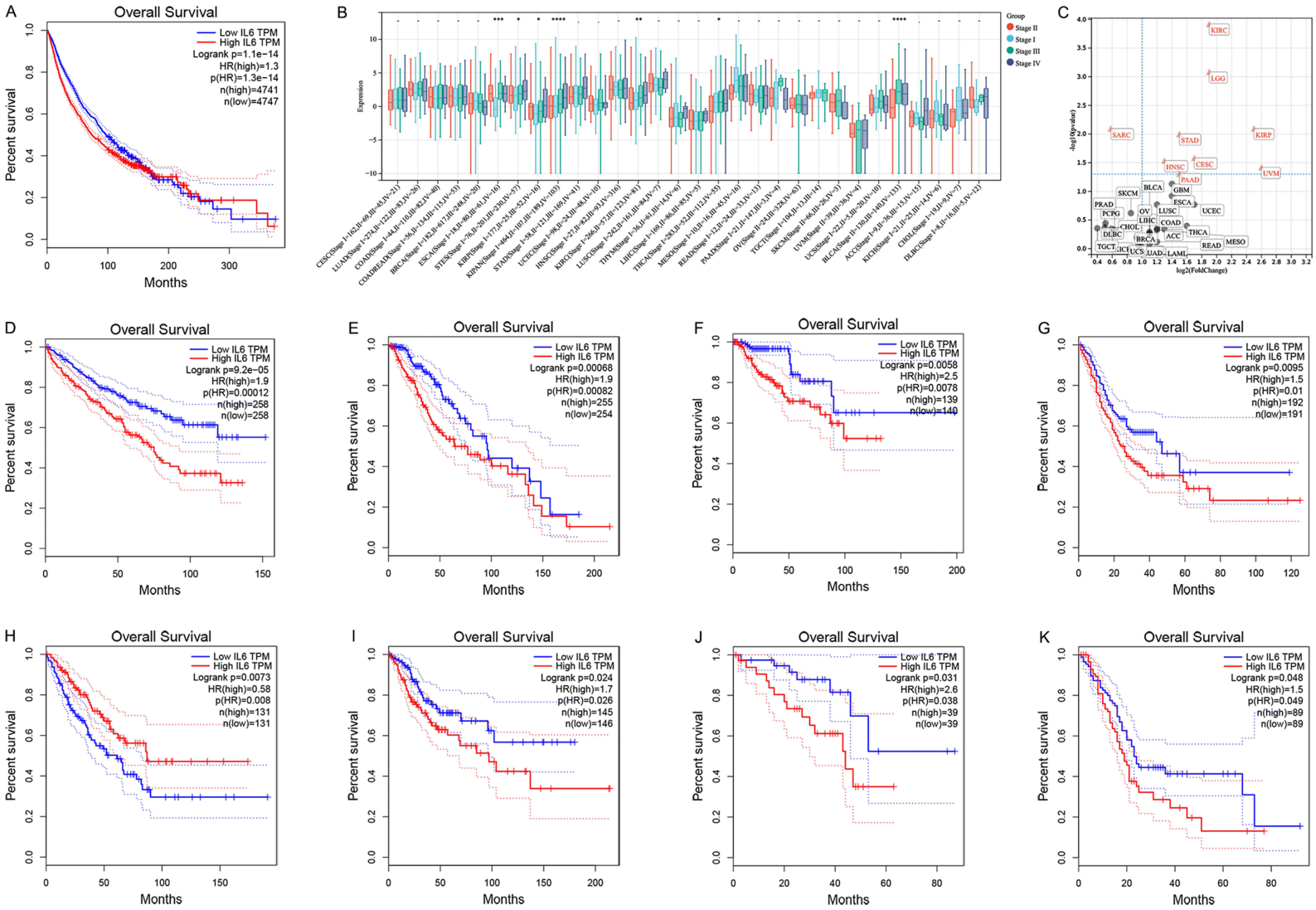

Figure 3 The correlation between IL-6 gene expression level and overall survival of cancer in TCGA.

Notes: (A), All tumor types; (**B**), IL-6 gene expression based on stage; (**C**), Association between IL-6 gene expression levels and survival of patients with different types of cancer in TCGA; (**D**), KIRC; (**E**), LGG; (**F**), KIRP; (**G**), STAD; (**H**), SARC; (**I**), CESC; (**J**), UVM; (**K**), PAAD.

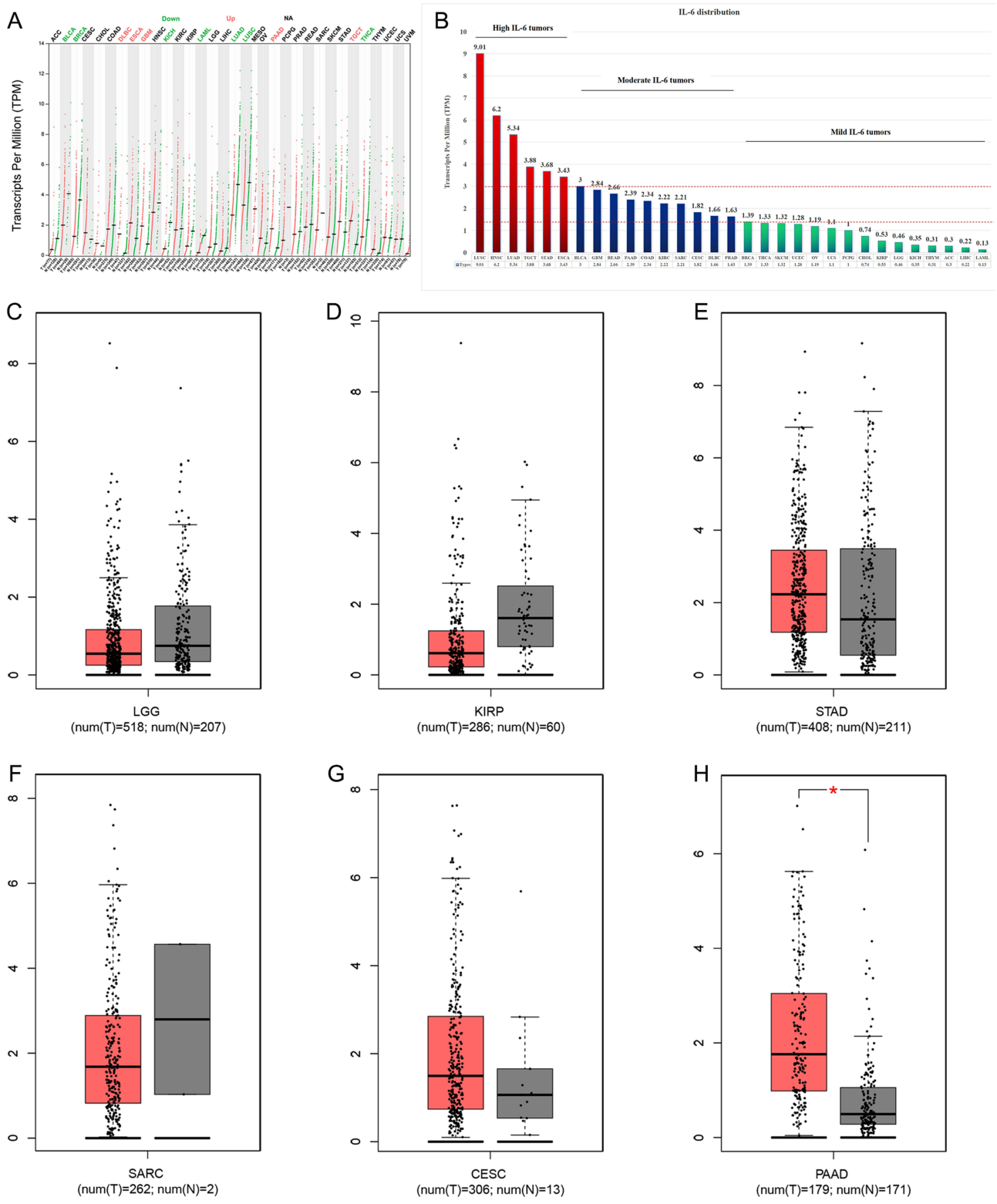

Figure 4 Expression of IL-6 gene in different cancer types.

Notes: (A), Expression of IL-6 gene in different cancer types; (**B**), The distribution level of IL-6 gene in different cancer types; (**C**), Expression of IL-6 gene in LGG; (**D**), Expression of IL-6 gene in KIRP; (**E**), Expression of IL-6 gene in STAD; (**F**), Expression of IL-6 gene in SARC; (**G**), Expression of IL-6 gene in CESC; (**H**), Expression of IL-6 gene in PAAD. *Represents $p<0.05$.

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significant differences in IL-6 gene expression between cancerous and adjacent tissues. Specifically, IL-6 was upregulated in LGG, KIRP, and SARC, while it was downregulated in STAD, CESC, and PAAD compared to adjacent tissues (Figure 4C–H).

IL-6 Expression is Related to Immune and Molecular Subtypes in Human Cancers

We investigated the role of IL-6 expression in the immune and molecular subtypes of human cancer. IL-6 expression correlated with different immune subtypes in BLCA, BRCA, CHOL, COAD, HNSC, KICH, KIRC, LGG, LIHC, LUAD, OV, PAAD, SARC, and UCEC. It exhibited differential expression among the different immune subtypes within a specific cancer type (Figure S5). Additionally, IL-6 expression was significantly associated with the molecular subtypes of BRCA, COAD, HNSC, KIRP, LGG, LIHC, LUSC, OV, and SKCM (Figure S6). These findings indicate that IL-6 exhibits distinct expression patterns in the immune and molecular subtypes of various cancer types.

IL-6 Gene Expression and Immune Infiltration

Pan-cancer and TIMER database analysis showed a significant correlation between IL-6 gene expression and immune infiltration, yielding six immune cell infiltration scores for 9,406 tumor samples across 38 cancer types (Figure S4). In KICH, neuroblastoma, BLCA, GBMLGG, PCPG, High-Risk Wilms Tumor, PAAD, READ, and COADREAD, higher IL-6 expression scores were associated with increased immune cell infiltration, indicating a strong positive correlation between IL-6 and ESTIMATEScore (Figure S7). Furthermore, IL-6 expression was closely associated with immune checkpoint genes such as TGFβ1, C10orf54, and IL1β in most cancer types, including STAD, COAD, GBM, and SARC (Figure S8).

IL-6 Gene Mutations in Human Cancers

MSI and TMB are novel biomarkers associated with immune therapy response. We investigated the relationship between IL-6 expression and MSI and calculated the Pearson correlation coefficient for each tumor type. Significant correlations were seen in eight cancer types. While IL-6 expression showed a significant positive correlation with MSI in COAD and COADREAD patients, it showed a significant negative correlation with MSI in GBMLGG, ACC, HNSC, LUSC, KIRP, ACC, and CHOL (Figure S9A). Additionally, IL-6 expression showed a significant negative correlation with TMB in KIRP (Figure S9B). An analysis of the IL-6 mutation sites and types in 18 cancers found missense mutations to be the predominant type of IL-6 mutation in most cancers. In particular, COAD and COADREAD exhibited significant missense mutations in IL-6. These findings suggest that IL-6 mutations may be associated with cancer and potentially related to subsequent treatment (Figure S9C).

Functional Enrichment Analysis of IL-6 in STAD

The results so far indicate a close relationship between IL-6 and STAD. Further, based on a LinkedOmics database analysis, we identified 9,815 and 10,409 genes that showed significant positive and negative correlations with IL-6, respectively (Figure 5A). Figure 5B–C presents heat maps displaying the top 50 genes that were positively and negatively associated with IL-6 (Figure 5B–C). A GSEA to investigate the regulatory role of IL-6 in STAD showed that the genes co-expressed with IL-6 were mainly associated with inflammatory pathways, such as the TNF, IL-17, NF-kappa B, and JAK-STAT pathways, cytokine-cytokine receptor interaction, and complement and coagulation cascades (Figure 5D). A GO analysis identified several enriched biological process categories, including biological regulation, metabolic processes, cell communication, and cell proliferation. In the cellular component category, IL-6-related genes were primarily involved in processes related to the membrane, nucleus, vesicles, and cytoskeleton. The molecular function category concentrated on protein binding, nucleic acid binding, and transferase activity (Figure 5E). Additionally, we utilized the cBioPortal database to analyze the genes co-expressed with IL6 in the STAD dataset. We identified 185 genes using p-value < 0.05 and Spearman correlation coefficient > 0.8 as the filtering criteria. We constructed and visualized a protein-protein interaction (PPI) network of these 185 genes using Cytoscape (Figure 5F). Moreover, we identified the top two core modules in the PPI network (Figure 5G–H).

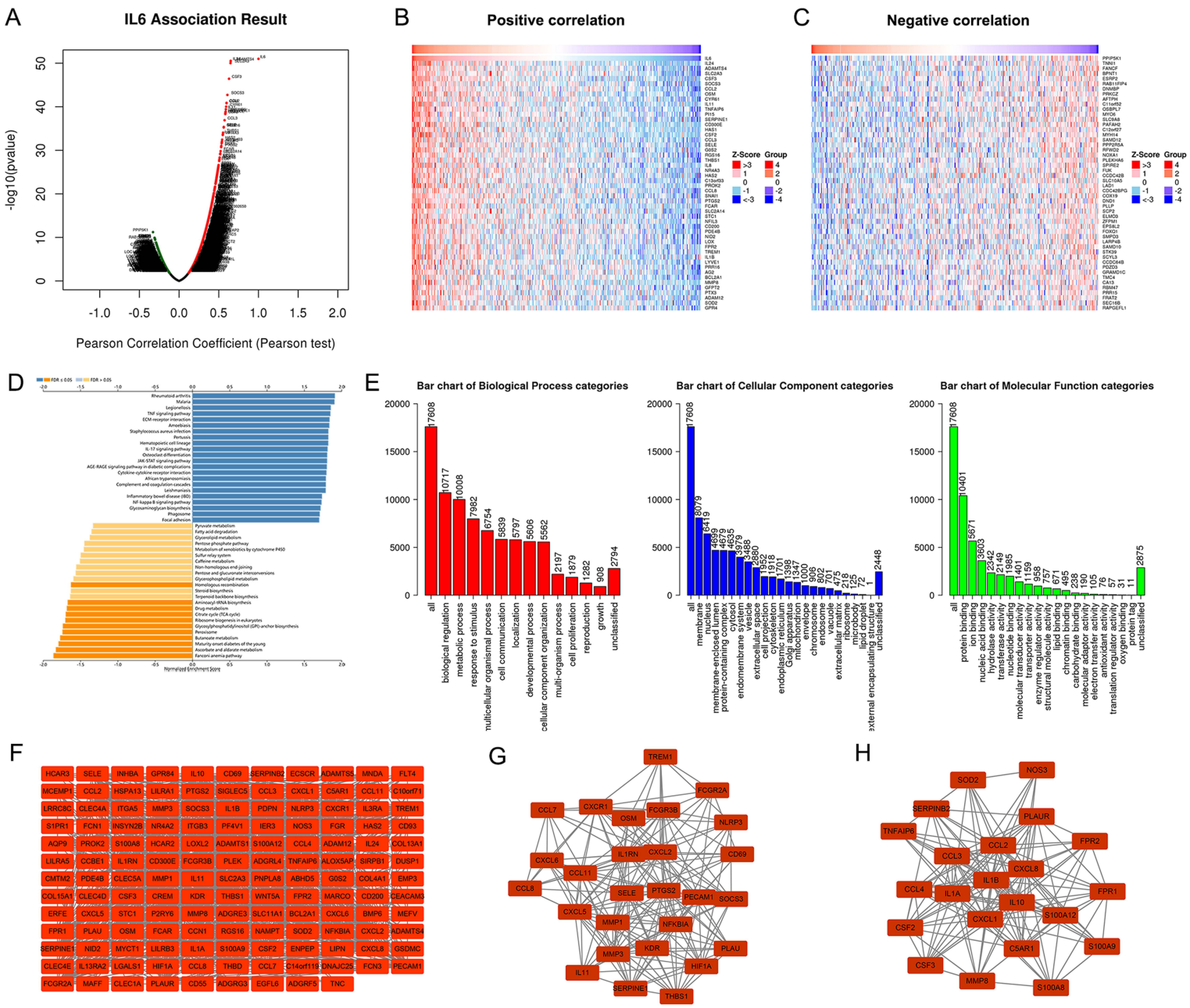

Figure 5 IL-6 co-expression gene in STAD.

Notes: (A), IL-6 co-expressed volcanic map in STAD; (**B**), Positive correlation gene in STAD; (**C**), Negative correlation gene in STAD; (**D**), KEGG in STAD; (**E**), Go analysis; (**F**), key gene; (**G**), Cluster 1; (**H**), Cluster 2.

Discussion

Inflammation is a significant risk factor for cancer. Prolonged low-grade inflammation can lead to cellular damage, DNA injury, and genetic mutations, thereby increasing the risk of cancer. In addition, inflammation can affect tumor growth, angiogenesis, and metastasis.[25–27] Cytokines and chemical mediators produced by inflammatory cells can promote the proliferation, invasion, and angiogenesis of tumor cells, creating a microenvironment favorable for tumor development. These factors may also interfere with the normal functioning of the immune system, reducing the body's antitumor immune response.[10,28] In addition to being a risk factor, inflammation also plays a significant role in the response of tumors to therapy. Tumors in an inflamed state are usually more resistant to radiotherapy and chemotherapy because inflammatory cells produce various anti-apoptotic molecules and antioxidants, protecting tumor cells from treatment-induced damage. Moreover, inflammation reduces the effectiveness of immunotherapy, limiting the immune system from attacking tumors.[5] Therefore, adequate control of inflammation can help improve tumor prognosis, and extensive research has been directed toward the application of therapeutic strategies targeting inflammatory cells or cytokines and modulating immune responses to improve the effectiveness of tumor treatment and prognostic outcomes.

Among the various inflammatory factors, IL-6 is considered the central factor linking inflammation and tumor development.[10] In patients with colon, breast, and other cancers, serum and tumor tissues showed elevated levels of IL-6, which is closely related to tumor invasion and poor prognosis.[29–31] In this study, we found that cancer patients with high serum IL-6 levels had a significantly worse prognosis, higher staging, higher mortality rates, and higher hospitalization costs. Multivariate COX regression analysis showed that high serum IL-6 level was an independent risk factor related to cancer prognosis. Analysis based on tumor types found that serum IL-6 level was an effective prognostic predictor for gastrointestinal cancer. In addition, compared to 17 common serum markers associated with systemic inflammation in clinical practice, IL-6 was found to be the best prognostic indicator of cancer.

Analysis of the pan-cancer database provides an excellent overview of cancer prevention and treatment strategies. Survival analysis was used to determine the correlation between high IL-6 gene expression and OS. Cancer subgroup analysis showed that IL-6 was associated with the prognosis of multiple tumors, including KIRC, LGG, KIRP, STAD, SARC, CESC, HNSC, UVM, and PAAD. We explored the IL-6 expression in different cancer types and found it was highly expressed in STAD, CESC, and PAAD and weakly expressed in LGG, KIRP, and SARC. Based on the expression of IL-6, cancers were divided into high IL-6 tumors, moderate IL-6 tumors, and mild IL-6 tumors.

IL-6 performs multiple functions in immune regulation. It promotes B lymphocyte proliferation and antibody production, participates in the activation and proliferation of T cells, regulates platelet production and bone marrow hematopoiesis, and influences inflammation and tissue repair processes.[32,33] We found significant differences in the expression of IL-6 in the different immune and molecular subtypes of most human cancers, indicating that IL-6 is a promising pan-cancer biomarker of immune regulation. Additionally, IL-6 expression is closely correlated with immune infiltration in various cancer types. Its expression strongly correlated with the immune scores in most cancers. IL-6 expression showed a positive correlation with the MSI status in COAD and COADREAD and a negative correlation in GBMLGG, KIPAN, HNSC, LUSC, ACC, and CHOL. In KIRP, IL-6 expression showed a negative correlation with TMB. In immune checkpoint analysis, the expression of IL-6 showed a correlation with immune checkpoint genes such as TGFβ1, C10orf54, and IL1β in most cancer types. These results strongly suggest that IL-6 is a potential target for anticancer immunotherapy.

Cohort studies have shown a close correlation between serum IL-6 levels and the prognosis of gastrointestinal cancer. Pan-cancer analysis revealed a significant association between high IL-6 expression and adverse outcomes in patients with STAD. Among gastrointestinal cancers, STAD is a high IL-6 tumor. Therefore, we hypothesized that IL-6 may be a molecular target for STAD treatment. To elucidate the regulatory mechanisms involved, we explored IL-6-related protein-coding and co-expressing genes in STAD. KEGG enrichment analysis indicated that IL-6-related proteins were mainly involved in pathways associated with inflammation, such as the TNF, IL-17, and JAK-STAT signaling pathways and cytokine-cytokine receptor interaction. GO enrichment analysis suggested that IL-6-related proteins were significantly associated with cell communication, proliferation, and membranes. Thus, we speculate that IL-6 may function as an inflammatory secretory protein involved in tumor cell communication. Previous studies have shown that IL-6 promotes the growth of tumor cells as an inflammatory secretory factor.[18,34] In the tumor microenvironment, IL-6 acts as a paracrine or autocrine growth factor and induces cancer-related inflammation.[35–37] These findings contribute to the understanding of the biological role of IL-6 in STAD.

The findings of this study further validate IL-6 as a reliable prognostic marker for the evaluation of patients with cancer. With a high degree of accuracy, IL-6 is expected to become an independent routine prognostic marker in clinical practice. However, it is important to note that despite the comprehensive systematic analysis of different pan-cancer databases, this study has certain limitations. The disparities in the sequencing data from various databases could introduce systematic biases. Although our evidence suggests the efficacy of IL-6 as a prognostic predictor of STAD, further in vivo and in vitro experiments are necessary to validate our findings.

Conclusion

The current study indicates that serum IL-6 can serve as an independent prognostic indicator of cancer. Compared to other clinical inflammatory biomarkers, IL-6 demonstrates superior prognostic efficacy. IL-6 has been identified as a prognostic biomarker in various cancers and is associated with immune infiltration, MSI, TMB, and immune checkpoints.

Abbreviations

ACC, Adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangiocarcinoma; COAD, Colon adenocarcinoma; COADREAD, Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA, Esophageal carcinoma; FPPP, FFPE Pilot Phase II; GBM, Glioblastoma multiforme; GBMLGG, Glioma; HNSC, Head and Neck squamous cell carcinomaKICH, Kidney Chromophobe; KIPAN, Pan-kidney cohort (KICH+KIRC+KIRP); 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; STAD, Stomach adenocarcinoma; SKCM, Skin Cutaneous Melanoma; STES, Stomach and Esophageal carcinoma; TGCT, Testicular Germ Cell Tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine Corpus Endometrial Carcinoma; UCS Uterine Carcinosarcoma; UVM, Uveal Melanoma; TARGET-OS, Osteosarcoma; TARGET-ALL, Acute Lymphoblastic Leukemia; TARGET-NB, Neuroblastoma; TARGET-WT, High-Risk Wilms Tumor.

Data Sharing Statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

This study followed the Helsinki declaration. All participants signed an informed consent form and this study was approved by the Institutional Review Board of each hospital (Registration number: ChiCTR1800020329). This study was also approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, with approval number (2024-S691-01).

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Disclosure

The authors declare that they have no competing interests in this work.

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