Multi-dimensional analysis reveals NCKAP5L is a promising biomarker for the diagnosis and prognosis of human cancers, especially colorectal cancer

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Abstract. The Nck-associated protein 5-like (NCKAP5L) gene, also known as Cep169, is associated with certain cancers. However, the diagnosis and prognosis value of NCKAP5L in several types of human cancer, including colorectal cancer, is not fully understood. In the present study, a comprehensive pan-cancer analysis of NCKAP5L was performed using several approaches, including gene expression and alteration, protein phosphorylation, immune infiltration, survival prognosis analyses and gene enrichment using the following: The University of California Santa Cruz Genome Browser Human Dec. 2013 (GRCh38/hg38) Assembly, Tumor Immune Estimation Resource (version 2), Human Protein Atlas, Gene Expression Profiling Interactive Analysis (version 2), University of Alabama at Birmingham Cancer Data Analysis portal, the Kaplan-Meier Plotter, cBioportal, Search Tool for the Retrieval of Interacting Genes/Proteins, Jvenn and the Metascape server. The role of NCKAP5L in colorectal cancer was further assessed by reverse transcription-quantitative PCR. The results demonstrated that NCKAP5L was upregulated in the majority of cancer types, including colorectal cancer. The high expression of NCKAP5L was significantly correlated with patient survival prognosis and immune infiltration of cancer-associated fibroblasts in numerous types of cancer, including colorectal cancer. Furthermore, Gene Ontology analysis identified that NCKAP5L may serve an important role in metabolic and cellular processes in human cancers. In summary, the data from the present study demonstrate that NCKAP5L is a potential tumor biomarker for the diagnosis and prognosis of human cancers, especially colorectal cancer.

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Introduction

Colorectal cancer constitutes a notable global public health challenge. It ranks as the 3rd most prevalent malignancy globally, following only lung and breast cancer, and concurrently represents the 4th leading cause of malignant neoplasm-related mortality. Thus, the adoption of comprehensive measures encompassing prevention, early detection, therapeutic intervention and prognosis is imperative to effectively address this substantial threat (1-3).

In recent years, next-generation sequencing (seq) technology has promoted cancer research progress at the genomic, transcriptomic and epigenetic levels (4). Consequently, a wealth of sequencing and clinical data for different types of cancer is now available (5). By using public databases such as The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO), bioinformatics can be used to perform pan-cancer analysis at the DNA, RNA, protein and epigenetic level to reveal the roles of genes in different cancers (6-9).

Nck-associated protein 5-like (NCKAP5L), also known as Cep169, is a centrosomal protein with a molecular weight of 169 kDa. Research has demonstrated the pivotal role served by NCKAP5L as a microtubule plus-end tracking protein, involved in the regulation of microtubule dynamics and stability (10-12). Previous studies have reported that NCKAP5L is involved in certain cellular processes associated with tumorigenesis and development including proliferation, invasion, and so on. (13-16). However, despite the abundance of available gene and clinical data, the value of NCKAP5L as a biomarker for the diagnosis and prognosis of human cancers, especially colorectal cancer, is not well understood. In the present study, the role of NCKAP5L in human cancers was assessed by databases including TCGA, GEO and The University of California Santa Cruz Genome Browser, Tumor Immune Estimation Resource (version 2) and Human Protein Atlas from multiple perspectives, including gene expression and alteration, immune infiltration, protein phosphorylation, and survival prognosis and gene enrichment analyses. The findings of the present study

may be valuable for understanding the role of NCKAP5L in colorectal and other human cancers.

Materials and methods

Analysis of gene expression and functions. The specific location of the NCKAP5L gene on the chromosome was identified, as well as its expression in 54 tissues using RNA-seq data from 17,382 samples and 948 donors [Genotype-Tissue Expression (GTEx) project; version 8, using the University of California Santa Cruz (UCSC) Genome Browser Human Dec. 2013 (GRCh38/hg38) Assembly (http://genome.ucsc.edu/) (17).

To assess the differences in NCKAP5L expression between distinct tumors of TCGA cohorts and their adjacent normal tissues, the 'Exploration' module of the Tumor Immune Estimation Resource version 2.0 (TIMER2.0) webserver (http://timer.cistrome.org/) (18) was used. The distribution of gene expression levels was displayed using a boxplot.

The online Human Protein Atlas (HPA) portal (version 21.0, https://www.proteinatlas.org/) was also used, and NCKAP5L was inputted in the 'Tissue' module to assess the normalized expression (NX) levels of 55 normal tissue types. The row data was TPM normalized, and the transcript expression values were represented as NX. 'Low specificity' indicated that NX was ≥1 in ≥1 cell/region/tissue type, but not significantly elevated in any cell/region/tissue.

The Gene Expression Profiling Interactive Analysis version 2 (GEPIA2) webserver (http://gepia2.cancer-pku.cn/#index) (19) was used to assess the difference in NCKAP5L expression between normal and tumor tissues for specific tumor types where a normal control group was not available in TCGA database, including cholangiocarcinoma (CHOL), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), glioblastoma multiforme (GBM), lung adenocarcinoma (LUAD), pancreatic adenocarcinoma (PAAD), stomach adenocarcinoma (STAD), thymoma (THYM), clear cell RCC, liver hepatocellular carcinoma (LIHC), testicular germ cell tumors (TGCT). Boxplots that compared the NCKAP5L expression levels between normal and tumor tissues were generated by configuring the primary parameters, such as P-value cutoff=0.01 and $\log_2$fold change cutoff=1 and selecting the option 'Match TCGA normal and GTEx data' in the 'Box Plots' module of GEPIA2. Additionally, the 'Pathological Stage Plot' module of GEPIA2 was used to generate violin plots that showed the NCKAP5L expression levels across different pathological stages of all TCGA tumors. The expression data for the boxplots and violin plots were $\log_2$-transformed transcripts per million (TPM) values with a '+1' offset.

Finally, the 'National Cancer Institute's Clinical Proteomic Tumor Analysis Consortium (CPTAC) analysis' module of the University of Alabama at Birmingham Cancer Data Analysis (UALCAN) portal (http://ualcan.path.uab.edu/analysis-prot.html), an interactive web portal for the analysis of TCGA gene expression, was used and the 'CPTAC analysis' module was applied to assess the difference in the total NCKAP5L protein expression level between tumors and normal tissues (20).

Survival prognosis analysis. To assess the potential role of NCKAP5L in cancer prognosis, multiple online tools were used. First, the 'Survival Map' module of GEPIA2 was used and cut-off high (50%) and cut-off low (50%) values were set to generate survival maps for NCKAP5L expression in all types of TCGA tumors, including overall survival (OS) and disease-free survival (DFS) significance map data. This allowed the identification of high- and low-expression cohorts of NCKAP5L in certain types of cancer (19).

Subsequently, the Kaplan-Meier Plotter (https://kmplot.com/analysis/) was used to assess the prognosis of NCKAP5L in four types of cancer: Breast, ovarian, lung and gastric cancer, at the level of gene chip or RNA-seq. 'NCKAP5L' was also entered into UALCAN in 'TCGA' module, and the 'Survival' section was used to obtain information on the effects of NCKAP5L expression levels on the survival of patients with cancer based on relevant cancer data in TCGA database.

Clinical tissue samples. In the present study, clinical samples of tumor tissue (n=3) and non-tumor tissue (n=3) were collected from patients with colorectal cancer who underwent surgical operations at The Second Affiliated Hospital of Guilin Medical College (Guilin, China). The clinical samples were collected from September to November 2022. The present study was approved (approval no. NO.ZLXM-2022001) by the Ethics Committee of The Second Affiliated Hospital of Guilin Medical College (Guilin, China) and all the patients have signed an informed consent form.. The clinical tissue samples used for reverse transcription (RT)-quantitative (q)PCR were stored at -80˚C after adding the RNA*later*™ Stabilization Solution (Invitrogen; Thermo Fisher Scientific, Inc.).

RT-qPCR. Total RNA was extracted from tumor and non-tumor tissues obtained from patients with colorectal cancer using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.). The extracted RNA was reverse transcribed into cDNA using RevertAid Master Mix, with DNase I (Invitrogen; Thermo Fisher Scientific, Inc.) and RT-qPCR was performed using the ViiA 7 real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reaction mixture consisted of 5 μl 2X SYBR® Green Real-time qPCR Master Mix (Arraystar Inc), 2 μl cDNA, 2 μl nuclease-free water and 0.5 μl primer, in a total volume of 10 μl. The RT-qPCR conditions included 40 cycles of amplification with the following parameters per cycle: 95˚C for 10 min, 95˚C for 10 sec and 60˚C for 60 sec. The primer sequences used were as follows: NCKAP5L, forward (F): 5'-AGATGCTGAGTGCCCTGTTTC-3' and reverse (R): 5'-GTGGCTGGAGTGGAGTGAGTG-3'; β-actin, F: 5'-GTGGCCGAGGACTTTGATTG-3' and R: 5'-CCTGTA ACAACGCATCTCATATT-3'. NCKAP5L expression was quantified using the $2^{-\Delta\Delta Cq}$ method (21), and statistical analysis was performed using GraphPad Prism (version 9; Dotmatics) and a paired Student's t-test.

Genetic alteration analysis. cBioportal (http://www.cbioportal.org) is a multidimensional cancer genomics dataset that provides interactive exploration, visualization and analysis of multivariate genetic alterations (22-24). 'TCGA Pan-Cancer Atlas Studies' section of the cBioPortal was used to obtain data on the type of NCKAP5L mutation, frequency of alteration and copy number alteration in different TCGA-based tumors.

Immune infiltration analysis. To assess the association between NCKAP5L expression and immune infiltration, the

'Immune-Gene' module of TIMER2.0 and specifically selected 'cancer-associated fibroblasts' were used. EPIC (18,25,26), MCPCOUNTER (18,25,27), XCELL (18,25,28) and TIDE (18,25,29) algorithms were then applied with the 'Purity Adjustment' option to assess the immune infiltration of NCKAP5L in several TCGA cancer types (30).

NCKAP5L-related gene enrichment analysis. The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING, version: 11.5) web server (https://string-db.org/) was used to identify proteins that interact with NCKAP5L by inputting 'NCKAP5L' as the single protein name for 'Homo sapiens' organism and setting the following parameters: 'Low confidence (0.150)' for minimum required interaction score; 'evidence' for the meaning of network edges; 'no more than 50 interactors' for max number of interactors to show; and 'experiments' for active interaction sources. The 'Similar Gene Detection' module of GEPIA2 was then used to obtain the top 100 NCKAP5L-correlated genes across all tumor and normal tissues of TCGA, and a correlation analysis was performed using the 'correlation analysis' module of GEPIA2 to visualize the results in a scatter plot with $\log_2$TPM values. Additionally, the 'Gene Corr' function of TIMER2.0 was used to obtain and visualize the association between NCKAP5L and the screened genes in a heatmap.

To further narrow down the candidate genes, the interactive Venn diagram viewer Jvenn (http://bioinformatics.psb.ugent.be/webtools/Venn/) was used to intersect the top 100 genes correlated with NCKAP5L, and the 10 genes that interacted with NCKAP5L-binding proteins, resulting in the identification of one gene (LZTS2).

To perform functional enrichment analysis of NCKAP5L, the Metascape web server (https://metascape.org/gp/index.html) and the Gene Ontology (GO) database were used. GO is a community-based bioinformatics resource that provides information on the function of gene products across different species (31,32).

Statistical analysis. The significance of differences in NCKAP5L expression was assessed using the Wilcoxon rank-sum test, comparing tumor and normal tissues from different TCGA cohorts. Additionally, NCKAP5L expression was analyzed in specific tumor tissues without normal controls using one-way ANOVA and Tukey's Honest Significant Difference post-hoc test, using combined data from TCGA and GTEx databases through GEPIA2. In UALCAN, the overall protein expression of NCKAP5L between phosphorylation sites in normal and tumor tissues was assessed, represented by Z-scores tailored to specific cancer types. Log_2 spectral count ratio values from the CPTAC dataset were normalized within and across samples, and Wilcoxon rank-sum test was used to assess the statistical significance of these differences. Furthermore, NCKAP5L expression analysis was conducted using GraphPad Prism with the paired t-test. Moreover, the Mantel-Cox test in GEPIA2 analysis was used to evaluate the prognostic association between NCKAP5L expression and both OS and DFS across certain tumor types. Kaplan-Meier analysis and log-rank tests were used to assess the prognostic significance of NCKAP5L expression in pan-cancer studies. Finally, Spearman's correlation coefficient was used to assess the correlation between NCKAP5L expression and immune cell infiltration levels. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Analysis of gene expression and functions. Based on the UCSC Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly, NCKAP5L was found to be located on chromosome 12 at position chr12:49791146-49828750 (Fig. 1A). Analysis of the NCKAP5L gene expression data from 54 normal tissues of GTEx RNA-seq from 17,382 samples and 948 donors, revealed that NCKAP5L expression was notably higher in tissues such as 'Nerve-Tibial', 'Cervix-Endocervix', 'Adrenal Gland' and 'Testis' compared with other normal tissues including liver and pancreas (Fig. 1B).

A consensus dataset on NCKAP5L was obtained from the online HPA, which included expression levels of 55 tissue types. This consensus dataset, which combined HPA and GTEx transcriptomics datasets, employed an internal normalization pipeline and color-coded tissue groups based on similar functional features. As a consensus dataset, NCKAP5L expression level was relatively high in the adrenal gland, lung, testis, prostate and myocardium, and was detected to varying degrees in the other 50 tissue types, indicating that NCKAP5L mRNA has low tissue specificity (Fig. 1C).

A boxplot was generated using TIMER2.0 to visualize the differences in NCKAP5L expression between different tumors and their corresponding adjacent normal tissues in TCGA (Fig. 1D). In colon adenocarcinoma (COAD), CHOL and bladder urothelial carcinoma tissue, the expression level of NCKAP5L was significantly increased compared with that in adjacent normal tissues. Additionally, head and neck squamous cell carcinoma (HNSC), GBM and esophageal carcinoma (ESCA) tissue also had notably increased levels of NCKAP5L expression, compared with their corresponding adjacent normal tissues. Moreover, ovarian serous cystadenocarcinoma (OV), kidney renal papillary cell carcinoma (KIRP), kidney renal clear cell carcinoma (KIRC), rectum adenocarcinoma (READ), thyroid carcinoma (THCA), uterine corpus endometrial carcinoma (UCEC), STAD and LIHC tissue demonstrated notably increased NCKAP5L expression compared with their adjacent normal tissue. However, the expression level of NCKAP5L in breast invasive carcinoma (BRCA), kidney chromophobe), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), LUAD, PAAD and pheochromocytoma and paraganglioma (PCPG) tissue had a notably lower expression level of NCKAP5L than the corresponding adjacent tissues (Fig. 1D).

Boxplots were generated using the 'Box Plots' module of GEPIA2 that compared the NCKAP5L expression levels in tumor and normal tissues for several cancer types. Fig. 2A demonstrates that the NCKAP5L gene was significantly upregulated in the tumor tissues of CHOL, DLBC, GBM, LUAD, PAAD, STAD and THYM, compared with that in normal tissues. The heightened expression of NCKAP5L in these tissues may be associated with the development and progression of these cancers, thereby suggesting the potential candidacy of NCKAP5L as a putative biomarker for these cancer types.

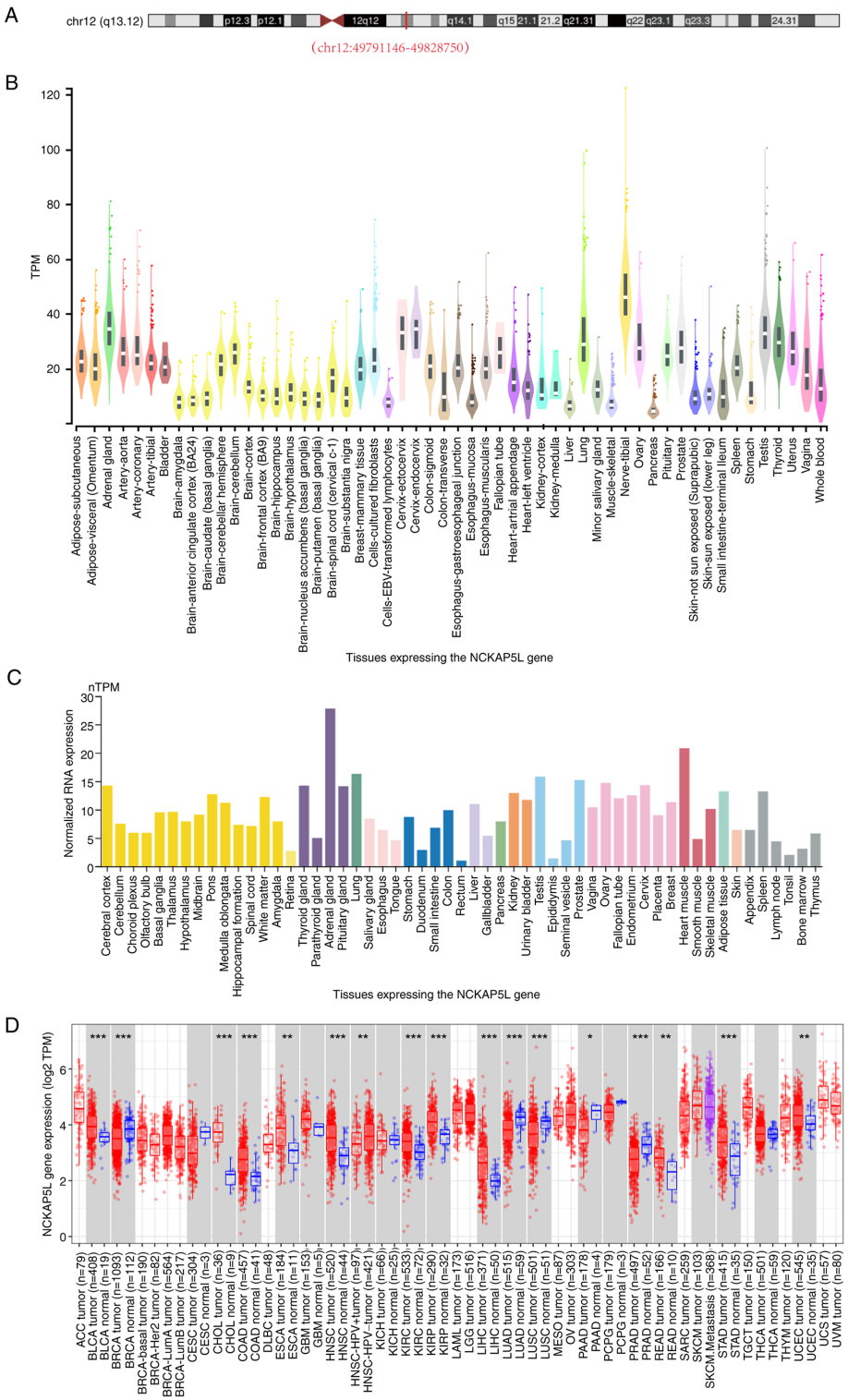

Figure 1. Analysis of NCKAP5L gene expression and genetic location in human tissues and tumors. (A) Genetic location of human NCKAP5L using data from The University of California Santa Cruz Genome Browser Genome Browser on Human Dec. 2013 (GRCh38/hg38) Assembly. (B) Expression of NCKAP5L gene using GTEx RNA-sequencing data from 54 tissues from 17,382 samples and 948 donors. (C) Consensus NCKAP5L dataset consists of normalized expression levels in 55 tissue types, created by combining the Human Protein Atlas and GTEx transcriptomics datasets; (D) Differences in NCKAP5L expression between distinct tumors in The Cancer Genome Atlas cohorts and their adjacent normal tissues using the Tumor Immune Estimation Resource, version 2. *P<0.05; **P<0.01; ***P<0.001. NCKAP5L, Nck-associated protein 5-like; GTEx, Genotype-Tissue Expression project; TPM, transcripts per million; nTPM, normalized TPM, ACC, adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangiocarcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute Myeloid Leukemia; LGG, Brain Lower Grade Glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular Germ Cell Tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine Corpus Endometrial Carcinoma; UCS, Uterine Carcinosarcoma; UVM, Uveal Melanoma.

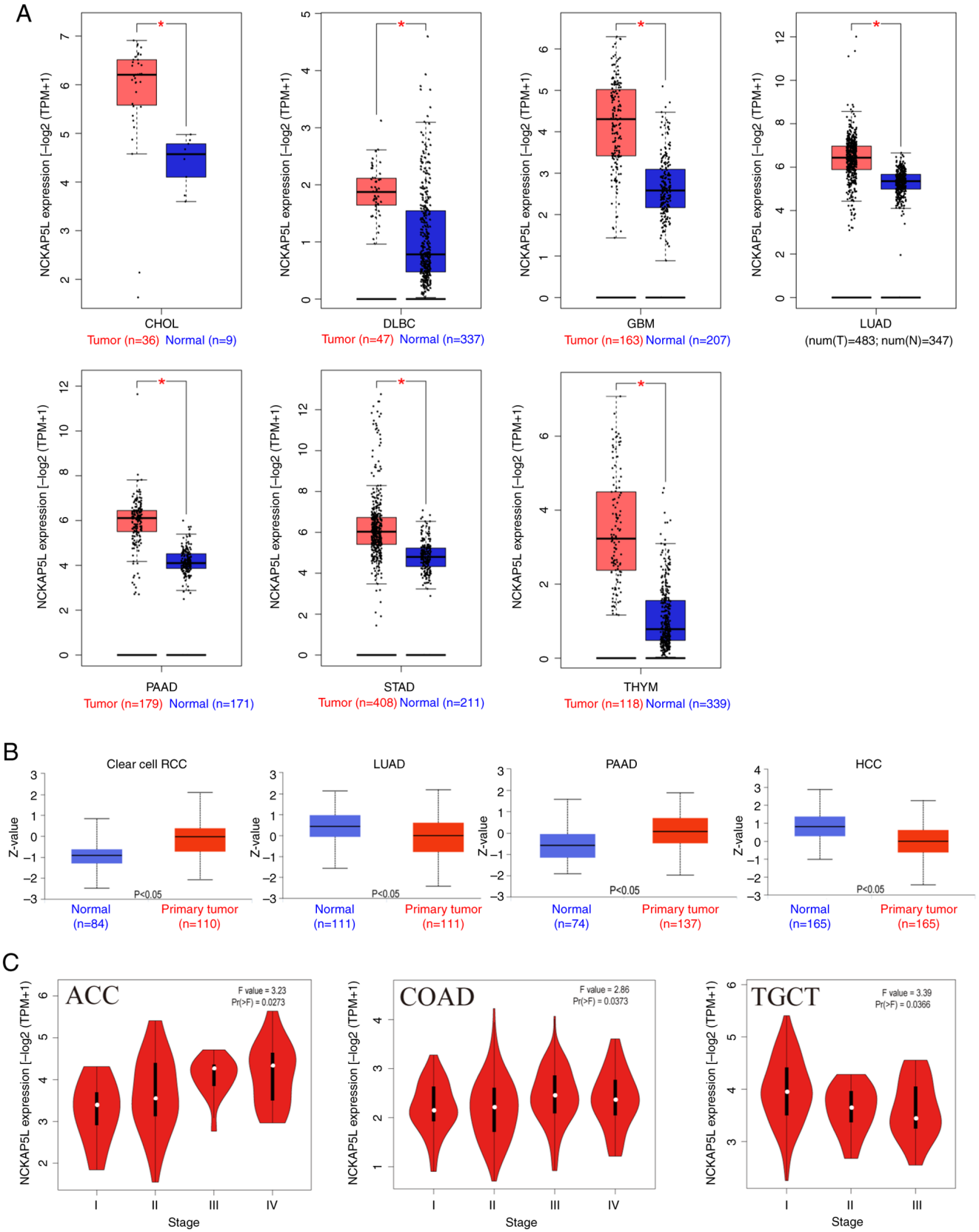

Figure 2. NCKAP5L gene expression patterns in tumor tissues and pathological stages across different cancer types. (A) Difference in NCKAP5L expression in certain tumor tissues (which have no normal control group in TCGA database) and normal tissue, obtained through the combined analysis of data from TCGA and GTEx database using the GEPIA2. (B) Protein expression levels of NCKAP5L in primary tumors and normal tissues in clear cell RCC, PAAD, HCCand LUAD using data from the University of Alabama at Birmingham Cancer Data Analysis portal. (C) Violin plots demonstrating NCKAP5L expression in different pathological stages of ACC, COAD and TGCT, assessed using the GEPIA2. *P<0.05. NCKAP5L, Nck-associated protein 5-like; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression project; GEPIA2, Gene Expression Profiling Interactive Analysis, version 2; CHOL, cholangiocarcinoma; DLBC, lymphoid neoplasm diffuse large B-cell lymphoma; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; STAD, stomach adenocarcinoma; THYM, thymoma; RCC, renal carcinoma; ACC, adrenocortical carcinoma; COAD, colon adenocarcinoma; TGCT, testicular germ cell tumors.

The difference in the total NCKAP5L protein expression level between tumors and normal tissues from the UALCAN portal was assessed. As shown in Fig. 2B, clear cell renal cell carcinoma and PAAD had significantly higher protein expressions of NCKAP5L in primary tumor tissues, compared with that in normal tissues, whereas hepatocellular carcinoma (HCC) and LUAD had significantly lower protein expression of NCKAP5L in primary tumor tissues, compared with that in normal tissues. The results indicate that the variation in NCKAP5L protein expression among primary tissues in different cancer types may serve a significant role in cancer progression. These findings of differential expression sets the stage for further investigations into the specific functional roles of NCKAP5L within numerous cancer subtypes and its potential use as a biomarker.

Furthermore, the 'Pathological Stage Plot' module of GEPIA2 was used to generate violin plots of the NCKAP5L expression in different pathological stages of all TCGA tumors, helping detect the clinical stage of the patient. As shown in Fig. 2C, NCKAP5L expression levels were demonstrated to significantly increase with increases in clinical stage in patients with adrenocortical carcinoma (ACC), but significantly decrease with increases in the clinical stage in patients with TGCT. Additionally, NCKAP5L expression levels were significantly higher in stage III of COAD, compared with that in stages I, II and IV.

Survival prognosis analysis. To further assess the association between the level of NCKAP5L gene expression and prognosis for patients with cancer, GEPIA2 was used to generate survival maps for NCKAP5L expression in all types of TCGA tumors. Subsequently, the association between high and low expression of NCKAP5L and OS and DFS was assessed. Fig. 3A demonstrates that high NCKAP5L expression was significantly associated with lower OS in several cancer types, including ACC, KIRC, OV, COAD, LIHC and skin cutaneous melanoma (SKCM). Similarly, high NCKAP5L expression was significantly associated with lower DFS in ACC, KIRC, SKCM, COAD, LIHC and STAD (Fig. 3B). Therefore, the results of the present study indicate a significant association between high expression of the NCKAP5L gene and unfavorable survival rates across multiple cancer types. Specifically, for cancer types such as ACC, KIRC, OV, COAD and SKCM, patients in the high-expression group consistently exhibited a markedly higher risk of mortality at any given time point compared with the low-expression group. Furthermore, in the case of patients with LIHC, even when the analyzed period of time was <100 months, the high-expression group also demonstrated an notably elevated risk of mortality. Additionally, the DFS analysis indicates that high expression of the NCKAP5L gene was associated with higher mortality rates in numerous cancer types, including ACC, SKCM, COAD, LIHC and STAD. Regardless of the time points of observation, patients in the high-expression group consistently displayed a significantly higher risk of mortality compared with those in the low-expression group. Moreover, for patients with KIRC, a notably elevated risk of mortality was also observed when the analyzed period of time ≤120 months. These findings suggest that the expression levels of NCKAP5L may represent a critical influencing factor in the survival rates of patients across multiple cancer types.

Furthermore, the association between OS, relapse-free survival (RFS), first progression (FP), post-progression survival (PPS), disease-specific survival and progress-free survival, and the level of expression of NCKAP5L in patients with breast, ovarian, lung and gastric cancer was assessed using Kaplan-Meier Plotter based on gene chip (Fig. 4A) or RNA-seq data (Fig. 4B). The findings obtained from gene chip data demonstrated significant associations between NCKAP5L gene expression and patient prognosis across several cancer types. It was demonstrated that for ovarian cancer, the high NCKAP5L expression group exhibited a significantly lower RFS, OS and PPS than that of the low-expression group. Conversely, for breast cancer, gene chip data demonstrated that low NCKAP5L expression was significantly associated with lower RFS, compared with that of the high-expression group. Gene chip data, analyzed within a period of ≤150 months, also demonstrated a significant association between high NCKAP5L expression and a worse prognosis in lung cancer, compared with that of the low-expression group. Furthermore, gene chip data from patients with gastric cancer demonstrated a signification association between high NCKAP5L expression and an adverse prognosis, compared with that in the low-expression group, a trend reflected in OS, FP and PPS outcomes. Lastly, OS results obtained from breast cancer RNA-seq data also indicated that low NCKAP5L expression was significantly associated with an unfavorable prognosis, corroborating the findings from the chip data.

UALCAN was then used to assess the effects of NCKAP5L expression levels on survival of patients with cancer based on relevant cancer data in TCGA database. As shown in Fig. 4C, high expression levels of NCKAP5L in ACC, KIRC, acute myeloid leukemia, PAAD and SKCM were significantly associated with a lower survival probability, compared with that in the low/medium expression group. Additionally, within a 3,000-day analyzed period, elevated NCKAP5L expression in patients with LIHC was significantly associated with reduced survival probabilities, compared with those that had low/medium expression levels. These results collectively indicate that the expression levels of the NCKAP5L gene may serve as a pivotal determinant of patient survival across multiple cancer types, underscoring the potential significance of NCKAP5L as a prospective biomarker for prognostic predictions in diverse cancer types.

Furthermore, the relative expression of NCKA5L in clinical tissue samples of patients with colorectal cancer was quantified using RT-qPCR, obtaining differential expression of NCKAP5L in tumor and non-tumor tissues. As shown in Fig. 4D, the level of NCKAP5L mRNA expression in tumor tissues was significantly higher than in non-tumor tissue (P=0.0301), which was in agreement with bioinformatics analyses.

Genetic alteration analysis. As gene changes were demonstrated to be associated with tumorigenesis, 'TCGA Pan Cancer Atlas Studies' module of cBioPortal was used to analyze the genetic changes of NCKAP5L in different TCGA-based tumors. As shown in Fig. 5A, SKCM had the highest NCKAP5L gene alteration frequency (>7%), mainly comprising of mutations. Across TCGA-based tumors, the types of NCKAP5L gene alterations were predominantly

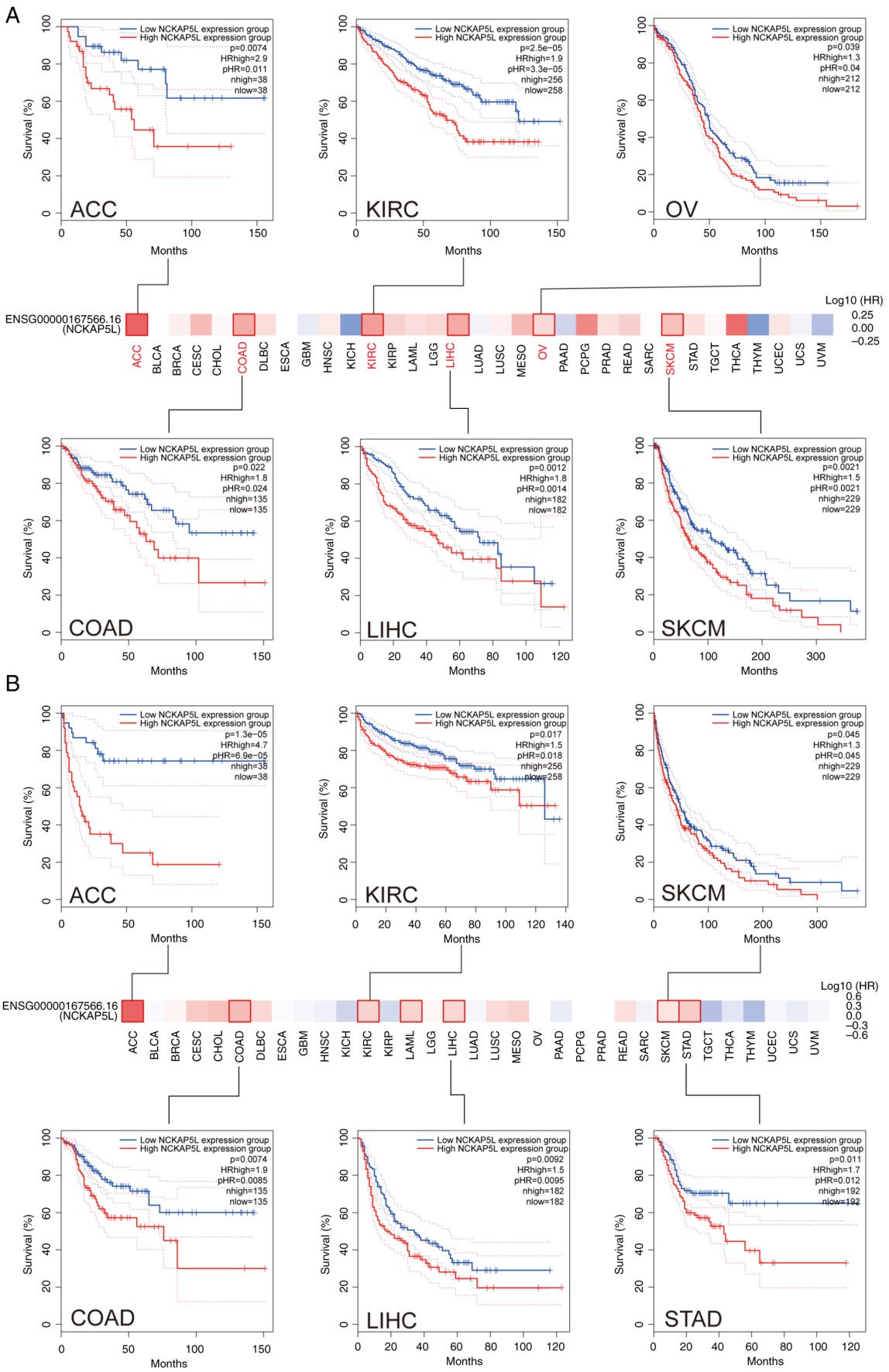

Figure 3. Prognostic association of NCKAP5L expression with overall and disease-free survival in different tumors from the insights of GEPIA2 analysis. Association between the level of NCKAP5L expression and the (A) overall survival prognosis and (B) disease-free survival prognosis in different tumors using the GEPIA2. ACC, adrenocortical carcinoma; KIRC, Kidney renal clear cell carcinoma; OV, Ovarian serous cystadenocarcinoma; COAD, Colon adenocarcinoma; LIHC, Liver hepatocellular carcinoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; NCKAP5L, Nck-associated protein 5-like; GEPIA2, Gene Expression Profiling Interactive Analysis version 2.

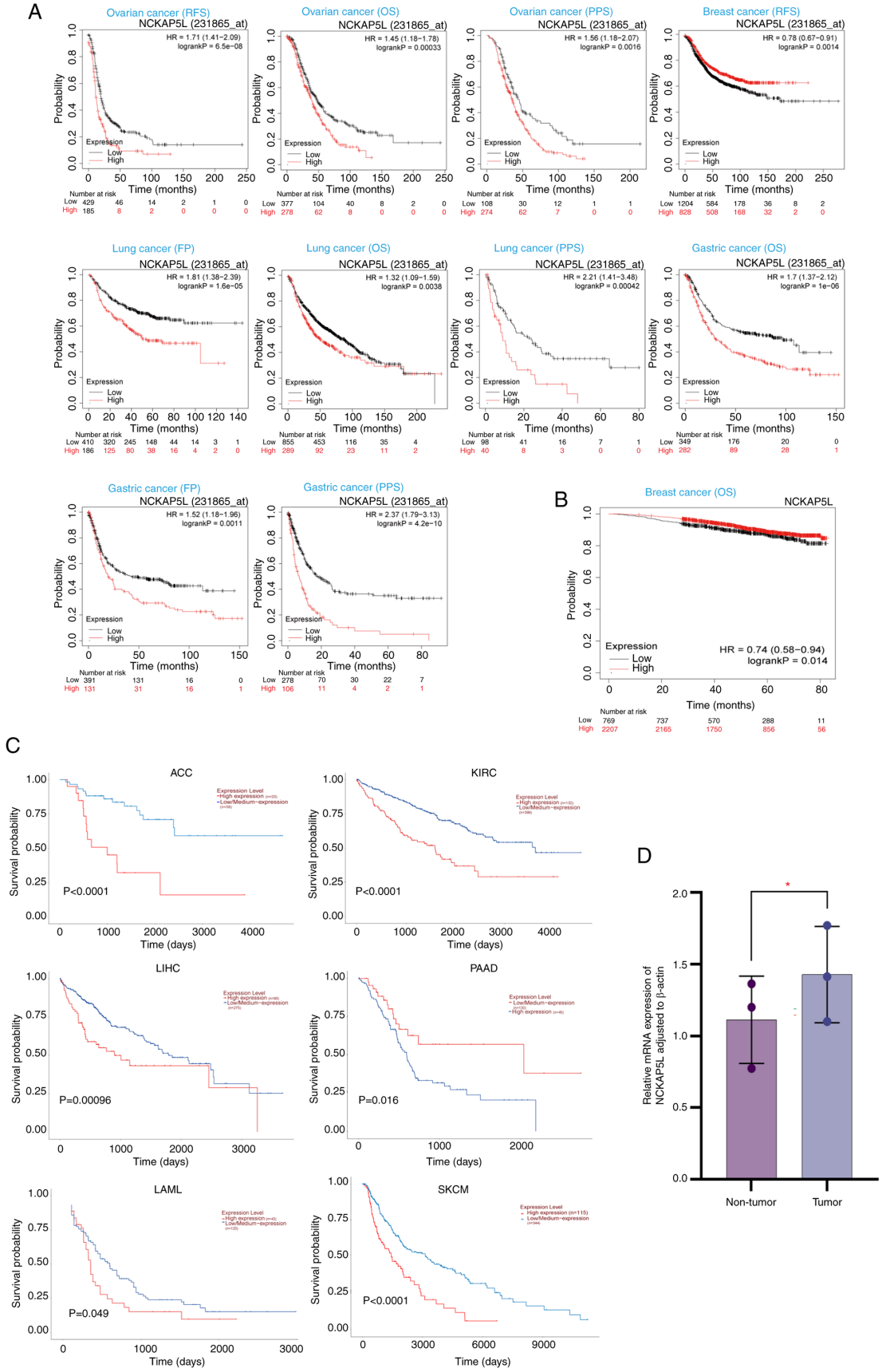

Figure 4. NCKAP5L expression and prognostic significance in pan-cancer studies from gene chip, RNA-seq and TCGA data analyses. Pan-cancer survival prognosis analysis of NCKAP5L expression from (A) gene chip and (B) RNA-seq data, assessed using the Kaplan-Meier Plotter. (C) Effect of NCKAP5L expression levels on survival of patients with cancer, based on relevant cancer data in the TCGA database. (D) NCKAP5L expression is significantly higher in colorectal cancer tumor tissues compared with non-tumor tissues from the same patients, assessed using reverse transcription-quantitative PCR. *P<0.05. NCKAP5L, Nck-associated protein 5-like; seq, sequencing; TCGA, The Cancer Genome Atlas; RFS, relapse-Free Survival; OS, Overall Survival; PPS, Post-Progression Survival FP, First Progression; ACC, Adrenocortical carcinoma; KIRC, Kidney renal clear cell carcinoma; LIHC, Liver hepatocellular carcinoma; PAAD, pancreatic adenocarcinoma; LAML, acute myeloid leukemia; SKCM, Skin Cutaneous Melanoma.

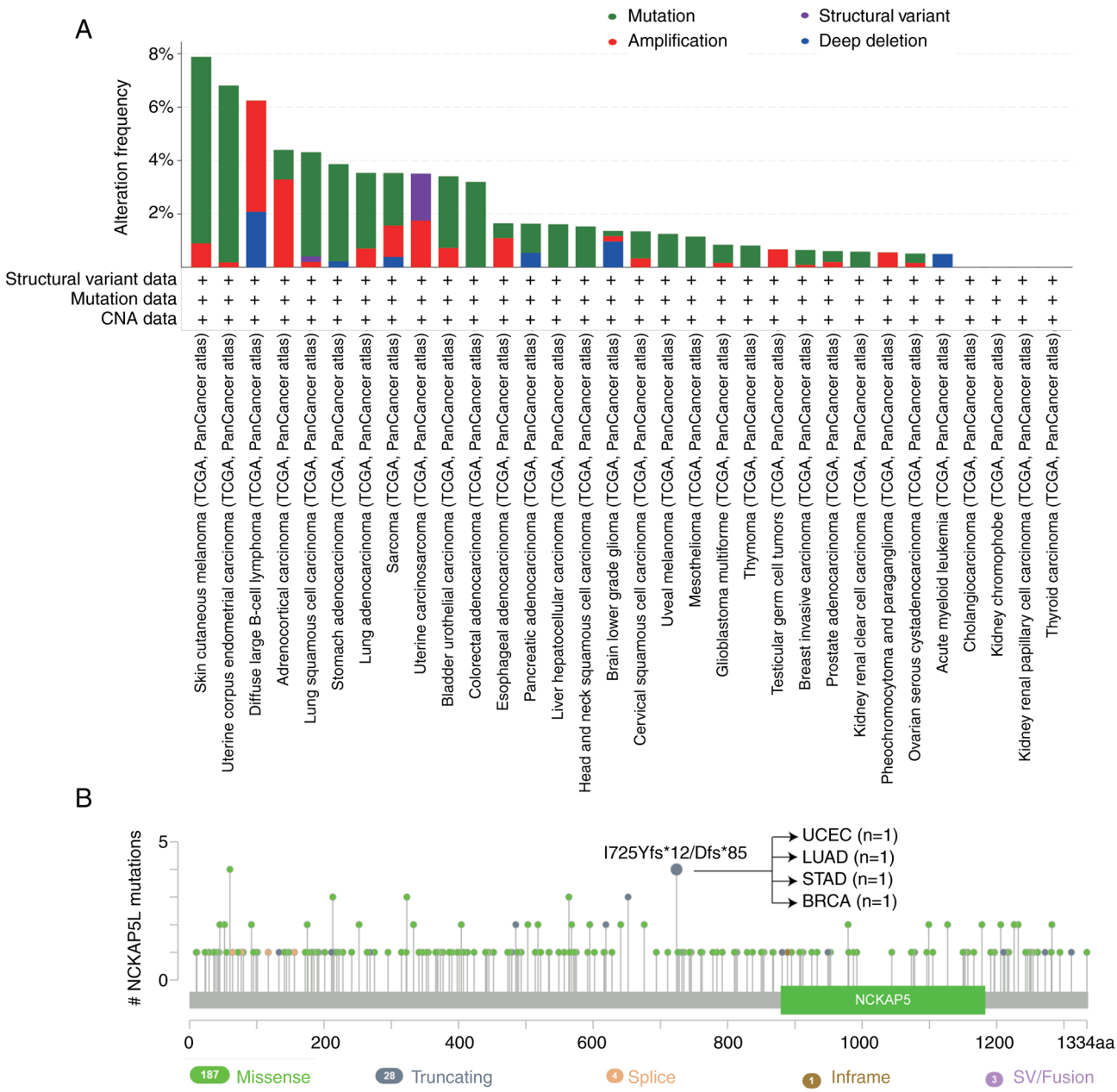

Figure 5. Mutation features of the NCKAP5L gene in different tumors based on data from TCGA, obtained from cBioPortal. (A) Frequency of types of NCKAP5L gene alteration. (B) Type, site and the number of cases of NCKAP5L gene mutations. NCKAP5L, Nck-associated protein 5-like; TCGA, The Cancer Genome Atlas; CNA, DNA copy-number alterations; UCEC, Uterine Corpus Endometrial Carcinoma; LUAD, Lung adenocarcinoma; STAD, Stomach adenocarcinoma; BRCA, Breast invasive carcinoma.

mutations, which could be found in nearly all TCGA tumors. COAD, LIHC, HNSC, uveal melanoma, mesothelioma, THYM and KIRC had only one type of gene alteration, mutations. In addition, amplification was the second most frequently observed genetic alteration, with the highest alteration frequency in DLBC and ACC. TGCT and PCPG only had one type of gene alteration, amplification. Moreover, Fig. 5B demonstrates the type, site and number of cases of NCKAP5L mutations. Missense mutations were the most common, and a translocation mutation due to an I725Yfs*12/Dfs*85 alteration in one case of UCEC, LUAD, STAD and BRCA was found.

Immune infiltration analysis. The progression of cancer is influenced by behavior of cancer cells and their interaction with the tumor microenvironment (33). Serving a significant role in the microenvironment, tumor-infiltrating immune cells also affect the development and metastasis of cancer (34). To assess the potential correlation between NCKAP5L expression and immune infiltration of cancer-associated fibroblasts across diverse TCGA cancer types, we utilized TIMER2.0. A total of four algorithms (EPIC, MCPCOUNTER, XCELL and TIDE) were used to generate a heatmap (Fig. 6A) and partial scatterplots (Fig. 6B). The heatmap revealed a statistically significant positive correlation between immune infiltration of cancer-associated fibroblasts and diverse TCGA cancer types, including BRCA, CESC, COAD, DLBC, ESCA, HNSC, KIRP, LUAD, LUSC, OV, PAAD, PRAD, READ, STAD, THCA, THYM and UCEC. The positive correlation

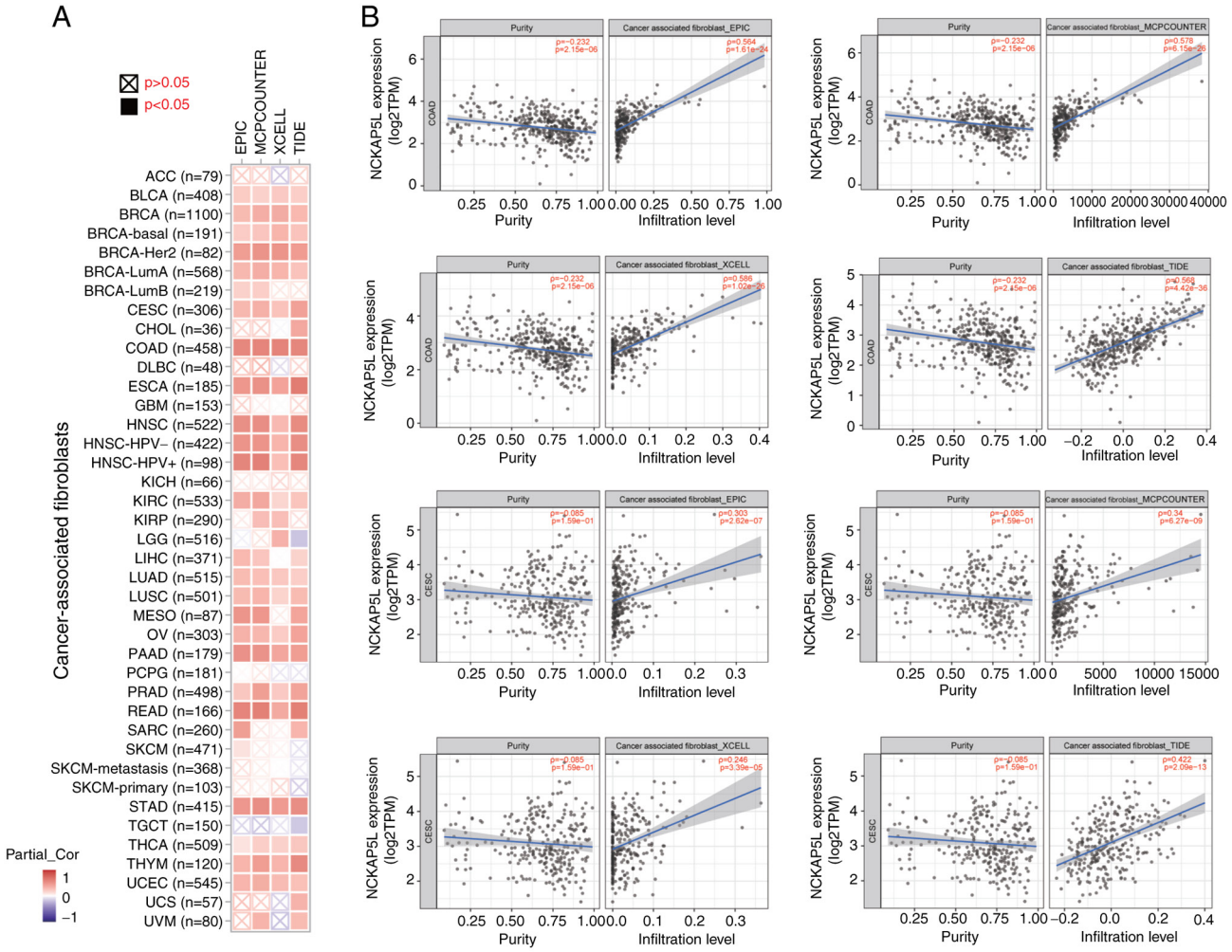

Figure 6. Correlation between NCKAP5L expression and immune infiltration, based on data from four algorithms: EPIC, MCPCOUNTER, XCELL and TIDE. (A) Immune infiltration of cancer-associated fibroblasts have a significant positive correlation with diverse TCGA cancer types. (B) Positive correlation between immune infiltration of cancer-associated fibroblasts and COAD and CESC, visualized by scatter plots. Analysis using four algorithms, EPIC, MCPCOUNTER, XCELL and TIDE, found that the NCKAP5L gene expression levels of COAD and CESC tumors were positively correlated with the level of cancer associated fibroblast infiltration. ACC, Adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangiocarcinoma; COAD, Colon adenocarcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LGG, Brain Lower Grade Glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; TGCT, testicular Germ Cell Tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine Corpus Endometrial Carcinoma; UCS, Uterine Carcinosarcoma; UVM, Uveal Melanoma, NCKAP5L, Nck-associated protein 5-like; TCGA, The Cancer Genome Atlas; COAD, colon adenocarcinoma; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; cor, correlation; TPM, transcripts per million.

found for COAD and CESC were also visualized using scatterplots (Fig 6B).

Protein phosphorylation analysis. In the present study, an in-depth analysis of variations in NCKAP5L phosphorylation levels across a spectrum of tumor types within the CPTAC dataset was performed. First, a schematic representation of the NCKAP5L protein domain was generated (Fig. 7A). Subsequently, alterations in phosphorylation sites and the corresponding protein phosphorylation levels of NCKAP5L in different tumor types was assessed (Fig. 7B). In comparison with normal tissue counterparts, there were distinctive patterns of NCKAP5L phosphorylation observed in the tumors from the base CPTAC dataset: The S477 site for PAAD, LIHC, HCC and HNSC demonstrated significantly higher phosphorylation levels; the S630 site had a significantly higher phosphorylation level for LIHC, GBM, HCC and HNSC; the T1235 site had a significantly higher phosphorylation level for PPAD; the T733 site had a significantly higher phosphorylation level for HNSC; the S735 site had a significantly higher phosphorylation level for GBM; the T1175 site had a significantly higher phosphorylation level for HNSC; the S440 had a significantly higher phosphorylation level for PAAD, but a significantly lower phosphorylation level for HNSC; the S767 site had a significantly lower phosphorylation level for PAAD; and the T1109 site had a significantly lower phosphorylation level for HNSC. These findings provide a deeper understanding of the intricate and multifaceted landscape of NCKAP5L phosphorylation in

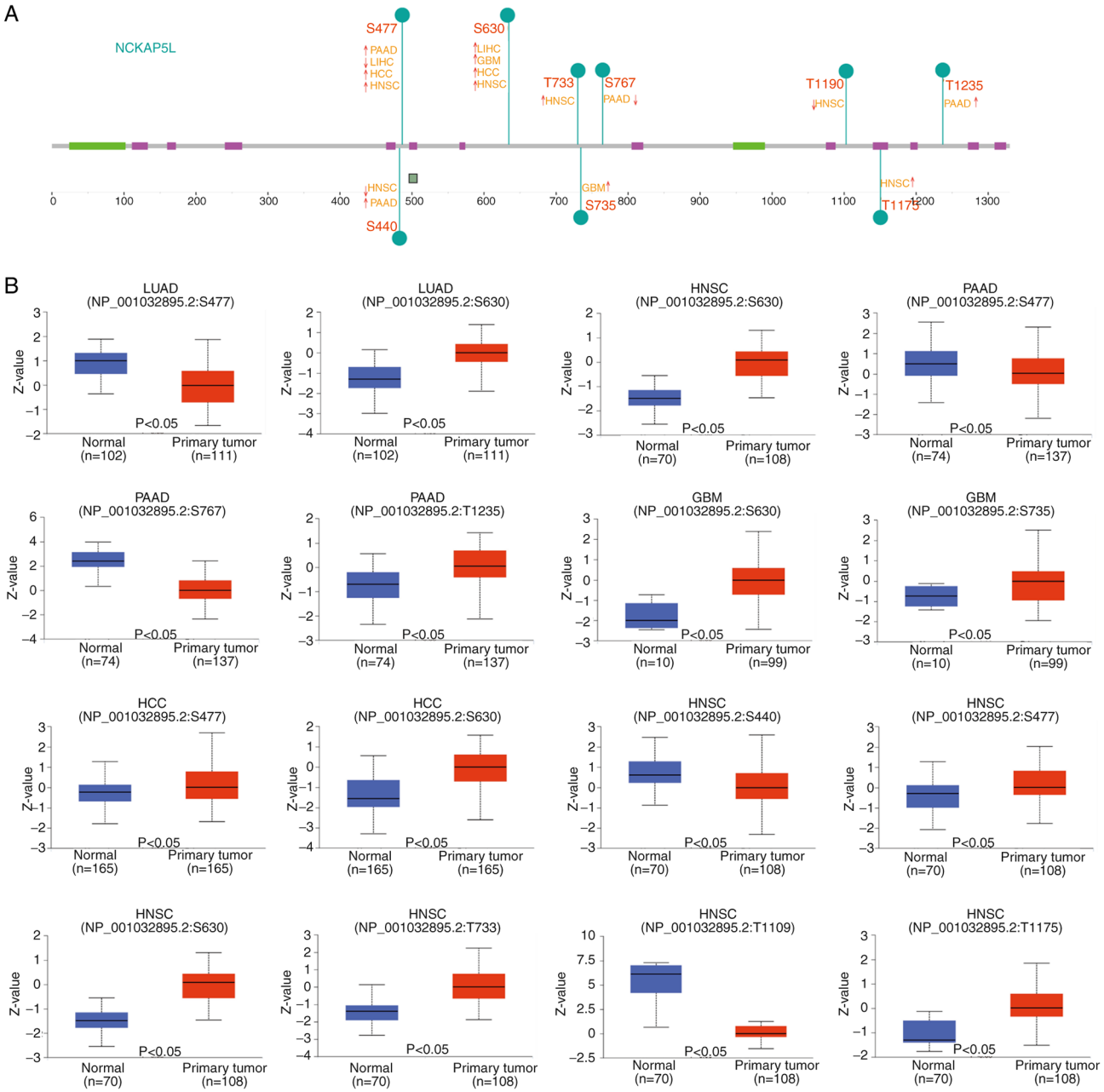

Figure 7. Phosphorylation analysis of NCKAP5L protein in different tumors. (A) Schematic diagram of phosphoprotein sites of the NCKAP5L protein. (B) Difference between the protein expression level of NCKAP5L in the phosphorylation sites of different normal and tumor tissues. PAAD, Pancreatic adenocarcinoma; LIHC, Liver hepatocellular carcinoma; HCC, hepatocellular carcinoma; HNSC, Head and Neck squamous cell carcinoma; GBM, Glioblastoma multiforme; NCKAP5L, Nck-associated protein 5-like.

a diverse array of tumor types. The phosphorylation status of NCKAP5L, as demonstrated by the findings of the present study, appears to be a finely tuned and dynamic process, showing distinct patterns and variations across different cancer types. This not only emphasizes the complexity of the involvement of NCKAP5L in numerous malignancies, but also underscores its potential significance in the context of cancer biology.

NCKAP5L-related gene enrichment analysis. The potential molecular mechanisms underlying the involvement of NCKAP5L in tumor occurrence and development was evaluated by performing gene function analysis, screening out the NCKAP5L-binding proteins and related genes for gene function analysis (31,32).

Using the STRING online database, a network of 10 NCKAP5L-binding protein interactions with 11 nodes and 13 edges was generated (Fig. 8A). Nodes represented genes, and edges represented associations between bound genes. Subsequently, the top 100 NCKAP5L-correlated targeting genes were obtained, based on data of tumor and normal tissues from TCGA with the help of GEPIA2. Fig. 8B demonstrates the intersection of NCKAP5L-correlated and co-expressed genes, showing one common member named LZTS2. In most TCGA tumors, NCKAP5L was significantly positively correlated with eight genes (RBM5, RBM6, DVL2,

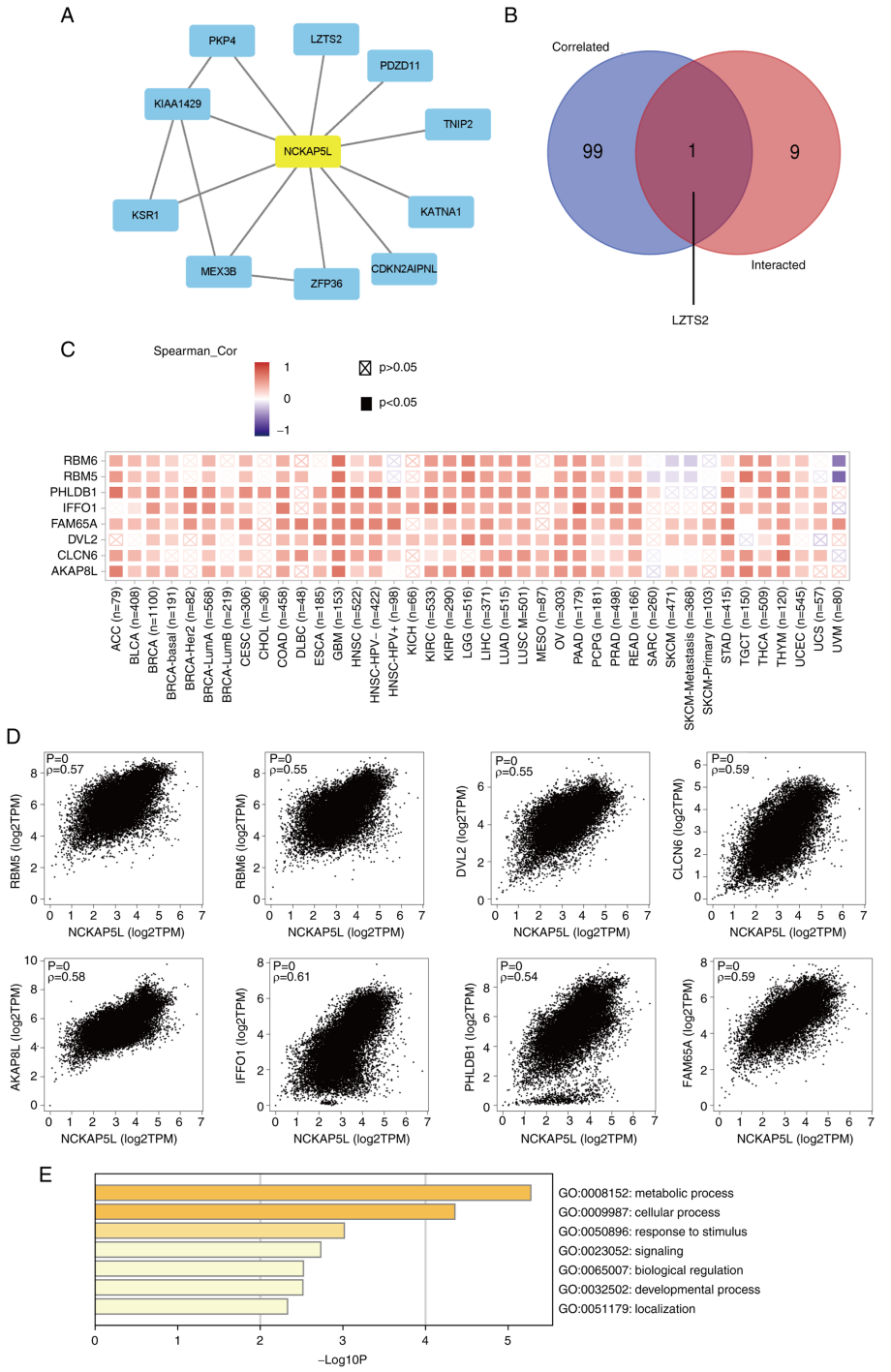

Figure 8. NCKAP5L-related gene enrichment analysis. (A) NCKAP5L-binding proteins obtained using the Search Tool for the Retrieval of Interacting Genes/Proteins web server tool. (B) Venn diagram demonstrating the intersection analysis of NCKAP5L-binded and -correlated genes. (C) Heatmap of detailed cancer types, based on data from the Cancer Genome Atlas. The partial correlation and P-value were determined using a purity-adjusted Spearman's rank correlation test. (D) Top 100 NCKAP5L-related genes acquired using the Gene Expression Profiling Interactive Analysis, version 2. The correlation between the expression of NCKAP5L and RBM5, RBM6, DVL2, PHLDB1, CLCN6, AKAP8L, IFFO1 and FAM65A is displayed. (E) Bar chart of the molecular functions of NCKAP5L, determined using GO analysis based on Metascape. Darker color signifies a more pronounced enrichment, suggesting a higher degree of significance for the association between the gene set and the specific biological process, cellular component, or molecular function represented by the Goterm. NCKAP5L, Nck-associated protein 5-like; Cor, correlation; GO, Gene Ontology; TPM, transcripts per million; ACC, Adrenocortical carcinoma; BLCA, Bladder Urothelial Carcinoma; BRCA, Breast invasive carcinoma; CESC, Cervical squamous cell carcinoma and endocervical adenocarcinoma; CHOL, Cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; ESCA, Esophageal carcinoma; GBM, Glioblastoma multiforme; HNSC, Head and Neck squamous cell carcinoma; KICH, Kidney Chromophobe; KIRC, Kidney renal clear cell carcinoma; KIRP, Kidney renal papillary cell carcinoma; LAML, Acute Myeloid Leukemia; LGG, Brain Lower Grade Glioma; LIHC, Liver hepatocellular carcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; MESO, Mesothelioma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; PCPG, Pheochromocytoma and Paraganglioma; PRAD, Prostate adenocarcinoma; READ, Rectum adenocarcinoma; SARC, Sarcoma; SKCM, Skin Cutaneous Melanoma; STAD, Stomach adenocarcinoma; TGCT, Testicular Germ Cell Tumors; THCA, Thyroid carcinoma; THYM, Thymoma; UCEC, Uterine Corpus Endometrial Carcinoma; UCS, Uterine Carcinosarcoma; UVM, uveal Melanoma.HCC, hepatocellular carcinoma, RBM5: RNA Binding Motif Protein 5; DVL2, Dishevelled Segment Polarity Protein 2; PHLDB1, Pleckstrin Homology Like Domain Family B Member 1; CLCN6, Chloride Voltage-Gated Channel 6, AKAP8L: A-Kinase Anchoring Protein 8-Like, IFFO1, Interferon-Induced Transmembrane Protein Family Member 1; FAM65A, Family with Sequence Similarity 65 Member A.

PHLDB1, CLCN6, AKAP8L, IFFO1 and FAM65A), as shown in Fig. 8C. In addition, Fig. 8D demonstrates the significant positive correlation between NCKAP5L and the screened related genes, underscoring their potential interplay in cellular processes.

For the functional enrichment analysis of NCKAP5L, the GO framework was used by accessing the Metascape web server. As shown in Fig. 8E, the results of the GO enrichment analysis revealed that the collective genes assessed were predominantly enriched in categories related to 'metabolic process' and 'cellular process'. This enrichment pattern suggests that NCKAP5L may serve a pivotal role in tumorigenesis and development through its involvement in these essential biological pathways. The enrichment of genes in these specific categories also implies that NCKAP5L may be intricately connected to cellular metabolism and general cellular activities, further highlighting its potential significance in cancer biology and disease progression.

Discussion

Pan-cancer analyses serve key roles in studying different types of tumors through a multi-dimensional approach and have notable implications for cancer biomarker discovery, treatment and prognosis. With advancement of human genome research, abnormal gene expression, which is caused by factors such as gene mutations and copy number alterations, has been found to be closely related to the development and progression of cancers (35,36). Therefore, the present study focused on a gene that was abnormally expressed in the tissues of different cancers.

NCKAP5L is a gene of which its roles in most types of human cancers remains unclear and therefore, a pan-cancer analysis of this gene from a comprehensive perspective is necessary. Furthermore, considering colorectal cancer is the 3rd most common cancer worldwide, the present study performed pan-cancer analyses and focused on colorectal cancer (1,2).

Using bioinformatics tools, analyses of numerous cancers, including colorectal cancer, were performed from multiple perspectives, including gene expression and mutation, immune invasion, and survival and prognosis analyses.

Data from the present study indicated that the both the gene and protein expression of NCKAP5L were significantly upregulated in the tissues of different cancers, in comparison with that in normal tissues, and the expression level of NCKAP5L was associated with the stage of colorectal cancer. RT-qPCR analysis of clinical tissue samples further demonstrated that the expression of NCKAP5L in colorectal cancer tissues was significantly higher than that in non-tumor tissues, which was consistent with the results of the bioinformatics analysis. Therefore, this indicates that the abnormal expression of NCKAP5L may serve as a promising biomarker for the diagnosis and prognosis of human cancers, especially in colorectal cancer.

Tubulin, encoded by NCKAP5L, is an important component of microtentacles, which are characteristic adhesion sites providing signaling nodes in epithelial-mesenchymal transition (EMT) (37). During EMT, rearrangements of cell adhesion may affect the proliferation and invasive capacity of cancer cells. Therefore, EMT may serve an essential role in cancer progression and metastasis by promoting tumor cell invasion and tissue infiltration (38-42). It has been reported that EMT can promote the invasion and metastasis of cancers such as lung, colorectal, liver and ovarian cancer (43-48). Therefore, NCKAP5L may serve a role in tumor promotion during EMT.

In addition, the bioinformatics analysis in the present study identified NCKAP5L mutations in certain cancers. Moreover, the high expression and mutations of NCKAP5L may closely associate with EMT and promote the development of cancer, invasion and metastasis. The present study found that patients with colorectal cancer with high expression of NCKAP5L had worse OS and DFS than those with low expression, and the expression level of NCKAP5L in patients with cancer was associated with the stage of cancer. Thus, this indicates that NCKAP5L may be a potential biomarker for the occurrence, development, invasion and metastasis of colorectal cancer from multi-dimensional analysis.

As an important component of the tumor microenvironment, fibroblasts are important mesenchymal stem cells that constitute tissues and organs of organisms and may serve roles in biological processes such as tissue fibrosis and collagen secretion (49-51). Cancer-related fibroblasts have been extensively studied and are associated with the occurrence and development of cancers (52-55). There is also an association between NCKAP5L and cancer-related fibroblast immune infiltration (52-55). However, the mechanism of the interaction between fibroblasts and cancer tissue is still unclear. It has been reported that the C-C chemokine ligand 5 (CCR5) secreted by tumor tissue recruits a large number of fibroblasts through the CCR5 solute carrier family 25 member 24 (SLC25A24) pathway, and further promotes the invasion and metastasis of colorectal cancer by using the function of fibroblasts to promote angiogenesis and collagen synthesis (2). The present study demonstrated that the expression of NCKAP5 was positively associated with the immune infiltration of cancer-related fibroblasts in colorectal cancer. Therefore, this indicates that NCKAP5L may promote the secretion of CCR5 in tumor tissue and then recruit a large number of cancer-related fibroblasts via the SLC25A24 pathway. Subsequently, cancer-related fibroblasts lead to invasion and metastasis of colorectal cancer by promoting tumor tissue to generate blood vessels and collagen (56). In the present study, GO enrichment analysis was performed on NCKAP5L-binding proteins and expression-related genes and it was demonstrated that most of them were enriched in 'metabolic process'- and 'cellular process'-related functions, which further indicated that NCKAP5L may be associated with the invasion and proliferation of related cancers. Therefore, the findings indicate that NCKAP5L may be a valuable biomarker for the prognosis of human cancers.

However, the present study has limitations. Firstly, databases such as TCGA, CPTAC and GTEx, have a limited number of cases for each cancer type. Secondly, the present study only assessed the expression of NCKAP5L in the tissues of patients with colorectal cancer and did not evaluate other types of tumors. Bioinformatics tools are limited by restricted customization and debugging options. These tools typically rely on external databases, which are constantly evolving.

Consequently, this dynamic nature of data can lead to variations in analysis results at different time points. Furthermore, online tools often provide limited analysis choices, constraining users in terms of personalized customization and debugging capabilities. For example, when conducting survival analysis for NCKAP5L using online analysis tools, the inability to set the observation time range prevents analyzing survival curves within specific time intervals. This, in turn, limits our ability to conduct in-depth analysis of time intervals where survival curve crossovers do not occur.

In conclusion, the present study combined bioinformatics with RT-qPCR to provide a comprehensive characterization of NCKAP5L in pan-cancer tumor types, which is beneficial to further understand the role of NCKAP5L in tumorigenesis, progression and prognosis. The results indicate that NCKAP5L may be a potential prognostic biomarker in pan-cancer, with particular relevance to colorectal cancer.

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Availability of data and materials

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

Authors' contributions

MO and YS were involved in the conception and design of the study. MO provided administrative support. YS, WL, CM and XH contributed to the provision of study materials or patients. YS, WL and CM were responsible for the collection and assembly of data. YS, MO, WL and XH performed the data analysis and interpretation. MO and YS participated in the writing of the manuscript. YS and MO confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Approval was granted by the Ethics Committee of the Second Affiliated Hospital of Guilin Medical University (Guilin; China; September 24, 2022; approval no. ZLXM-2022001) and all the patients have signed an informed consent form.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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