Pan-Cancer Analysis Identifies YTHDF2 as an Immunological and Prognostic Biomarker

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Research

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Abstract

Background

N6-methyladenosine (m6A) modification is a dynamic and reversible post-transcriptional RNA modification prevalent in eukaryotic cells. As a member of m6A reader protein, YT521-B homology domain family 2 (YTHDF2) has been demonstrated involving in many vital biological process, whereas its role in cancers remains unclear.

Methods

We evaluated the immunohistochemical staining of tissue sections from 51 hepatocellular carcinoma patients and TIMER database to investigate the association between the YTHDF2 expression and the infiltration of immunocytes. TCGA, GEPIA2, GEO and GTEx databases were used to analyze the differential expression of YTHDF2 in various cancers. R 3.6.3 is used for Data visualization.

Results

YTHDF2 is overexpressed in most tumors, and the increased YTHDF2 expression is associated with poor OS, PFI, and DSS. YTHDF2 expression is also positively correlated with MMR, TMB and MSI. Moreover, GSEA revealed that functions associated with cell adhesion, cell cycle and immune regulation were enriched in YTHDF2 overexpression tumors. Further study verified the participation of YTHDF2 in immune regulation through its correlation with ICP genes and the infiltration of immunocytes in tumor microenvironment. Notably, we demonstrated a positive correlation between YTHDF2 expression and the infiltration of $CD8^+$ T cells and macrophages, as well as exhausted T lymphocytes.

Conclusion

YTHDF2 can be used as a new therapeutic target and a potential biomarker for cancer immune evasion and poor prognosis.

1. Introduction

Cancer is a leading cause of death worldwide and seriously affects the quality of life [1]. To date, there is no absolute cure for cancer. In recent years, the emerging cancer immunotherapy has shown its potential in revolutionizing cancer treatment, among which the immune checkpoint blockage therapy has been proved to be a prominent approach [2]. With the continuous development and improvement of public databases such as The Cancer Genome Atlas (TCGA), it is possible to predict new immunotherapy targets by performing pan-cancer expression analysis and evaluating their correlation with clinical prognosis and related signaling pathways [3].

N^6-methyladenosin (m^6A) is one of the most pervasive mRNA modifications in eukaryotes[4, 5], and has been reported involving in many biological processes, such as mRNA stability[6], protein translation[7], embryonic development [8], and immunoregulation[9]. Recently, emerging evidences showed that dysregulation of m^6A modification and aberrant expression of m^6A-associated proteins can promote the initiation and progression of cancers [10, 11]. For instance, the aberrant expression of fat mass and obesity-associated protein (FTO), a demethylase that can decrease the systemic m^6A levels, leaded to the downregulation of tumor suppressor genes and upregulation of tumor promoter genes[12]. The upregulation of another demethylase, α-ketoglutarate-dependent dioxygenase alkB homolog 5 (ALKBH5), tended to stimulate cancer progression through stabilizing stemness-related transcripts [13, 14]. Moreover, increased RNA methylation catalyzed by methyltransferase-like 3 (METTL3) was required for cancer development [15, 16]. The fate of m^6A-modified mRNAs was dependent on the m^6A selective binding proteins [17]. YTH-Domain Family Member 2 (YTHDF2) is the first identified m^6A-binding protein and its function in mRNA stabilization has been well-studied [18]. It has been reported that YTHDF2 played a dual role in pancreatic cancer cells by promoting proliferation, whereas suppressing migration and invasion[19]. YTHDF2 might function as a tumor suppressor to inhibit cell growth and proliferation in HCC [20]. On the contrary, YTHDF2 could also act as a tumor oncogene to promote prostate cancer cell proliferation and migration [21]. However, there is insufficient scientific evidence to conclude a consistent pathogenic role of YTHDF2 in the development and progression of various cancers, let alone to summarize its role and underlying mechanisms in regulating the immune microenvironment and modulating therapeutic responses. Therefore, we used numerous databases to explore and evaluate the associations between YTHDF2 expression and prognosis, tumor mutation load (TMB), microsatellite instability (MSI), immune checkpoint (ICP) genes, tumor microenvironment (TME), immune cell infiltration, and immune-related genes, hoping to uncover the underlying mechanisms.

2. Materials And Methods

TIMER Database Analysis

The Tumor Immune Estimation Resource (TIMER) database (https://cistrome.shinyapps.io/timer/)[22-23] includes 10,897 samples covering 32 cancer types from TCGA. We analyzed the expression of YTHDF2 in different cancer types via different expression modules. The TIMER2 server was used to analyze the correlations between YTHDF2 expression and the infiltration of six types of immune cells, including B cells, cluster of differentiation 8-positive ($CD8^+$) T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells (DCs). The correlation analysis was conducted using the Partial Spearman's correlation coefficient and the purity-corrected partial Spearman's rho value along with the corresponding p values ($p < 0.05$).

GEPIA Database Analysis

Gene Expression Profiling Interactive Analysis (GEPIA) (http://gepia.cancer-pku.cn/index.html)[24-25] is an interactive web server that provides the RNA sequencing expression analysis results of 9,736 tumor

and 8,587 normal samples from the TCGA and the GTEx projects, using a standard processing pipeline. GEPIA was used to analyze the expression of YTHDF2 in 33 different cancer types.

Correlation Analysis of YTHDF2 Expression and Prognosis

Survival data for each sample was extracted and downloaded from the TCGA. Three indicators, including overall survival (OS), disease-specific survival (DSS), and progression-free interval (PFI), were selected to study the relationship between the YTHDF2 expression and prognosis of patients. The Kaplan-Meier method and log-rank test were used for survival analyses ($p < 0.05$) of each cancer type. Survival curves were drawn using the R packages “survival” and “survminer.” Moreover, Cox analysis was conducted using the R packages “survival” and “forestplot” to determine the pan-cancer relationship between YTHDF2 expression and the survival.

Correlation Analysis of YTHDF2 Expression and Multiple Tumor-related Factors

The relationship between YTHDF2 expression and TMB, MSI, ICP genes, and ESTIMATE score in the TME was explored via the SangerBox website (http://sangerbox.com/Tool)

Immunohistochemistry (IHC) Staining and the Evaluation of YTHDF2 Protein Expression

Anonymously archived tissue chips of human hepatocellular carcinoma were obtained from Department of Pathology, Southern Medical University. For immunohistochemical (IHC) staining, sections were dewaxed, rehydrated by ethanol series, followed by a high-pressure antigen repair with TRIS-EDTA buffer for seven minutes. After Incubating in 3% H_2O_2 for 15 min to block endogenous peroxidase, slices were blocked with 5% normal goat serum at room temperature for 60 min. Then, the slices were incubated with appropriate primary antibody of YTHDF2 (1:1000, A15616, Abclonal), CD3 (ZA-0503, ZSGB-BIO), CD8 (ZA-0508, ZSGB-BIO) and CD68 (ZM-0060, ZSGB-BIO) at 4°C overnight. IHC staining was performed using Horseradish peroxidase (HRP) conjugated with DAB. IHC staining results of HCC samples and adjacent normal samples were examined under double-blind conditions, and the semi-quantitative analyses were performed according to the scores of intensity and degree. The intensity scores were defied as 0 (no staining), 1 (weak), 2 (medium) and 3 (strong). The percentage of positive staining area was defined as 0 (no staining), 1 (1-25%), 2 (26-50%), 3 (51-75%) and 4 (>=75%). IHC scores are calculated by multiplying the intensity and degree scores of each sample (scale range from 0 to12). YTHDF2 level was divided into "low YTHDF2" group (score <6) and "high YTHDF2" group (score >=6).

The Biological Significance of YTHDF2 in Tumors

Gene Set Enrichment Analysis (GSEA) was conducted to investigate the biological functions of YTHDF2 in tumors.

3. Results

The Expression of YTHDF2 in Various Cancer Types

We used the TIMER database to study the differential expression of YTHDF2 in tumor tissues and adjacent normal tissues. Figure 1A shows that YTHDF2 was overexpressed in bladder urothelial carcinoma, cholangiocarcinom, esophageal carcinoma, head and neck squamous cell carcinoma, lung adenocarcinoma, prostate adenocarcinoma, liver hepatocellular carcinoma, stomach adenocarcinoma, and uterine corpus endometrial carcinoma tissues, compared with that in the adjacent normal tissues. However, the expression of YTHDF2 was lower in kidney chromophobe, kidney renal clear cell carcinoma, kidney renal papillary cell carcinoma, skin cutaneous melanoma, and thyroid carcinoma tissues, compared with that in the adjacent non-tumorous tissues. There was no expression difference of YTHDF2 between tumor tissues and adjacent normal tissues in breast invasive carcinoma, lung squamous cell carcinoma, and rectum adenocarcinoma. Unfortunately, we could not compare the expression of YTHDF2 between tumor and non-tumor tissues in adrenocortical carcinoma, lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), glioblastoma multiforme (GBM), acute myeloid leukemia (LAML), lower-grade glioma (LGG), mesothelioma, ovarian serous cystadenocarcinoma (OV), pancreatic adenocarcinoma (PAAD), pheochromo- cytoma and paraganglioma, sarcoma (SARC), skin cutaneous melanoma, testicular germ cell tumor, thymoma, uterine carcinosarcoma, and uveal melanoma, for the unavailable of adjacent normal tissues.

To provide a more comprehensive evaluation of YTHDF2 expression in various cancer types, we also used the online GEPIA database to compare the expression of YTHDF2 in 33 cancer types paired with normal tissues in TCGA and GTEx database. Figure1B shows that the expression of YTHDF2 was elevated in many cancer types, especially in DLBC, GBM, PAAD, and THYM. In addition, when only the tumor tissues was evaluated from the TCGA cohort, we found that the expression of YYTHDF2 was the highest in LUAD tumor tissues, while lowest in the PCPG tumor tissues (Figure 1C). Whereas studying the normal tissues from the GTEx database, we found that YTHDF2 expression was highest in Fallopian tissues and lowest in the heart (Figure 1D).

Prognostic Analysis of YTHDF2 in Cancers

To study the association between YTHDF2 expression and prognosis, we performed a series of survival-associated analysis, including OS, DSS, and PFI. Cox proportional hazards model analysis showed that the expression of YTHDF2 was associated with OS in LIHC ($p = 0.005$), KIRC ($p = 0.023$), KICH ($p = 0.038$), ACC ($p = 0.028$), LGG ($p < 0.001$), READ ($p = 0.05$) and SARC ($p < 0.001$) (Figure2A). Furthermore, YTHDF2 was a high-risk factor in LIHC, LGG, ACC, SARC, and KICH, while a low-risk gene in other cancer types, particularly in READ. Kaplan-Meier survival analysis also demonstrated a significant negative correlation between YTHDF2 expression and OS in patients with LIHC ($p = 0.005$), KICH ($p = 0.043$), ACC ($p = 0.013$), LGG ($p < 0.001$), and SARC ($p = 0.029$), whereas in patients with KIRC ($P = 0.02$) and READ ($p = 0.05$) (Figure 2B), high YTHDF2 expression was associated with a longer survival time.

Moreover, analysis of PFI data (Figure 3A) revealed an association between high YTHDF2 expression and poor prognosis in patients with LIHC ($p = 0.016$), KIRC ($p = 0.008$), KICH ($p = 0.034$), ACC ($p = 0.002$), LGG ($p < 0.001$) and KIRP ($p < 0.027$). However, in patients with CHOL ($p = 0.017$) and KIRC ($p = 0.008$),

YTHDF2 expression did not follow such a relationship with prognosis. Kaplan-Meier survival analysis revealed a negative correlation between YTHDF2 expression and prognosis in patients with ACC (p = 0.001), KICH (p = 0.04), KIRP (p =0.03), LIHC (p = 0.016) and LGG (p < 0.001) (Figure 3B).

Regarding the association between YTHDF2 expression and DSS, forest plots showed a negative correlation between YTHDF2 expression and PFI in ACC (p =0.031), LGG (p < 0.001) and SARC (p =0.001), while a positive correlation in patients with KIRC (p = 0.007) (Figure 3C). KM analysis showed that individuals with in ACC (p = 0.014), LGG (p < 0.001) and SARC (p = 0.002) had a high YTHDF2 expression level, but a poor PFI. On the contrary, patients with high YTHDF2 expression had a longer survival time in KIRC (p = 0.006) (Figure 3D).

YTHDF2 Expression Is Related to Immune Checkpoint Genes in Human Cancers

ICP genes have been demonstrated to have significant influences on immune cells infiltration and the outcomes of immunotherapy [26]. Hence, we explored the association between YTHDF2 expression and ICP genes in human cancers to explore the potential role of YTHDF2 in immunotherapy. Forty-seven ICP genes were verified closely related to expression in most cancer types (Figure 4A). The expression of YTHDF2 was positively correlated with immune checkpoint genes in COAD, KICH, KIRC, KIRP, LGG, LIHC, PAAD, PRAD, PCPG and UVM, especially in KICH, LGG, LIHC and UVM. These results suggested that the high expression of YTHDF2 might predict the efficiency of therapeutic effects of immunotherapies targeting ICP genes. In BLCA, BRCA, GBM, LUAD, LUSC, and THYM, YTHDF2 is inversely correlated with the ICP genes, suggesting that a poor immunotherapy outcome would get when YTHDF2 is highly expressed in patients.

Correlations Between YTHDF2 Expression and MMR, TMB, and MSI in Cancers

Microsatellites (MS) are simple repetitive sequences of nucleotide bases that are liable to make errors during DNA replication, which could be recognize and repair by MMR genes. Tumors with defective MMR systems are susceptible to microsatellite mutations, which lead to high levels of MSI, and in turn cause the accumulated mutations in cancer-related genes and the aggravated TMB. Therefore, we investigated the relationship between YTHDF2 expression and several MMR genes, including MLH1, MSH2, MSH6, PMS2 and EPCAM. YTHDF2 was positively correlated with MMR gene expression in all the cancer types, excluding CHOL and UCS (Figure 5A). As TMB is one of the biomarkers that can predict the therapeutic effects of immune checkpoint blockers (ICBs), we examined the association between YTHDF2 expression and TMB in cancers. The results showed that the expression of YTHDF2 and TMB were positively correlated in GBMLGG, COAD, COADREAD, STAD and LIHC, but negatively correlated in THCA (Figure 5B). We also studied the association between the expression of YTHDF2 and MSI, another immune checkpoint inhibitor (ICI) reaction, and found that they are positively correlated in GBM, CESC and STAD, while negatively correlated in BRCA, PRAD, HNSC, THCA and DLBC (Figure 5C). As the MMR, TMB, and MSI have all proven to be correlated with the sensitivity to ICP-related immunotherapy,the above results further confirmed our hypothesis that YTHDF2 might influence the anti-tumor immunity.

The influence of YTHDF2 on TME

TME plays a vital role in modulating malignant progression and influencing the response of immunotherapy. To assess the association between the expression of YTHDF2 expression and the composition of TME, we calculated the estimate core, stromal core, Immune score, and tumor purity in 33 cancers. As shown in this study, YTHDF2 expression was inversely correlated with the estimate score, stromal core and immune score in most cancers except LGG and PPAD, and a positively correlated with tumor purity in most cancers. (Figure 6A-D). These results demonstrated that the expression of YTHDF2 was closely related to the composition of TME in cancer.

Correlation Between YTHDF2 Expression and Immune Infiltrating Levels in Cancers

It has been proven that immune cells in the TME could affect the survival of cancer patients. Accompanied with the prognostic role of YTHDF2 identified in our pan-cancer study, it would be meaningful to explore the association between the expression of YTHDF2 and immune infiltration. We detected the correlation between YTHDF2 expression and immune infiltration levels in 39 different cancers by calculating their correlation coefficients in TIMER. The results indicated that YTHDF2 expression was significantly correlated with tumor purity in 12 cancer types. Furthermore, YTHDF2 expression was also significantly correlated with the infiltration levels of B cells, $CD4^+$ T cells, $CD8^+$ T cells, dendritic cells, macrophages, and neutrophils in 19, 18, 20, 22, 13, and 23 cancer types, respectively (Figure 7A). To better understand the relationship between YTHDF2 expression and differential infiltration of immune cells, we analyzed the correlations between the YTHDF2 and different immune cell markers in KICH, KIRP, LGG, LIHC, PPAD, THYM, UVM, KIRC, READ, ACC and SARC, using the TIMER database. We found that after tumor purity adjustment, YTHDF2 expression was positively correlated with most immunocell-marker genes in most tumors (Figure 7B). We also selected hepatocellular carcinoma (HCC) tissues with obvious immune cells infiltration, from which we confirmed a positive correlation between the protein expression of YTHDF2 and the infiltration of $CD3^+CD8^+$ T cells and $CD68^+$ macrophages using immunohistochemistry (Figure 7C-D).

$CD8^+$ T cells are the most significant and effective anti-tumor T cells. After infiltrating in tumor tissues, $CD8^+$ T cells gradually turn into a dysfunctional exhaustion state under a long-term continuous stimulation of tumor-associated antigens. We call this process T-cell exhaustion, which is an important mechanism for the weakening of anti-tumor effect [27]. Interestingly, we also found the expression of YTHDF2 was significantly correlated with T cell exhaustion markers, including PDCD1, CTLA4, TIGIT, LAG3 and HAVCR2 (Figure 7E). HAVCR2 is a vital gene that can mediate T cell exhaustion. In this study, we detected a positive correlation between YTHDF2 and HAVCR2, which indicated that YTHDF2 might play an important role in HAVCR2 mediated T cell exhaustion. Moreover, we also confirmed the positive correlation of YTHDF2 and other immunosuppressive molecules, suggesting the relevance between YTHDF2 and T cell exhaustion, as well as a vital role of YTHDF2 in the TME mediated immune escape.

GO/KEGG/GESA

We also conducted the biological significance of YTHDF2 expression in different cancers. The GO functional annotation and KEGG pathway analysis are shown in Figure 8A. Our data indicate that overexpression of YTHDF2 can positively regulate cell adhesion, cell cycle, and several immune-related functions. Cell cycle related pathways are also enriched in LIHC, KICH and ACC, while PD1, CTLA4, IL-10 and B cell related pathways are enriched in LGG, SRAC, READ and KIRC (Figure 8B).

4. Discussion

In the present study, we performed a pan-cancer analysis to identify the expression of YTHDF2 and its predictive value on prognosis in various cancer types. We demonstrated that YTHDF2 is overexpressed in most of the cancer types. These results suggest that YTHDF2 can promote the occurrence and development of tumors. Moreover, the aberrant expression of YTHDF2 is also proposed to be a prognostic factor in some types of cancers, including ACC, LGG, KICH, KIRP, LIHC, KIRC and READ, based on both Cox and KM survival analyses.

The development of ICIs is a revolutionary milestone in the field of cancer therapy. Tumor cells can evade immunosurveillance to achieve malignant progression through different mechanisms, one of which is to active the immune checkpoints to suppress antitumor immune responses [28]. Notably, we found that YTHDF2 expression was positively correlated with ICP genes in most tumors, especially in KICH, LGG, LIHC and UVM. These results suggest that YTHDF2 may promote immune escape.

MMR is an important factor related to genome stability and integrity [29, 30]. In addition, TMB and MSI are important new sensitive predictors of immunotherapy [31–33]. Our study also found that YTHDF2 expression was positively correlated with MMR genes in all cancers (excluding CHOL and UCS). In addition, YTHDF2 is positively correlated with TMB and MSI in some types of cancer. It is suggested that YTHDF2 may play an important role in tumor immunity and serve as a marker of immunotherapy.

TME plays an important role in tumor genesis, development, metastasis and clinical treatment, as well as affects tumor development, immune escape and angiogenesis [34–36]. It has been reported that the aberrant infiltration of immune cells in normal tissues might enhance the tumor development and progression [37, 38]. Some oncogenic proteins can also regulate the infiltration of immune cells in the TME. Our results showed that the expression of YTHDF2 was negatively correlated with estimate score, stromal score, and immune score in human generalized cancer, but positively correlated with tumor purity, suggesting the important role of YTHDF2 in TME composition. However, the role of YTHDF2 in immune infiltration needs to be further validated. Thus, we further investigated the role of YTHDF2 on immune infiltration levels in cancer. The results indicated that YTHDF2 is associated with immune cells infiltration in BLCA, BRCA, COAD, KICH, LGG, LICH, PPAD, PCPG, KIRP, PRAD, SKCM, and THCA. Moreover, a co-expression of YTHDF2 and immune cell-related genes in those cancer types further confirmed the positive correlation between YTHDF2 and tumor immune infiltration, which was confirmed by immunohistochemistry in HCC. $CD8^+$ cytotoxic T lymphocytes (CTLs) can kill their target cells directly by releasing perforin, granzyme A and granzyme B, in concert with activating the Fas/FasL mediated

apoptosis via the secretion of IFN-γ and TNFα [39]. Exhaustion of $CD8^+$T lymphocytes in TME has frequently been reported, which is closely relevant to the activation of immune checkpoints [40]. Most malignancies lead to tumor progression by enhancing the expression of inhibitory ligands to disrupt T cell function and escape the immunological surveillance. This is a key mechanism underlying tumor progression and immune escape.

The GESA analysis in several tumors indicated that the YTHDF2-related genes are enriched in cell cycle, DNA repair, cell adhesion and immune-related signaling pathways. Since the conclusion was based on the bioinformatics analysis of TCGA or GEO data sets, further biological experiments are required to verify our hypothesis.

In summary, we provide a comprehensive bioinformatics analysis on the expression of YTHDF2 and its prognostic value in various human cancers. Our results suggest that YTHDF2 plays a key role on the TME mediated immune cell infiltration, tumor prognosis, and therapeutic response. In general, YTHDF2 can serve as a potential biomarker for tumor detection, therapeutic response and prognostic analysis.

Abbreviations

m6A N6-methyladenosine

YTHDF2 YT521-B homology domain family 2

TIMER Tumor Immune Estimation Resource

GEPIA Gene Expression Profiling Interactive Analysis

TCGA The Cancer Genome Atlas

GTEx The Genotype-Tissue Expression

GEO Gene Expression Omnibus

OS Overall survival

PFI Progress Free Interval

DSS Disease-specific survival

GSEA Gene Set Enrichment Analysis

TMB Tumor mutation burden

MSI Myeloid-derived suppressor cells suppressor cells

MMR Mismatch Repair

ICP Immune Checkpoint

IHC Immunohistochemistry

LGG Lower Grade Glioma

LAML Acute Myeloid Leukemia

OV Mesothelioma, Ovarian Serous Cystadenocarcinoma

HNSC Head and neck squamous cell carcinoma

KIRC Kidney renal clear cell carcinoma

BLCA Bladder urothelial carcinoma

BRCA Breast invasive carcinoma

KICH Kidney chromophobe

KIRP Kidney renal papillary cell carcinoma

LIHC Liver hepatocellular carcinoma

ESCA Esophageal carcinoma

LUAD Lung adenocarcinoma

LUSC Lung squamous cell carcinoma

PRAD Prostate adenocarcinoma

READ Rectum adenocarcinoma

UCEC Uterine corpus endometrial carcinoma

CHOL Cholangial carcinoma

COAD Colon adenocarcinoma

CECS Cervical squamous cell carcinoma and endocervical adenocarcinoma

STAD Stomach adenocarcinoma

DLBC Lymphoid Neoplasm Diffuse Large B-cell Lymphoma

THCA Thyroid carcinoma

GBM Glioblastoma multiforme

PAAD Pancreatic adenocarcinoma

THYM Thymoma

ACC Adrenocortical carcinoma

BLCA Bladder urothelial carcinoma

PCPG Pheochromocytoma and Paraganglioma

UCEC Uterine corpus endometrial carcinoma

UCS Uterine Carcinosarcoma

OSCC Oral squamous cell carcinoma

UVM Uveal Melanoma

MESO Mesothelioma

TME Tumor Microenvironment

Declarations

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Authors' contributions

Conceptualization, W.L. and C.L.; methodology, L.Z.; software, W.L.; validation, Z.C., W.L. and C.Q.; formal analysis, R.Z.; investigation, W.L.; resources, L.Z.; data curation, R.Z.; writing—original draft preparation, W.L.; writing—review and editing, R.Z.; visualization, W.L.; supervision, L.Z.; project administration, L.Z.; All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

The original data used to support the findings of this study are available from TCGA Research Network (https://www.cancer.gov/tcga).

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

All authors declare that they have no competing interests.

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Figures

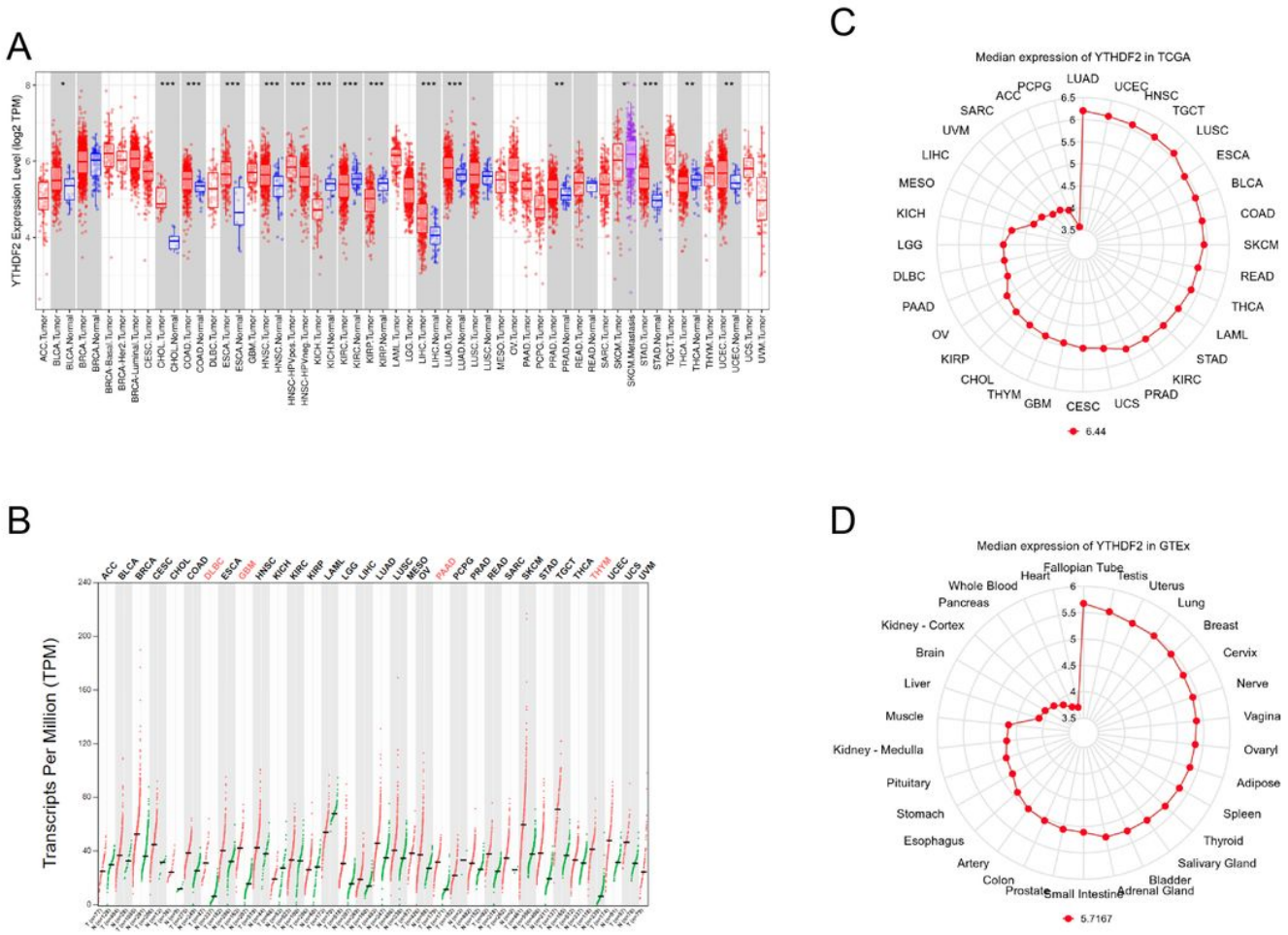

Figure 1

Differential expression of YTHDF2 in various cancer types. (A-B) YTHDF2 expression analysis using TIMER and GEPIA. (C) YTHDF2 expression in tumor tissues from TCGA cohort. (D) YTHDF2 expression in normal tissues from GTEx cohort.*P < 0.05, **P < 0.01, ***P < 0.001.

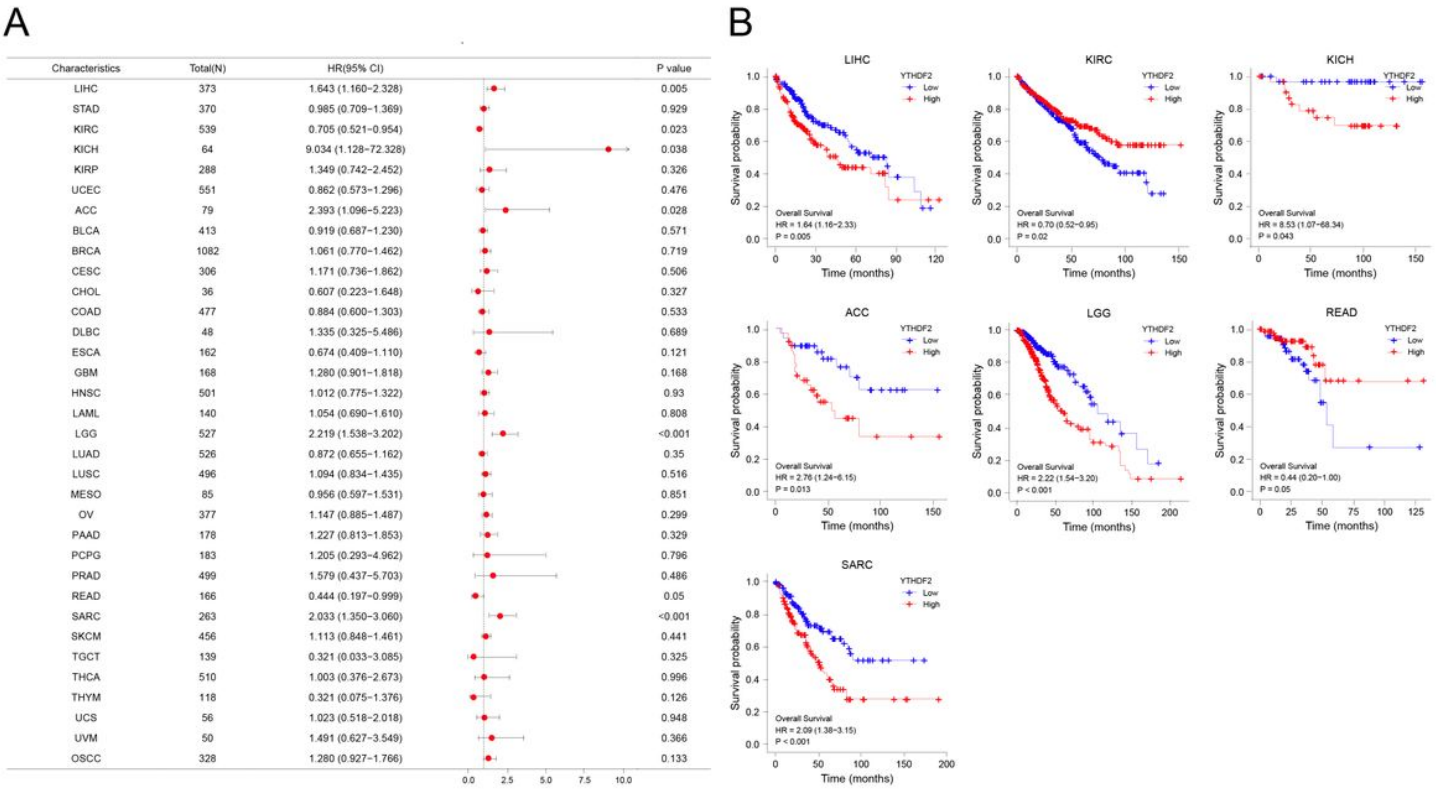

Figure 2

YTHDF2 expression is associated with overall survival time (OS). (A) Forest plot of OS associations in 34 cancer types. (B) Kaplan-Meier analysis of the association between YTHDF2 expression and OS.

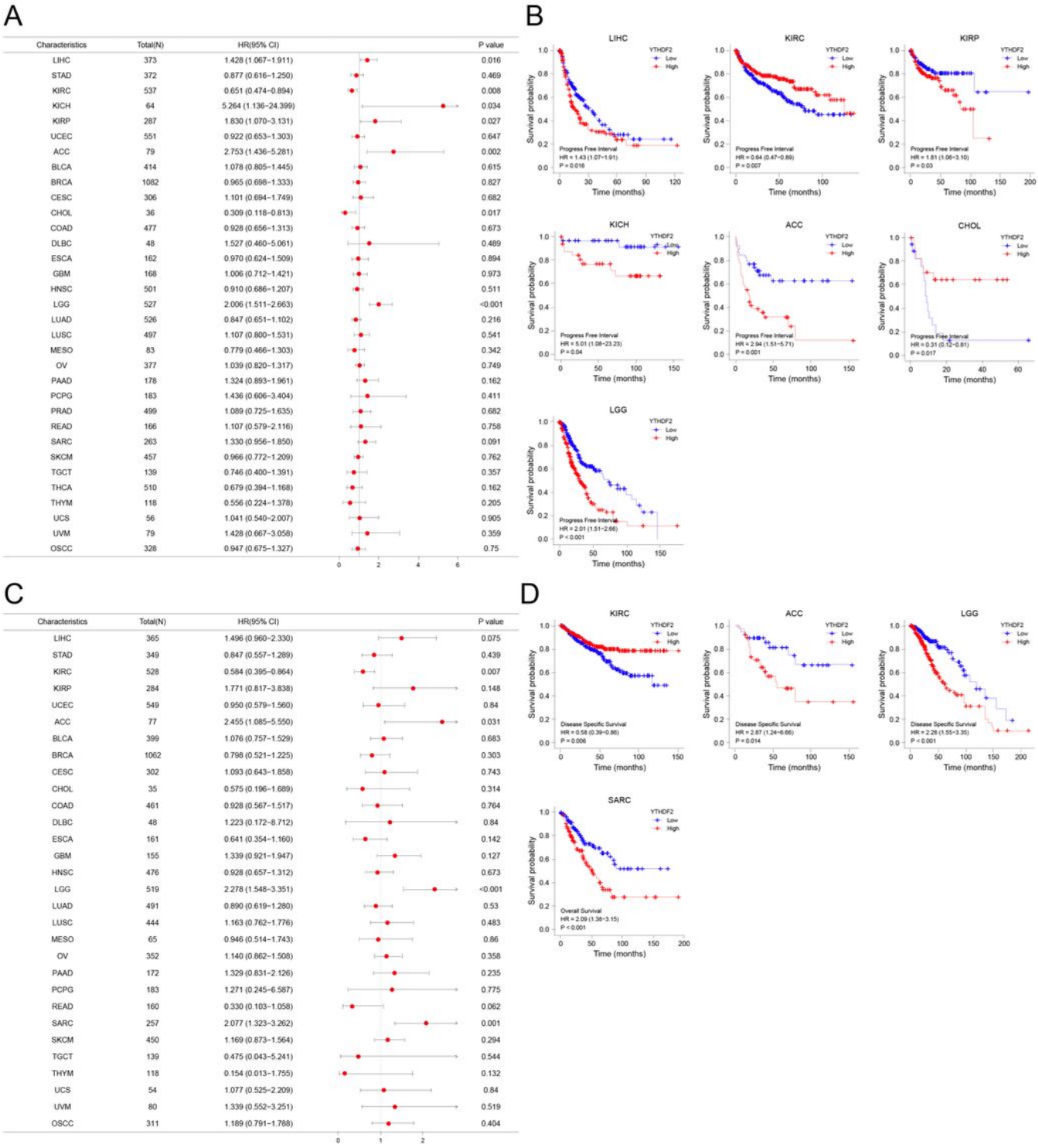

Figure 3

YTHDF2 expression is associated with progression-free interval (PFI) and disease-specific survival (DSS). (A) Forest plot indicates the association between PFI and YTHDF2 expression in 30 cancer types. (B) Kaplan-Meier analysis indicates the association between YTHDF2 expression and PFI. (C) Forest plot indicates the association of YTHDF2 expression and DSS in 33 cancer types. (D) Kaplan-Meier analysis indicates the association between YTHDF2 expression and DSS.

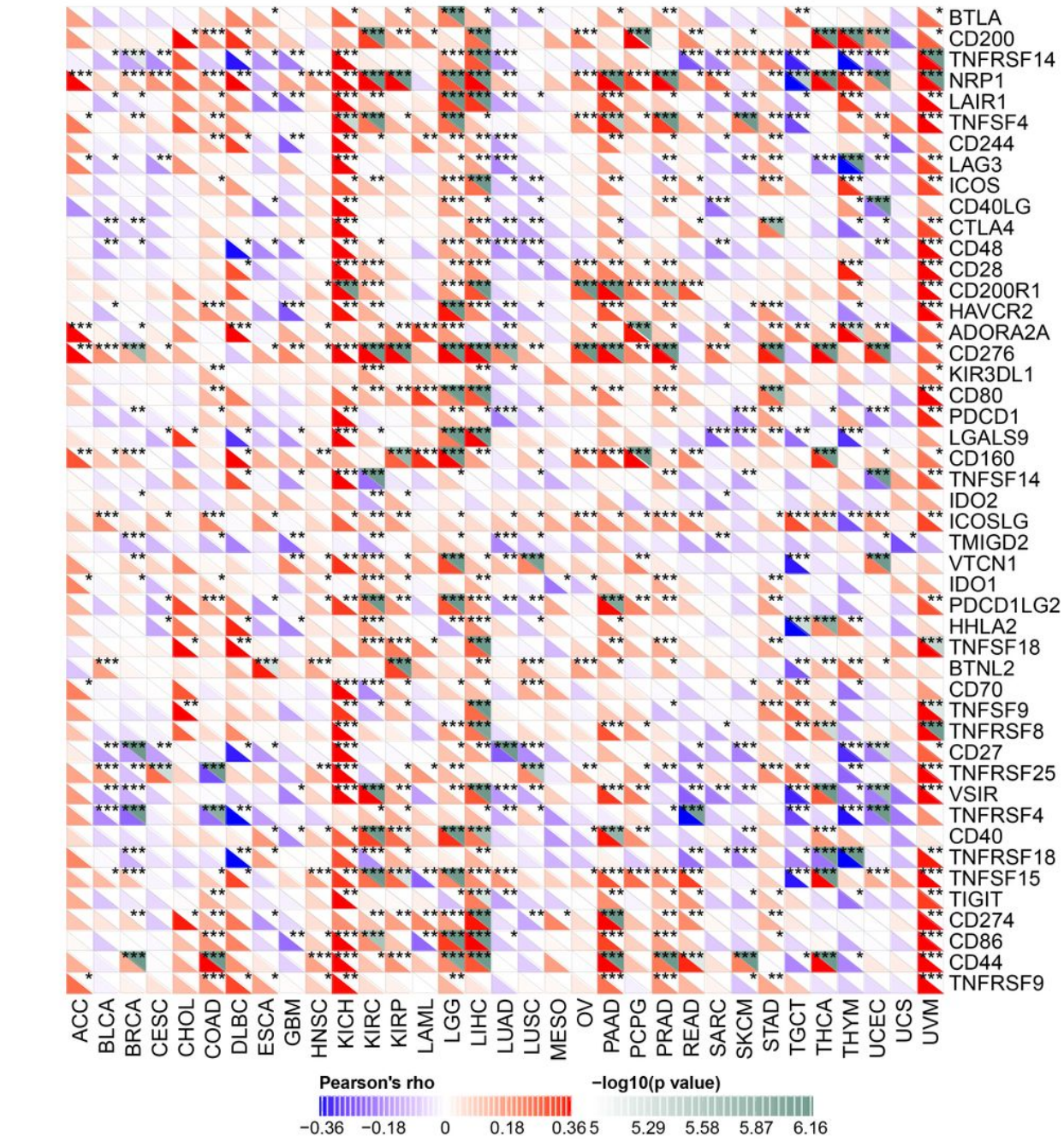

Figure 4

The correlation between YTHDF2 expression and pan-cancer immune checkpoint genes. *P < 0.05; **P < 0.01; ***P < 0.001.

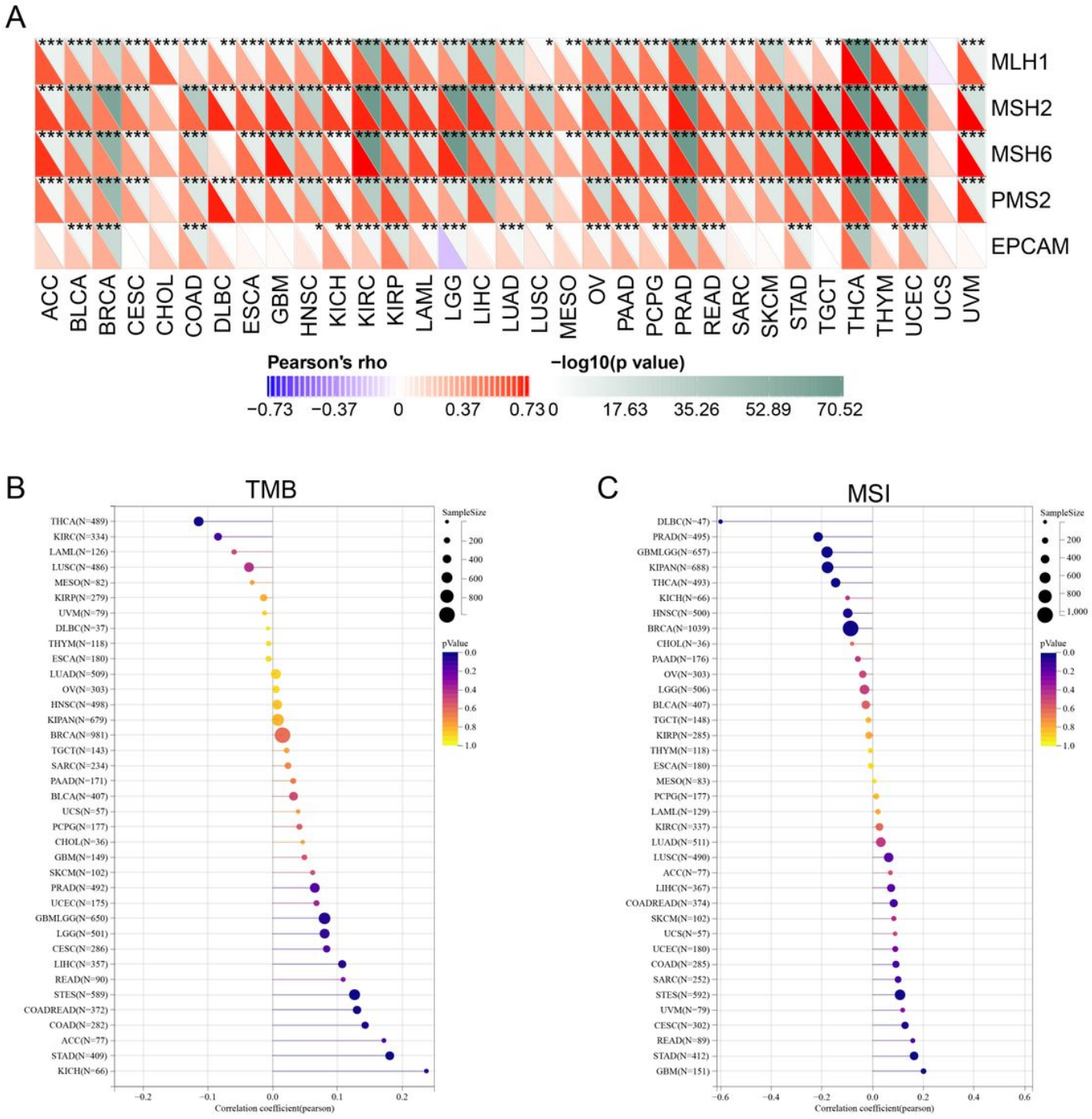

Figure 5

The correlation between YTHDF2 expression and the MMR (A), TMB (B), and MSI(C) . *P < 0.05; **P < 0.01; ***P < 0.001.

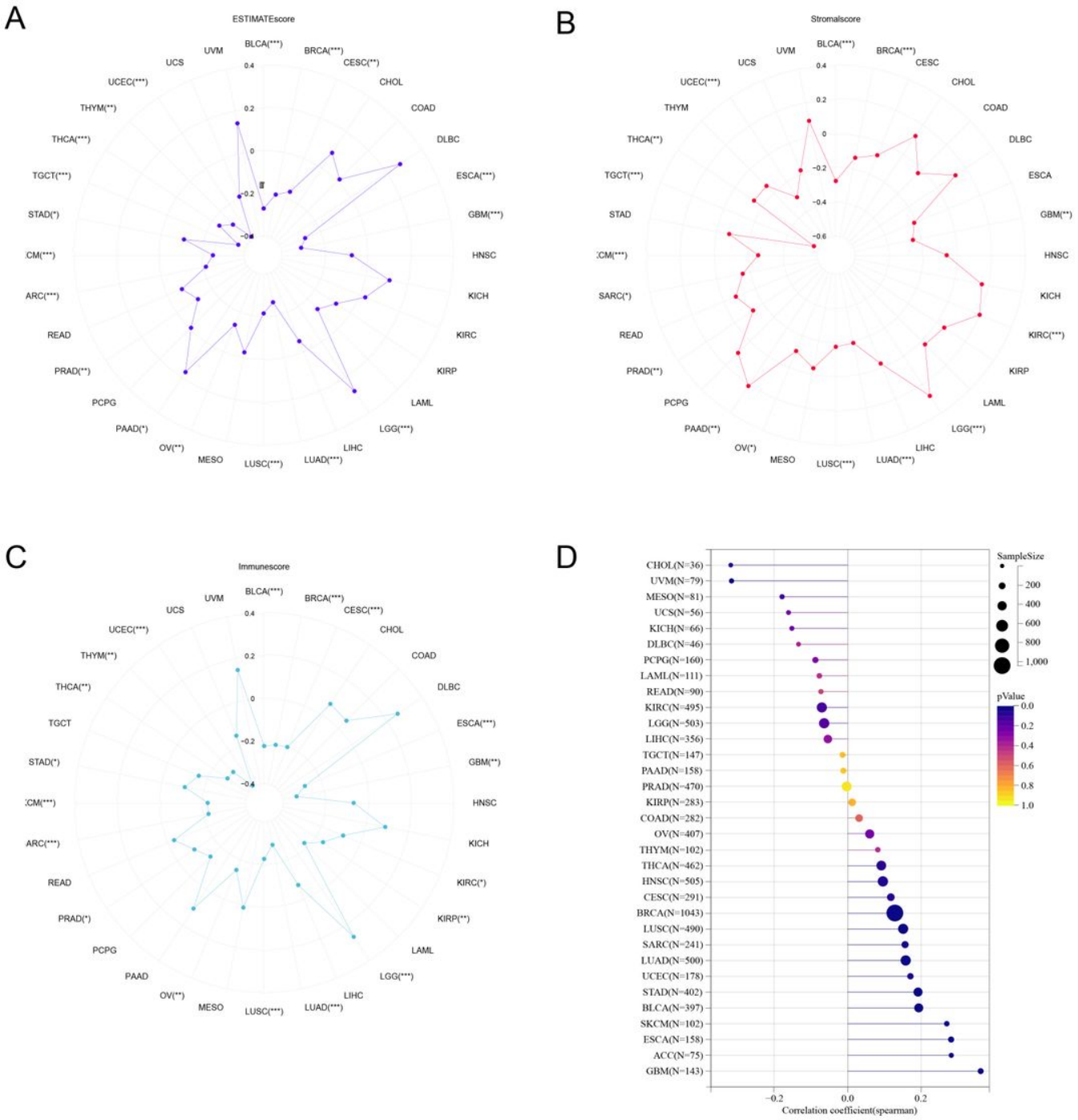

Figure 6

Correlation of YTHDF2 expression with EstimateScores（A),StromalScore (B), ImmuneScore(C) and Tumor purity in various cancers. *P < 0.05; **P < 0.01; ***P < 0.001.

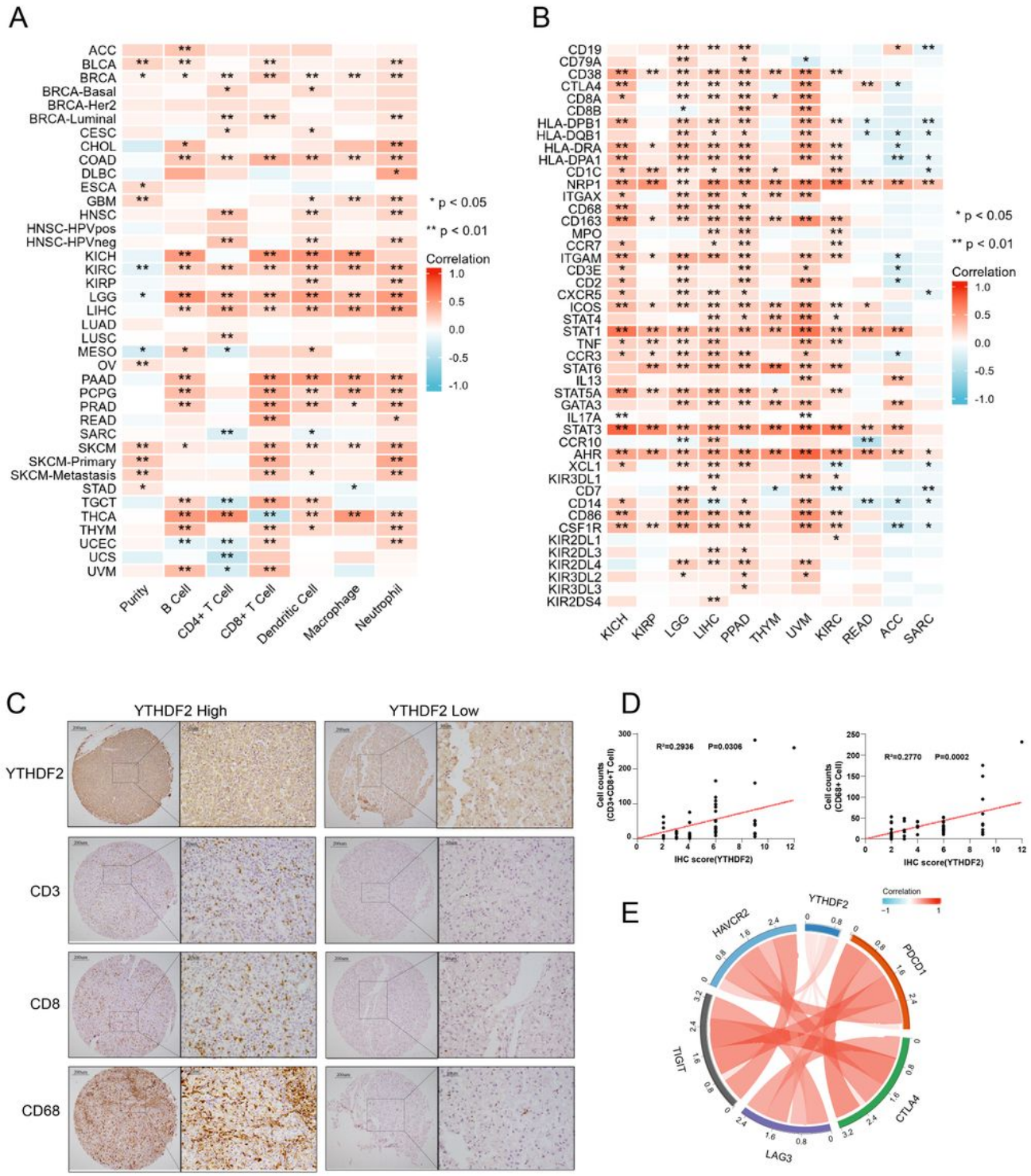

Figure 7

Correlation Between YTHDF2 Expression and Immune Infiltrating Level in Cancers. (A) Relationship between YTHDF2 expression and tumor infiltration of six immune cells. (B) Co-expression of YTHDF2 and immune cells related genes in various cancers. (C) Immunohistochemical images of hepatocellular carcinoma show intra - tissue characteristics of CD3+CD8+ T cells /CD68 macrophages with high and low expression of YTHDF2. (D) Correlation analysis of YTHDF2 protein level with CD3+CD8+ T cells and

CD68 macrophage infiltration in hepatocellular carcinoma validation cohort. (E) Correlation circle diagram of YTHDF2 with PDCD1, CTLA4, LAG3, TIGIT and HAVCR2. *P < 0.05; **P < 0.01; ***P < 0.001.

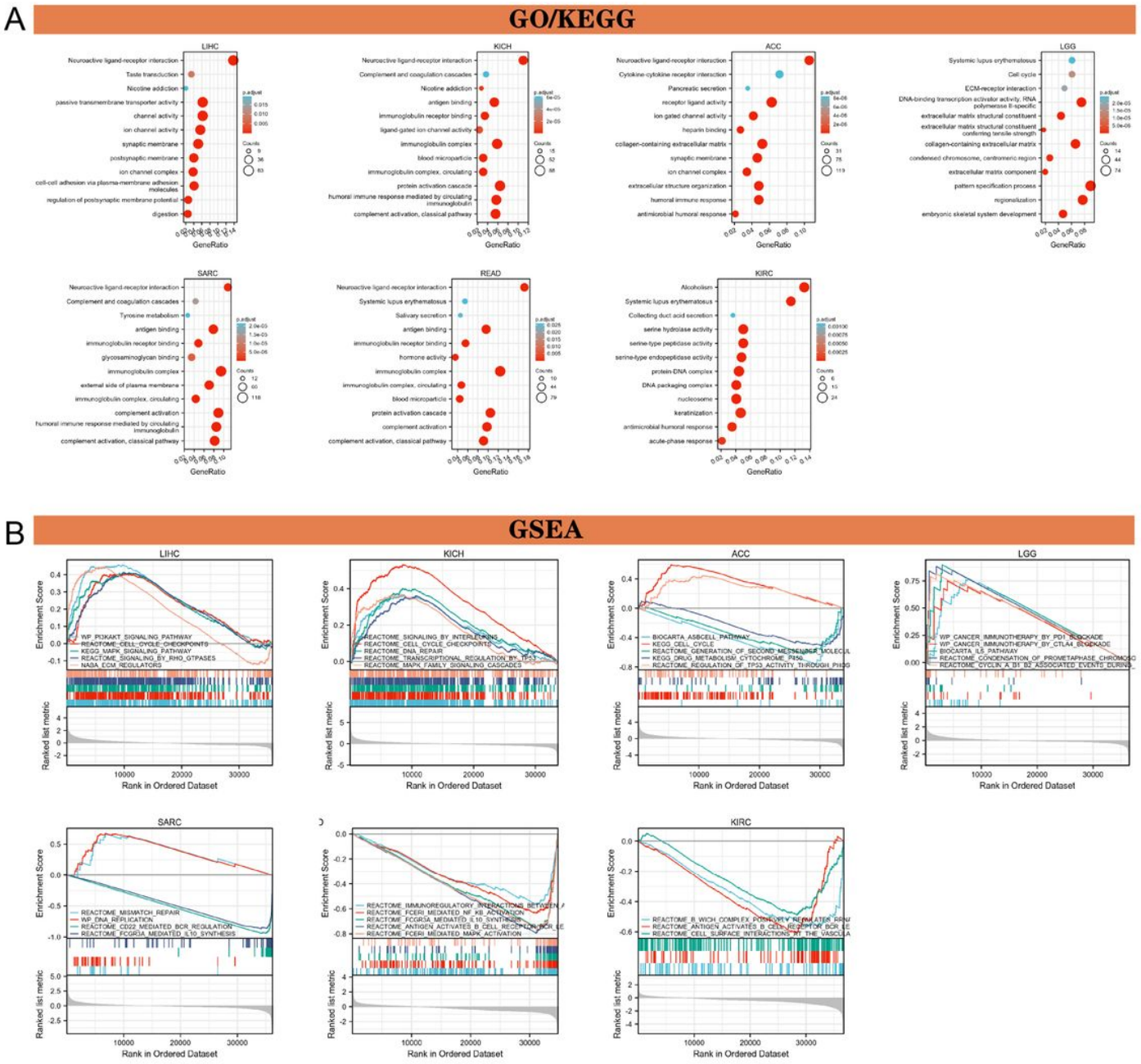

Figure 8

The pathway enrichment of YTHDF2 in various cancer types Enrichment results (A) GO/KEGG functional annotation of YTHDF2 in various cancers. (B) GSEA pathway analysis of YTHDF2 in multiple cancers.