Research Square

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YTHDC1 serves as a potential therapeutic target and is linked with the immune microenvironment in Pan cancer

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Research Article

Keywords: YTHDC1, Pan-cancer, Immune microenvironment, Bioinformatics

Posted Date: April 21st, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1546856/v1

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Abstract

Previous studies have shown that m6A is involved in many aspects surrounding tumor progression, but the role of classic m6A reading protein YTHDC1 in the progression and immune microenvironment among multiple cancers remains unclear. The aim of the present study was to investigate the role of YTHDC1 in the Pan cancer tumor progression and immune microenvironment. The YTHDC1 expression pattern and prognostic value were first detected in Pan cancer in the public data. The role of YTHDC1 in Pan cancer was analyzed by bioinformatic analysis. The association between YTHDC1 expression and immune cell infiltration and immune inhibitory markers was analyzed. The expression of YTHDC1 in hepatocellular carcinoma was verified by independent datasets and clinical samples, and its function was explored in vitro. YTHDC1 is positive expression in all of 33 types tumors, and its expression and phosphorylation modification have abnormal changes across varieties tumors based on the analysis of TCGA and CPTAC databases. Prognostic analysis also showed that YTHDC1 was closely related to the prognosis of patients in some tumors such as BLCA and KIRC ($p < 0.05$). Finally, independent data sets, clinical samples and in vitro experiments verified the high expression of YTHDC1 in liver cancer and its ability to promote tumor and immune microenvironment. In conclusion, YTHDC1 has potential functions in tumors, can be used as a prognostic marker for a variety of tumors, and is related to the formation of the immune microenvironment. Out of pan-cancer, YTHDC1 promotes liver cancer progression.

Introducation

With the development of scientific research and clinical practice, many advanced achievements have been made in the field of cancer [1]. However, the incidence of cancer is still increasing year by year. Cancer is the second leading cause of death in the United States which accounts for more than 60000 people death [2]. Therefore, it is necessary to further explore the molecular mechanism of tumorigenesis and development, find intervention targets and related prognostic markers, so as to systematically clarify the tumorigenesis process and help design the system personalized medical scheme.

RNA modification is a research hotspot in recent years. M6A methylation is the most common modification of RNA in eukaryotes [3]. Intracellular RNA methylation is directly regulated by methylase (METTL3, METTL14 and WTAP), demethylase (FTO and ALKBH5) and methylated reading protein (YTHDF1-3, YTHDC1-2 and IGF2BPs) [4]. At present, it has been reported to be closely related to tumor progression in recently studies. Zhang S et.al. found that ALKBH5 can promote the stemness of glioma [5]. ALKBH5 was also reported to inhibit the progression of esophageal cancer by Xiao D et.al. [6]. These studies suggested that the function of m6A methylation may not be completely consistent in different tumors. Pan cancer analysis of m6A methylation related genes can help us understand their role in a variety of tumors. Furthermore, immunosuppressive microenvironment has always been considered as one of the key factors for tumor growth in vivo. With the achievements of tumor immunotherapy, the study of the mechanism of tumor immune microenvironment has significant scientific and clinical significance. In previous studies, m6A has been reported to be involved in the formation of a variety of

tumor immune microenvironments, such as PD-L1 regulation, macrophage recruitment, T cell depletion and so on [7].

In this study, we systematically analyzed the function of YTHDC1 in Pan cancer using bioinformatics. YTHDCs family is the only m6A binding reading protein found to function in the nucleus. Previous studies reported that YTHDC1 was mainly involved in mRNA nuclear transport with m6A label [8]. Besides, YTHDC1 is also involved in the regulation of mRNA stability, suggesting its complex function. YTHDC1 has been reported its important role in some tumors, but its mechanism is not completely consistent [9]. At present, there is a lack of systematic analysis of a variety of tumors for YTHDC1. Therefore, Pan cancer analysis based on YTHDC1 in this study is necessary.

Materials And Methods

Data acquisition and processing

MRNA, tumor mutation load and clinical data of 33 types tumor were downloaded from the TCGA database including ACC, BLCA, BRCA, CESC, CHOL, COAD, DLBC, ESCA, GBM, HNSC, KICH, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, MESO, OV, PAAD, PCPG, PRAD, READ, SARC, SKCM, STAD, TGCT, THCA, THYM, UCEC, UCS and UVM [10]. The validation liver cancer dataset is downloaded from CPTAC database and ICGC database (LIHC-JP dataset). The data form is FPKM. Gene difference analysis was performed using Limma package.

Prognostic correlation analysis

Prognostic correlation analysis is to use tumor patients to be divided into two groups based on the intermediate value of YTHDC1 expression and match the corresponding prognostic information. Survival analysis and univariate COX regression analysis were conducted using R package "survival".

Tumor mutation load (TMI) and microsatellite Instability (MSI) correlation analysis

Pan cancer TMB and microsatellite instability data were downloaded from TCGA database. Correlation analysis was performed by combining the gene mutation data and expression data, using R package “Fmsb”, based on Spearman statistical method.

Tumor microenvironment and immune cell infiltration correlation analysis

The immune microenvironment and matrix scores were scored by using the estimate R package with reference to the previous literature. Immune cell infiltration is based on TCGA database gene expression data. The classical CIBERSORT method was used for evaluation [11], and the p value filtering condition was 0.05. Spearman analysis was used for correlation analysis

Gene set enrichment analysis (GSEA)

The KEGG7.4 pathways was used for GSEA analysis. Path analysis using “enrichplot” R package. Filter condition p value is less than 0.05. R package ggplot2 is used for drawing.

RT-qPCR, western blot and Immunohistochemistry

Clinical tissue samples were collected from the Second Affiliated Hospital of Chongqing Medical University. The protocols were reviewed and supported by the Ethics Committee of The Second Affiliated Hospital of Chongqing Medical University Approval Number: (2020) Institutional Review Board (IRB) (STUDY) No. 88. RNA extraction is the use of RNAiso from fragmented clinical tissues. RNA reverse transcription to cDNA was performed using the MCE reverse transcription kit. RT-PCR was performed and the data were analyzed by 2 ^ - △ CT method. Proteins were extracted from clinical samples using RIPA lysate with protease inhibitor. Separation of target protein by electrophoresis. Electroporation transfers protein to PVDF membrane. The primary antibody was incubated overnight at 4 ℃, the secondary antibody was incubated at room temperature for 1 hour, ECL solution was exposed, and the statistical results of imge J software were obtained. Paraffin sections are made of clinical samples. After gradient dehydration, sodium citrate solution is used for thermal antigen repair. Goat serum blocking nonspecific antigen with 1 hour. The primary antibody was incubated for 24 hours at 4 ℃. After cleaning with PBS solution for three times, incubate the secondary antibody. After DAB color development, observe the results under an upright microscope.

SiRNA intervention

After the cells grew to 30–40%, the mixture of siRNA and transfection reagent was added, and the transfection efficiency was detected three days later.

CCK8 and Transwell co-culture

Huh-7 cells were purchased from Procell Life Science&Technology Co.,Ltd [12]. Put an appropriate amount of liver cancer cells on 96 well plate. According to CCK8 liquid and medium 1; 25 configuration, detect the absorbance at OD450 nm, quantify the cells according to the standard curve, and detect the cell growth status for 72 hours. THP-1 cells were used as human macrophage model [13]. After PMA activation and adhesion, they were placed in the upper layer of 8um transwll chamber to detect the chemotaxis of lower hepatoma cells to THP-1. More detail can be found in supply material.

Statistical analyses

SPSS 24.0 and GraphPad Prism 8.0 (GraphPad, La Jolla, CA) were used for statistical analyses. The measured data are represented as means ± SEM. One-way analysis of variance (ANOVA) or two-tailed Student’s t test was conducted to compare quantitative data, whereas nonparametric $\chi 2$ test was used to analyze qualitative data for which $P < 0.05$ seemed significant (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

Results

YTHDC1 is widely expressed in a variety of tumors

Based on the TCGA database, we recognize that the expression of YTHDC1 exists in all 33 tumors (FPKM > 2), of which the highest expression was in LAML and THYM (Fig. 1A). Gene differential analysis showed that YTHDC1 was differentially expressed in 9 tumors, of which 5 tumors (CHOL, COAD, ESCA, LIHC and STAD) were significantly up-regulated and 4 tumors (BRCA, KICH, UCEC, THCA) were significantly down-regulated, suggesting its potential function in tumorigenesis (Fig. 1B). Furthermore, we analyzed the correlation between YTHDC1 expression and tumor stage. It was found that YTHDC1 expression was up-regulated in advanced liver cancer and down-regulated in five tumors (BLCA, KIRC, LUSC, SKCM and THCA) (Fig. 1C). In recent years, protein post-translational modifications (PTMs) has been found to play an important role in tumorigenesis and development, and phosphorylation modification is the most extensive PTMs in vivo. Based on CPTAC database, we found that the phosphorylation of YTHDC1 was abnormally modified in a variety of tumors (Fig. 1D) [14]. In conclusion, the above results suggest that YTHDC1 may be involved in the development of a variety of tumors.

YTHDC1 is associated with multiple tumor prognosis

Univariate Cox regression analysis showed that high expression of YTHDC1 was associated with better prognosis in patients with four tumors (BLCA, KIRC, LGG and SKCM) (Fig. 2A). KM survival analysis also suggested that the high expression of YTHDC1 in BLCA KIRC CESC and LGG was associated with better overall survival (Fig. 2B). These results also suggest that YTHDC1 may be a potential prognostic marker for some tumors.

Prediction of YTHDC1 related functions by gene enrichment analysis

We performed gene enrichment analysis (GSEA) to predict the potential function of YTHDC1 in all the above tumors (Fig. 3A). Interestingly, YTHDC1 is associated with NK cell-mediated cytokines in BRCA. In LUSC and MESO, it is closely related to toll like receptor signal. In PRAD, YTHDC1 is associated with cytokine transmission. Among the tumors identified above which YTHDC1 may play an important role, YTHDC1 is related to calcium signal transduction in LIHC and fatty acid metabolism signal in BLCA which are closely related to tumor immune regulation [15, 16]. Therefore, we speculate that YTHDC1 may be involved in the shaping of tumor immune microenvironment.

Correlation analysis between YTHDC1 and immune marker

Using TISIDB database, we found that YTHDC1 was closely related to a variety of immune markers [17]. MHC complex is an important signal for the body to initiate immune response. In the process of tumorigenesis and development, the abnormal expression of HLA-A and HLA-B signals promotes immunosuppression [18]. We found that YTHDC1 was negatively correlated with MHC complex expression in a variety of tumors including BLCA, BRCA, LIHC and etc. (Fig. 4A). Interestingly, the expression of YTHDC1 is closely related to the expression of a variety of inflammatory activators and immunosuppressive molecules (Fig. 4B and 4C). This seemingly contradictory result may be attributed to

the synergistic formation of pro-inflammatory and anti-inflammatory microenvironment in the tumor and YTHDC1 is involved in promoting the formation of tumor inflammatory microenvironment, which further supports the role of YTHDC1 in tumor immune microenvironment [19].

Correlation analysis between YTHDC1 and tumor microenvironment

Tumor growth in the body is inseparable from the support of tumor matrix and immune microenvironment [19]. Therefore, we performed correlation analysis between YTHDC1 and tumor microenvironment. The results showed that YTHDC1 are closely associated with matrix scores of eight tumors (ACC, BLCA, GBM, LGG, LUSC, SARC, TGCT, UCS) (Fig. 5A) and immune scores of ACC, BLCA, GBM, KIRP, PCPG, SARC, THYM and UCS (Fig. 5B). These results also support the aforementioned results from another perspective.

YTHDC1 is closely related to tumor mutation burden, microsatellite instability and macrophages in a variety of tumors

Tumor mutation burden (TMB) and microsatellite instability (MSI) have been recognized to play an important role in tumor immune signal transduction and microenvironment formation [20, 21]. The analysis results showed that YTHDC1 are positively associated with TMI in LAML, READ and LIHC, and negatively associated with TMI in BRCA and THCA (Fig. 6A). YTHDC1 was positively correlated with MSI in eight tumors (LUAD, LUSC, LGG, ACC, STAD, READ, SARC and COAD) and negatively correlated in DLBC (Fig. 6B). Tumor associated macrophages (TAMs) are not only involved in the formation of tumor immunosuppressive microenvironment, but also directly involved in tumor growth and metastasis. Our results showed that YTHDC1 are closely associated with TAMs infiltration in multiple tumors such as BRCA and LIHC (Fig. 6C). These results further support the role of YTHDC1 in tumor immune microenvironment.

Independent data sets and clinical samples were used to verify the expression of YTHDC1 in hepatocellular carcinoma

Furthermore, we implemented bioinformatics analysis and experiments to partially verify the above results. Previous results have suggested that YTHDC1 may play an important role in a variety of tumors. YTHDC1 is not only abnormally expressed in liver cancer, but also related to the formation of immune microenvironment of liver cancer. In the early stage, we mainly engaged in liver cancer related research. Therefore, we focused on the role of YTHDC1 in liver cancer. First we analyzed the expression of YTHDC1 in hepatocellular carcinoma in two independent datasets (ICGC-JP and CPTAC-LIHC datasets) which YTHDC1 is high expression in LIHC (Fig. 7A and 7B). We also collected 20 pairs of clinical samples for RT-PCR which the results showed that the mRNA of YTHDC1 was highly expressed in hepatocellular carcinoma (Fig. 7C). Western blot and IHC experiments showed that the results showed that the protein of YTHDC1 was highly expressed in hepatocellular carcinoma (Fig. 7D and 7E).

YTHDC1 promotes liver cancer proliferation and macrophage recruitment

To verify the function of YTHDC1 in hepatocellular carcinoma, we used siRNA to intervene in Huh-7 cells (Fig. 8A and 8B). CCK8 experiment showed that the intervention of YTHDC1 damaged the growth ability of Huh-7 cells (Fig. 8C). Co-culturing Huh-7 and THP-1 cells, after inhibiting YTHDC1, the ability of Huh-7 cells to recruit macrophages decreased (Fig. 8D). These results support the malignant function of YTHDC1 in hepatocellular carcinoma to a certain extent.

Discussion

M6A is a research hotspot in current scientific research. M6A methylation has been confirmed to be the most common modification on mRNA in eukaryotes [22]. At present, M6A seems to play an important role in almost all diseases such as tumors, immunity, and inflammation. Unlike other YTH domain family proteins that have relatively clear functions, YTHDC1 has been proven to be one of the reading proteins of m6A, but due to the diversity of its functions, such as variable splicing, nuclear transport, etc., the current research is relatively few [23]. However, there is no doubt that with the current deepening of m6A research, the role of YTHDC1 in a variety of diseases will be gradually revealed. To our knowledge, this study is the first pan-cancer bioinformatics analysis study of YTHDC1.

The TCGA database is currently the most widely used and recognized database, so we used the data set to analyze the expression of YTHDC1 in 33 types of tumors. This result shows that YTHDC1 is positively expressed in all tumors, and there are significant differential expressions in 9 types of tumors. Compared with tumor occurrence, tumor patient death is often due to its development from early stage to late stage and metastasis [24]. Our analysis shows that YTHDC1 is differentially expressed in different stages of 6 types of tumors. It is worth noting that the high expression of YTHDC1 in liver cancer is relative to normal liver tissue, and the expression of advanced liver cancer is further up-regulated, highlighting its potential function. Compared with other studies that only focus on changes in gene expression, functional changes after protein modification have gradually attracted the attention of scientists in recent years. Our study found that phosphorylation modified YTHDC1 has abnormal changes in a variety of tumors, suggesting that the changes in YTHDC1 function are in tumors. Patient survival time is not only a key endpoint to measure the success of treatment, but also an important support for clinicians to formulate personalized medical plans. Survival analysis suggests that YTHDC1 is significantly related to the survival of type 5 tumors. Although these results do not all appear in the same tumor, considering the complexity of tumor research, this is entirely attributable to the difference in the function of YTHDC1 in different tumors. In conclusion, the above studies all suggest that YTHDC1 has potential malignant functions in a variety of tumors.

Consistent with our above speculation, by using GSEA to predict the function of YTHDC1, we found that the function of YTHDC1 differs greatly in different tumors. For example, in BLCA, YTHDC1 is enriched in fatty acid metabolism pathway, in CHOL, it is enriched in cell cycle pathway, and in ACC, it is enriched in neural ligand pathway. It has been recognized that the immune microenvironment plays a pivotal role in the occurrence and development of tumors. The formation of the immune microenvironment is essentially caused by the differential conduction of signals within the tumor [25]. In BRCA, GBM, KIRP

and etc., GSEA analysis results show that YTHDC1 is closely related to immune signals. In other tumor such as LIHC, GSEA-enriched signal pathways such as calcium ion pathways have also been confirmed by a large number of studies to participate in immune-inflammatory signal transmission. Furthermore, our analysis found that YTHDC1 is significantly associated with MHC complex, immune stimulation, and activating factors which participate in the formation of the inflammatory microenvironment inside the tumor in almost all tumors. The tumor sample microenvironment score is based on previous classic research. Analysis of the correlation between YTHDC1 expression and immune microenvironment score further suggests that YTHDC1 may be involved in the formation of some tumor immune microenvironments [26]. TMB and MSI are also important mediators for initiating tumor immunity, and can reflect the applicability of some tumors to immunotherapy. Macrophages are the most abundant stromal cells in tumors [27]. They not only participate in the growth and metastasis of tumor cells, but also promote the formation of the immune microenvironment. Our analysis results show that YTHDC1 is closely related to the above-mentioned markers in some tumors, such as LIHC. Although these studies pay more attention to the influence of changes in YTHDC1 expression, but lack of attention to changes in function, and were mainly derived from bioinformatics analysis, we used a variety of analytical methods to demonstrate our results. In summary, our results highlight the potential function of YTHDC1 in pan-cancer immunity.

Furthermore, the above results suggest that YTHDC1 expression is elevated in liver cancer, especially advanced liver cancer, and univariate COX regression analysis also suggests that YTHDC1 is a poor prognostic (HR: 1.428) molecule. Although the P value is 0.068, it may be due to the small sample size. The ICGC and CPTAC databases are the most widely used and standardized databases except TCGA [28]. The two liver cancer data sets from the above databases both suggest that YTHDC1 is highly expressed in liver cancer. In addition, the clinical liver cancer samples collected by ourselves also suggest that YTHDC1 is highly expressed. By intervening in the expression of YTHDC1, we found that the proliferation ability of liver cancer cells was weakened, and the ability to recruit macrophages was weakened, which to a certain extent verified the potential of YTHDC1 in the growth of liver cancer and the formation of the immune microenvironment. However, like the previous bioinformatics research, our research also has some limitations [27–29]. Our data mainly comes from online data analysis, but we have selected the most authoritative TCGA database and used a variety of analyses to support our conclusions. The important thing is, compared with other bioinformatics studies, we also verified our results to a certain extent in independent data sets, external collection of samples, and liver cancer cell function experiments. In addition, there are a few functional experiments, and this research mainly focuses on the effect of YTHDC1 expression changes on Pan cancer rather than the change of function such affected by phosphorylation modification. Further experiments are necessary for the function of YTHDC1 in future research.

Conclusions

In conclusion, in this study, we conducted a pan-cancer-related bioinformatics analysis of YTHDC1 for the first time, and found that YTHDC1 is positively expressed in all tumors, and there are abnormal

modifications and differential expressions in a variety of tumors, and it is closely related to the prognosis of some tumors which suggested its potential malignant function in pan-cancer. We implemented functional analysis to predict the potential function of YTHDC1 in pan-cancer, and used a variety of bioinformatics analysis methods to support its function in the tumor immune microenvironment. Independent data sets, clinical samples and cell function experiments have verified our conclusions to a certain extent. In conclusion, YTHDC1 may serve as a promising prognostic marker and therapeutic target for some types of tumors.

Abbreviation

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; OV Ovarian serous cystadenocarcinoma; PAAD Pancreatic adenocarcinoma; PRAD Prostate adenocarcinoma; READ Rectum adenocarcinoma; SKCM Skin Cutaneous Melanoma; STAD Stomach adenocarcinoma; THCA Thyroid carcinoma; THYM Thymoma; UCEC Uterine Corpus Endometrial Carcinoma; UCS Uterine Carcinosarcoma; UVM Uveal Melanoma;

Declarations

Declaration of Competing Interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

Acknowledgments

No.

Funding

This project was supported by the National Science Foundation of China (No. 8207034238, 81470899, 81702357).

Ethics approval and consent to participate

Clinical tissue samples were collected from the Second Affiliated Hospital of Chongqing Medical University. The study was approved by the ethics committee of the Second Affiliated Hospital of Chongqing Medical University.

Consent for publication

All authors agree to publish.

Availability of data and materials

All data of the article can be obtained from the corresponding author with reasonable request.

Competing Interests

The authors have declared that no competing interest exists.

Author Contributions

Diguang Wen, Yue Li and Zuojin Liu performed research and wrote the first draft. Diguang Wen and performed experiment and collected data. Jiao Lu analyzed data. All authors contributed to the design and interpretation of the study and to further drafts. Yue Li and Zuojin Liu is the guarantor.

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Figures

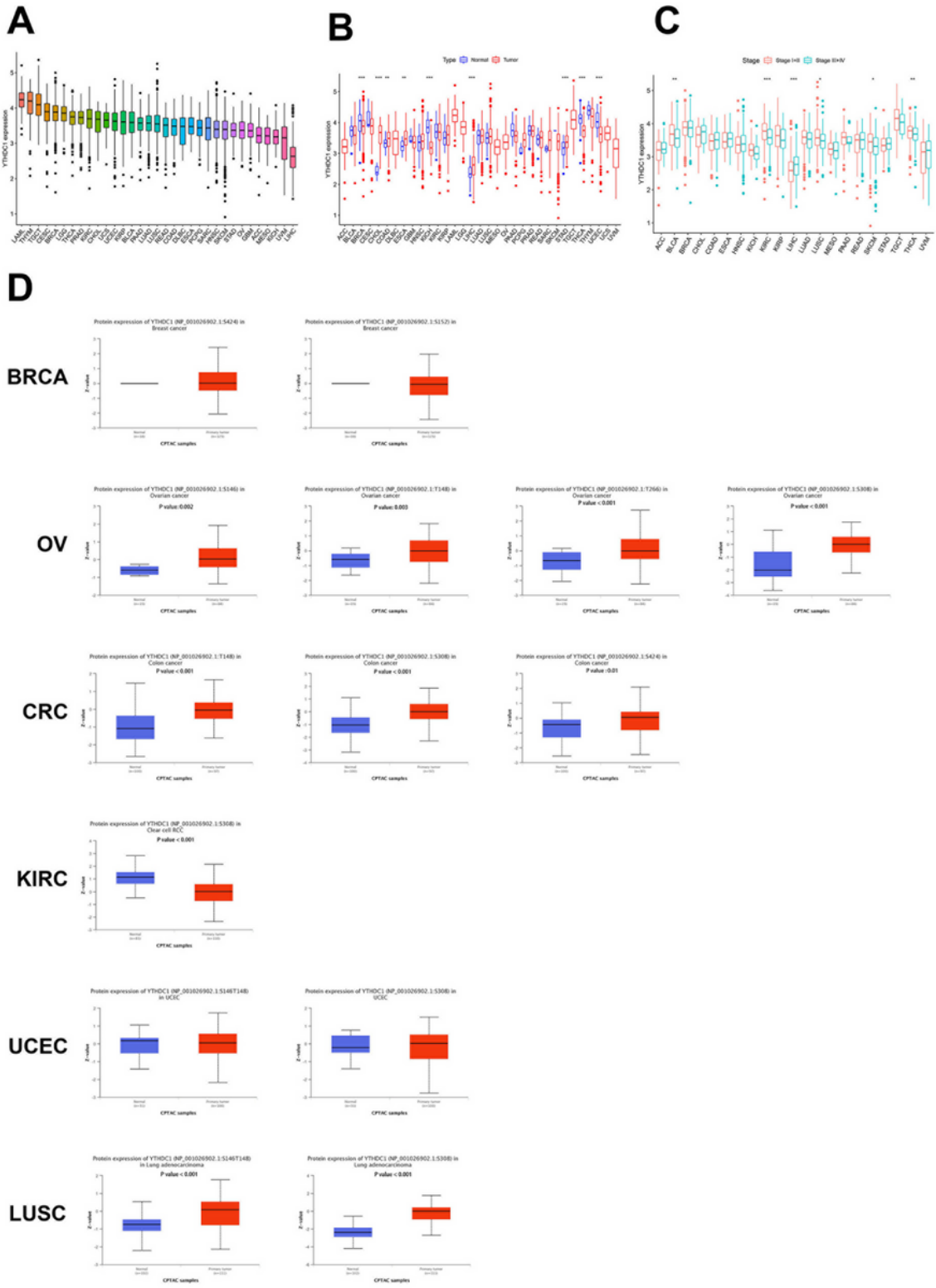

Figure 1

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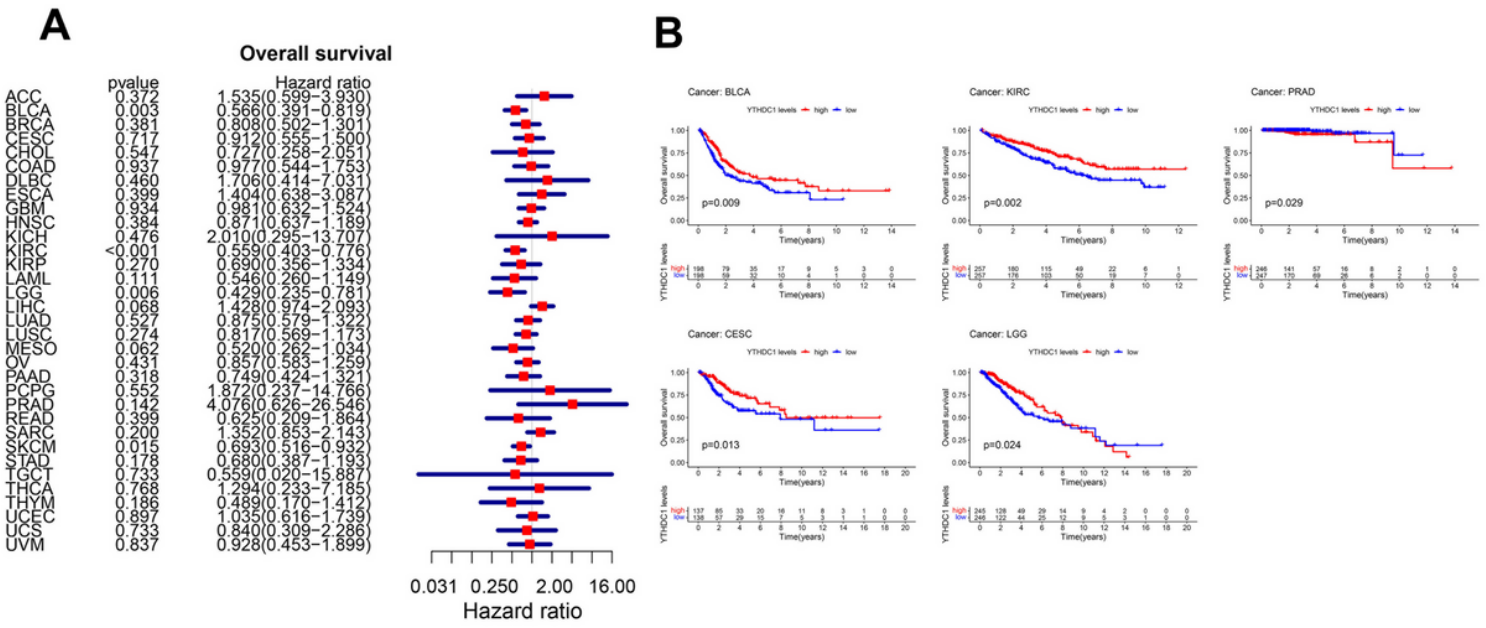

Figure 2

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Figure 3

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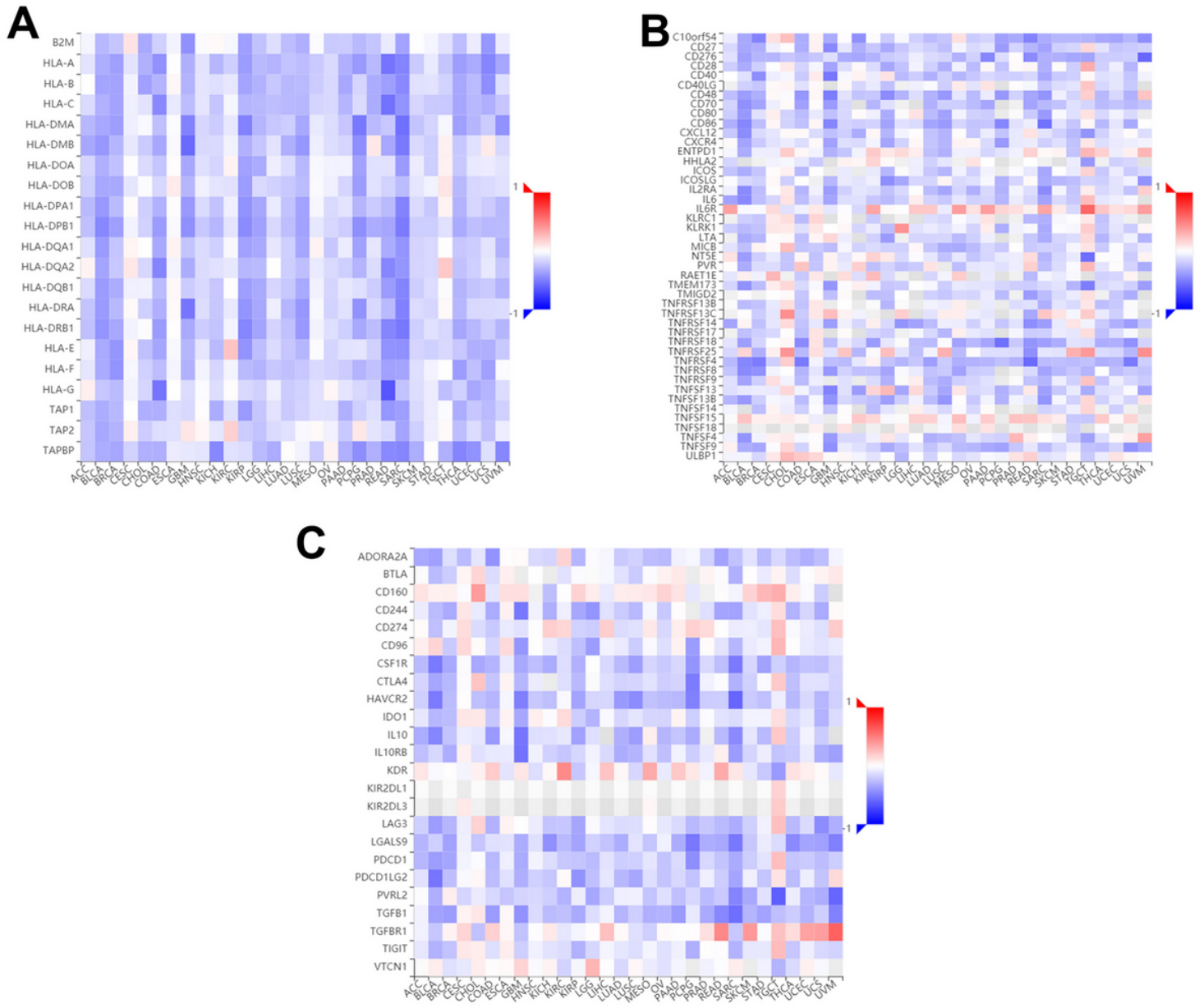

Figure 4

Legend not included with this version.

Figure 5

Legend not included with this version.

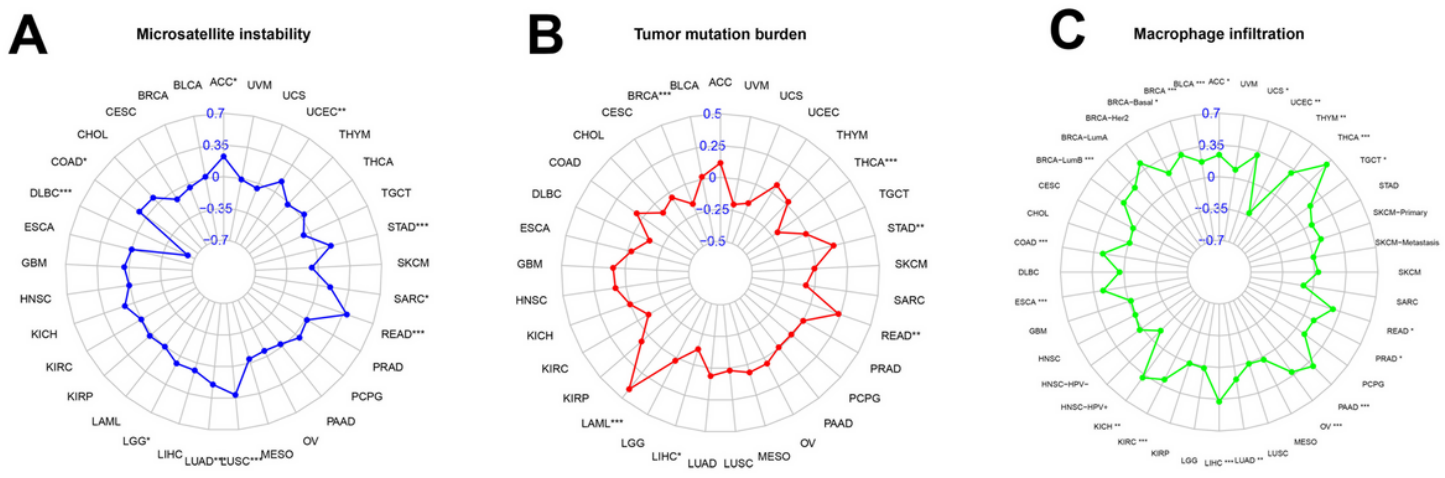

Figure 6

Legend not included with this version.

Figure 7

Legend not included with this version.

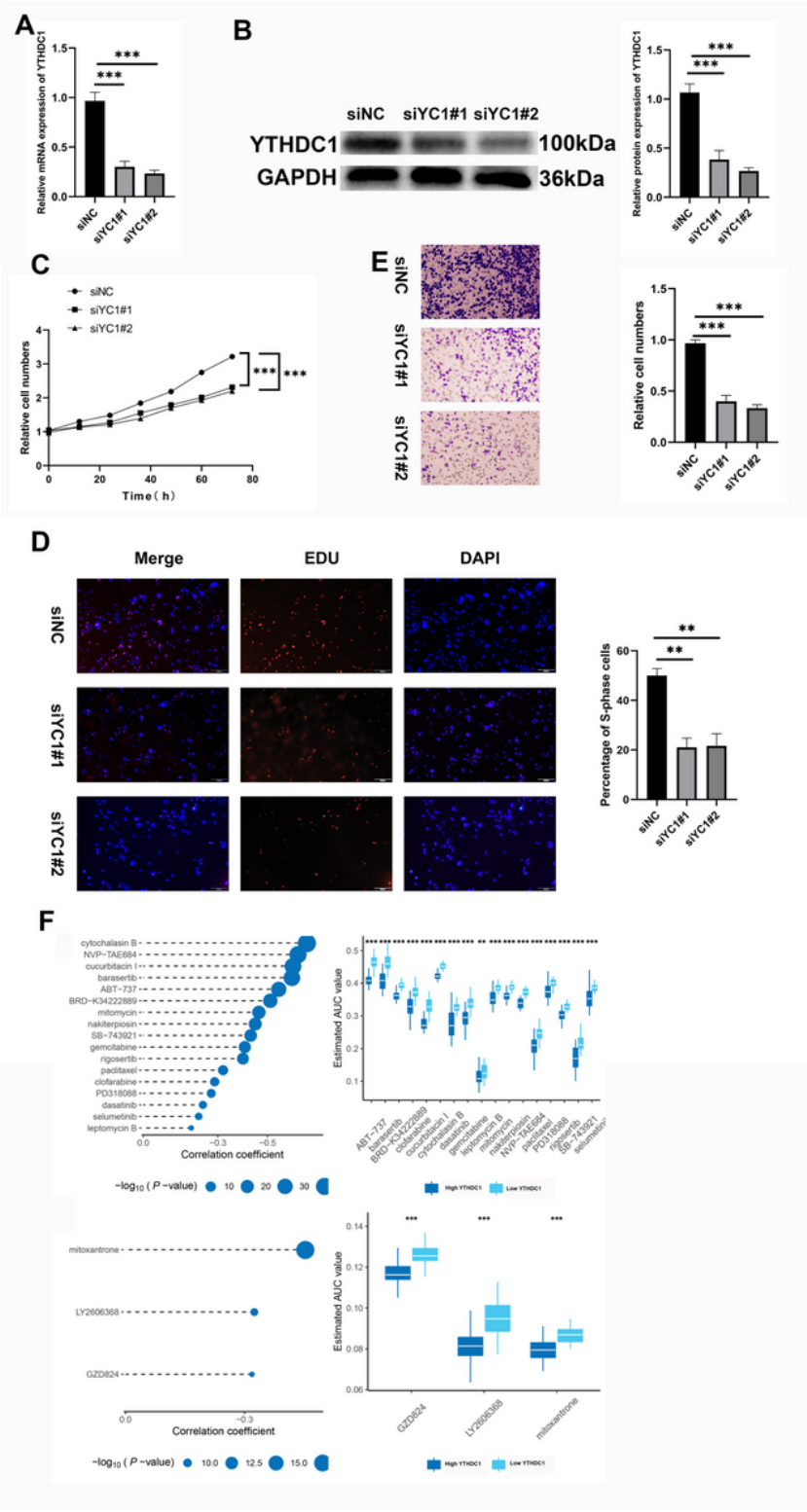

Figure 8

Legend not included with this version.

Supplementary Files

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- SupplyMaterial.pdf