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ORIGINAL RESEARCH

Pan-Cancer Analysis Identifies YKT6 as a Prognostic and Immunotherapy Biomarker, with an Emphasis on Cervical Cancer

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Background: Soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-mediated membrane fusion is crucial for autophagy, making YKT6, a key modulator of cell membrane fusion, a potential target for cancer therapy. However, its oncogenic role across different cancers remains unclear. This study was to investigate the prognostic value and potential immunological functions of YKT6, including cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC).

Methods: Multiple bioinformatics databases, including The Cancer Genome Atlas (TCGA), Cancer Cell Line Encyclopedia (CCLE), and Genotype-Tissue Expression (GTEx) databases, were used to investigate the correlation of the YKT6 expression pattern with the pathological stage and survival rate across cancers. Furthermore, ImmuCellAI, the UCSC Xena platform, and the ESTIMATE algorithm were subsequently utilized to explore the potential relationship between YKT6 expression, the tumor microenvironment, and tumor immune infiltration. Profiling of YKT6 gene mutation and amplification, methylation, and copy number alteration (CNA) was performed on the basis of the TCGA database. Moreover, q-PCR, TMA staining, and siRNA assays were used to validate the cancer-promoting role of YKT6 in CESCs.

Results: Our results reveal that YKT6 is a potential prognostic and cancer immunity biomarker. Elevated YKT6 expression is correlated with poor overall survival (OS) and disease-free survival (DFS). Distinct gene mutation, methylation, and CNA patterns for YKT6 were found in certain types of cancers. The correlation of YKT6 expression with tumor-infiltrating immune cells was verified by analyzing the StromalScore, ESTIMATEScore, ImmuneScore, and tumor purity. In vitro analysis confirmed that YKT6 was highly expressed in advanced-grade CESCs and that the knockdown of YKT6 inhibited the proliferation of cervical cancer cells.

Conclusion: The SNARE protein YKT6 serves as a biomarker and candidate oncogene with actionable mutations. Moreover, YKT6 has the potential to be a prognostic indicator in CESCs. Targeting YKT6 could enhance autophagy regulation and improve therapeutic strategies for personalized cancer treatment.

Keywords: YKT6, pan-cancer analysis, CESC, TCGA, immune infiltration

Introduction

The morbidity and mortality rates of cancer are increasing worldwide.[1] Scientists and researchers focusing on global public health have also become increasingly aware of this problem. Thus, therapeutic tumor vaccines, which can prevent an infection that might lead to cancer, are strongly recommended for adolescents. For example, the human papillomavirus (HPV) vaccine is usually employed to prevent HPV infection and HPV-associated cancers.[2] However, in accordance with the latest cancer statistics from 2023, the incidence of advanced-stage cervical cancer and cervical adenocarcinoma, which are often undetected by cytology, continues to increase among young women.[1] In principle, because HPV plays a significant role in the development of these cancers, the immune system is expected to eliminate HPV-induced tumors relatively quickly. However, these HPV-associated tumors have evolved to escape immune

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detection. Thus, novel immunotherapy targets, which can serve as potential prognostic and predictive indicators of virus-driven cancer, need to be identified by pan-cancer expression analysis.

Autophagy is a self-degradative process in which intracellular and extracellular stimuli trigger the degradation of cellular components to maintain homeostasis.[3] A large body of evidence from extensive studies has revealed the crucial functions of autophagy in immunity.[4–6] In fact, recent evidence has suggested that in pancreatic cancer, the inhibition of autophagy restores resistance to immune checkpoint blockade (ICB) therapy by degrading MHC-I.[7] In parallel, there is strong evidence that PD-L1 expression in gastric cancer is strongly regulated by pharmacological modulation of autophagy.[8] During this self-protective process, SNARE proteins have been proven to play important roles in cellular activities, such as transporting substances and fusing membranes, offering insight into the molecular mechanisms that govern the normal and physiological processes of cells.[9,10] SNARE proteins play crucial roles in the process of autophagy. SNARE proteins play irreplaceable regulatory roles in various stages of autophagy: early onset, autophagosome formation and maturation, and autophagosome–lysosome fusion.[11–14] Deletion of the SNARE protein results in a defect in autophagy, which has a significant effect on the normal physiological functioning of cells.

Therefore, we aimed to determine the underlying prognostic and immunotherapeutic value of SNARE proteins in cervical cancer through pan-cancer analysis. We identified YKT6, a type of SNARE protein that participates in the process of cell membrane fusion, as a potential indicator of autophagy and immunotherapy regulation.

YKT6 is a common SNARE protein that plays an important role in mediating cell membrane fusion; it is responsible for the positioning and fusion of transport vesicles with target membrane layers and participates in a variety of membrane fusion processes.[15,16] Previous studies have shown that the inhibition of autophagosome–lysosome fusion can prevent cancer progression and resistance to chemotherapy. YKT6 is an important protein in autophagosome–lysosome fusion.[17] Abnormal YKT6 activity hinders the synthesis of lysosomal hydrolases. However, little research has focused on the detailed role of multifunctional YKT6 in cancer therapy.

In the present study, in-depth experiments revealed that YKT6, a novel gene, is associated with immunotherapy efficacy and patient prognosis. Furthermore, on the basis of multiple databases, including data retrieved from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) database, an in-depth bioinformatics analysis was performed to determine the functional significance of YKT6 in various cancers; we utilized expression data from the TCGA and GTEx databases, gene copy number alteration data from the cBioPortal database, and gene mutation data for the high and low YKT6 expression groups from the UCSC Xena database. We also performed tumor immunity, GO, and KEGG analyses. We found that YKT6 was specifically correlated with cervical cancer in both tumor tissues and cell lines.

Materials and Methods

Differentially Expressed Genes from the Pan-Cancer Analysis

Thirty-three tumor types were included in the pan-cancer analysis. Gene expression array data and the associated clinical survival information were derived from TCGA and GTEx for analysis. All the data were downloaded from the UCSC Xena database (https://xenabrowser.net/datapages/). All tumor tissue data were taken from the TCGA database, whereas the normal tissue data were taken from the TCGA and GTEx databases.

Gene Alterations Across Cancers

Gene mutation and amplification were explored via the cBioPortal database (https://www.cbioportal.org/). By using Pearson correlation coefficients, a correlation analysis of gene expression changes and copy number changes was conducted.

Prognosis Analysis

The relationship between the YKT6 value and overall survival was investigated via the Kaplan–Meier method, which is based on the survival and survminer R packages.

Immune Cell Infiltration Analysis

The relationships between YKT6 expression and immune/stromal scores in multiple tumors were determined via the ESTIMATE algorithm. Furthermore, characteristics and profiling of tumor-infiltrating immune cells were performed via the ImmuCellAI (http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/) portal.

Cell Culture

The cervical cancer cell lines HeLa and C33a and normal human embryonic kidney (HEK) cells were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). HeLa and C33a cells were cultured in DMEM according to the manufacturer's instructions. HEK cells were maintained in a DMEM/F12K medium. The media for both the cervical cancer cell lines and the normal human embryonic kidney cells were supplemented with 10% heat-inactivated fetal bovine serum (FBS) and 1% penicillin/streptomycin, and the cells were kept at 37°C in a humidified environment with 5% CO2. Tests for mycoplasma contamination were performed routinely.

Real-Time Quantitative PCR

Total RNA was extracted from cells via TRIzol according to the manufacturer's instructions. cDNA was synthesized via a SynScript III cDNA Synthesis Mix (cat. no. TSK322S; Tsingke Biotechnology, Beijing, China). The primer pairs for YKT6 were as follows: forward primer, TGGTCACCTCAGTAGATACCAG; reverse primer, CTCGCTCTAACAGAGACTCCA. GAPDH was used as an internal control.

Immunohistochemical Staining

Human cervical cancer tissue microarrays (TMAs), including 26 cervical cancer tissue samples, were purchased from Bioaitech Company (F261301, Xian, China). YKT6 (1:50, #ab236583, Abcam) was used. The H-score method was applied to evaluate high and low expression levels according to the intensity of immunostaining. This method was based on the staining intensity scores and the percentage of positive cells.

RNAi and Cell Transfection

The sequences of the human YKT6 siRNAs used were as follows: YKT6-siRNA-1, forward: 5'-GGTGTGGTCATTGCTGACAATGAAT-3' and reverse: 5'-ATTCATTGTCAGCAATGACCACACC-3'; YKT6-siRNA -2, forward: 5'-GCUCAAAGCCGCAUACGAU-3' and reverse: 5'-ATCGTATGCGGCTTTGAGC-3'; and YKT6-siRNA -3, forward: 5'-AUACCAGAACCCACGAGAA-3' and reverse: 5'-TTCTCGTGGGTTCTGGTAT-3'. Lipofectamine™ 3000 reagent (Thermo Fisher Scientific) was used according to the manufacturer's instructions. HeLa cells were transfected with YKT6 siRNA and harvested for subsequent experiments.

Colony Formation Assay

The colony formation experiment was conducted by seeding cells in 6-well plates. After a period of 2 weeks, the cells were treated with methanol to immobilize them and then subjected to staining with crystal violet. The colonies were quantified via ImageJ software. The data are reported as the means±SDs obtained from three separate tests, with each experiment performed in triplicate wells.

Statistical Analysis

The data are presented as the means ± SDs. Statistical analyses and graphical production were performed via GraphPad Prism software (version 9.3).

Results

YKT6 Expression from Multilevel Analysis

Since there are few or no normal tissue samples in the TCGA database, a comparison was made between the expression levels of tumors and normal tissues in various cancerous tissues by combining TCGA and GTEx data. Detailed

information is listed in Supplementary Table 1. All the data were downloaded from the UCSC Xena database (https://xenabrowser.net/datapages/); tumor tissue data were derived from the TCGA database, and data on the expression level of YKT6 in normal tissues were obtained from the TCGA database and GTEx database. A schematic representation of the workflow used in this study is shown in Figure 1.

Each cancer type was evaluated for the differential expression of YKT6 in tumor and normal tissues. As shown in Figure 2A, YKT6 expression was relatively higher in the tumor tissue than in the corresponding paratumor tissue among the 33 tumors, except for kidney renal clear cell carcinoma (KIRC), acute myeloid leukemia (LAML), and thyroid carcinoma (THCA).

Next, the mean expression levels of YKT6 in the 33 tumors from the TCGA database (Figure 2B) and major organs from the GTEx database (Figure 2C) were listed in order of lowest to highest expression. YKT6 was strongly expressed in most of the tumors. These results indicate that YKT6 may play a vital role in the tumor initiation stage. The highest expression level was found in GBM, and the lowest was found in LIHC. Different expression patterns were found among the organs; the YKT6 expression level was the highest in the bone marrow and the lowest in the liver. YKT6 mRNA expression was enriched in the uterus, nerve, skin, pituitary, testis, and bone marrow. Furthermore, we detected the expression of YKT6 in diverse cancer cell lines from the Cancer Cell Line Encyclopedia (CCLE) database (Figure 2D). Unlike the universally high expression trend in the 33 tumors and major organs, there were significant differences in expression among the different tumor cell lines. The expression of YKT6 in the glioblastoma multiforme (GBM) cell line was approximately half as high as that in the chronic myelogenous leukemia (CML) cell line. On the basis of the CCLE database, the heterogeneity of YKT6 expression both within and between cell lines was demonstrated. In the LCML and LAML cell lines, YKT6 mRNA was detectable but the levels remained low.

Assessment of YKT6 Expression in Various Stages of Various Types of Cancer

We downloaded the related pathological stage and grade information from the UCSC Xena database. A comprehensive analysis of the relationship between YKT6 expression and different pathological statuses in various cancers was performed. We found that 12 cancers, including adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), kidney chromophobe (KICH), kidney clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), ovarian serous cystadenocarcinoma (OV), skin cutaneous melanoma (SKCM), thyroid carcinoma (THCA), and uterine carcinosarcoma (UCS), were closely associated with the aggressive tumor stage (Figure 3, all $P<0.05$).

Assessment of the Prognostic Value of YKT6 Expression in Multiple Cancers

A comparison of the overall survival (OS), disease-free interval (DFI), disease-free survival (DSS), and progression-free interval (PFI) rates was conducted to assess the prognostic value of YKT6 expression levels in multiple cancer types. To better understand the association between YKT6 expression and survival, further research was conducted by utilizing the "survminer" package to classify the 33 cancers from the TCGA database into either the high-expression YKT6 group and the low-expression YKT6 group. By calculating the hazard ratios through univariate Cox regression analysis, we found that the hazard ratios for YKT6 were significant for ACC, BLCA, BRCA, CESC, GBM, HNSC, KICH, LGG, LIHC, LUAD, MESO, and THCA (Figure 4A). The highest and lowest risk effects were found for the KICH (HR = 6.964, p=0.026) and HNSC (HR=1.337, p=0.013) cancers, respectively. Furthermore, in terms of OS, there was a negative correlation between a high expression of YKT6 and a longer life expectancy, and a K–M plot was used to further analyze the correlation between YKT6 expression and prognosis in various cancers, including ACC, BLCA, CESC, HNSC, LGG, LIHC, LUAD, MESO, and UVM (Figure 4B–J).

Next, to determine the correlation between YKT6 expression and the DFI, we conducted a univariate Cox regression analysis (Figure 5A). Obvious differences were found for the Cox regression analysis correlated with the DFI. No correlation was detected between YKT6 expression and the DFI in any type of cancer. A survival study was subsequently performed, and the results revealed that cancer types with high YKT6 expression had, once again, a worse prognosis than those with low YKT6 expression (Figure 5B and C). A high YKT6 expression level in BLCA patients (Figure 5B, p=0.041)

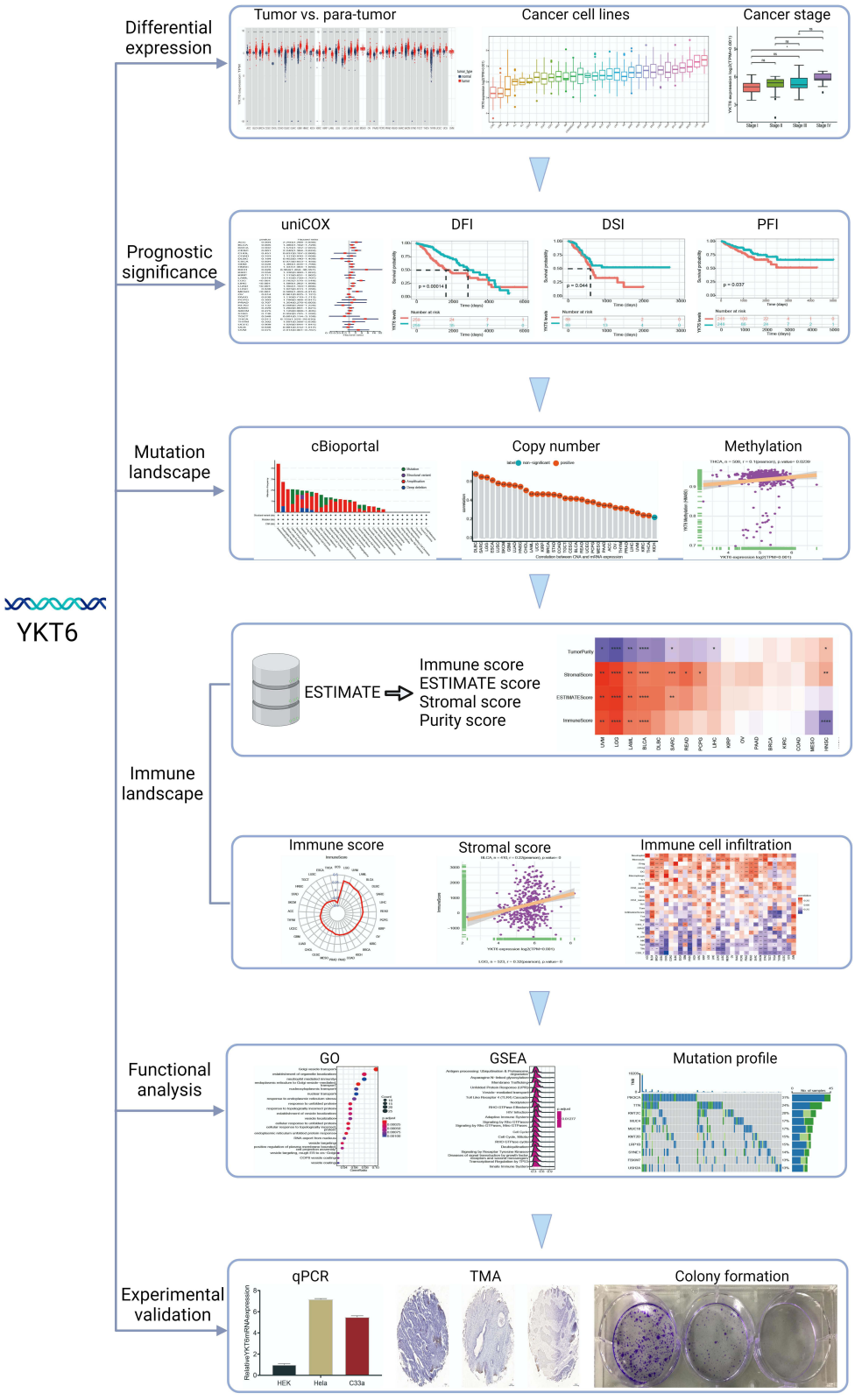

Figure 1 Overview of the pan-cancer analysis of YKT6. Schematic representation of the workflow used in this study. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns—not statistically significant.

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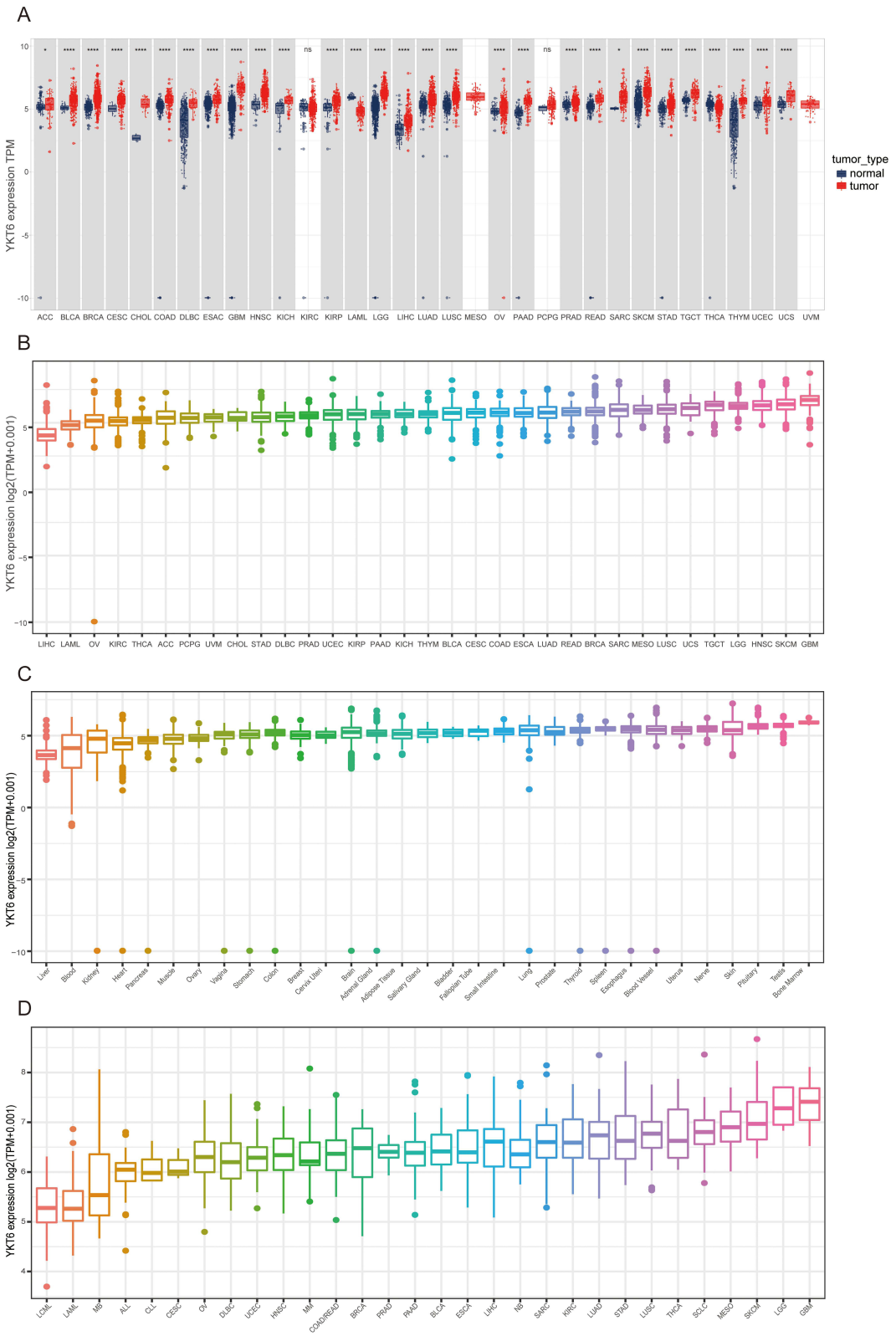

Figure 2 YKT6 expression levels in normal tissues and cancers. (**A**) YKT6 expression in the tumor tissue compared with corresponding paratumor tissue among the 33 tumors; (**B**) Mean expression of YKT6 in the 33 different tumor types based on the TCGA database; (**C**) Mean expression levels of YKT6 in different organs based on the GTEx database; (**D**) Mean expression levels in the diverse cancer cell lines from the Cancer Cell Line Encyclopedia (CCLE) database. *P < 0.05, ****P < 0.0001, ns—not statistically significant.

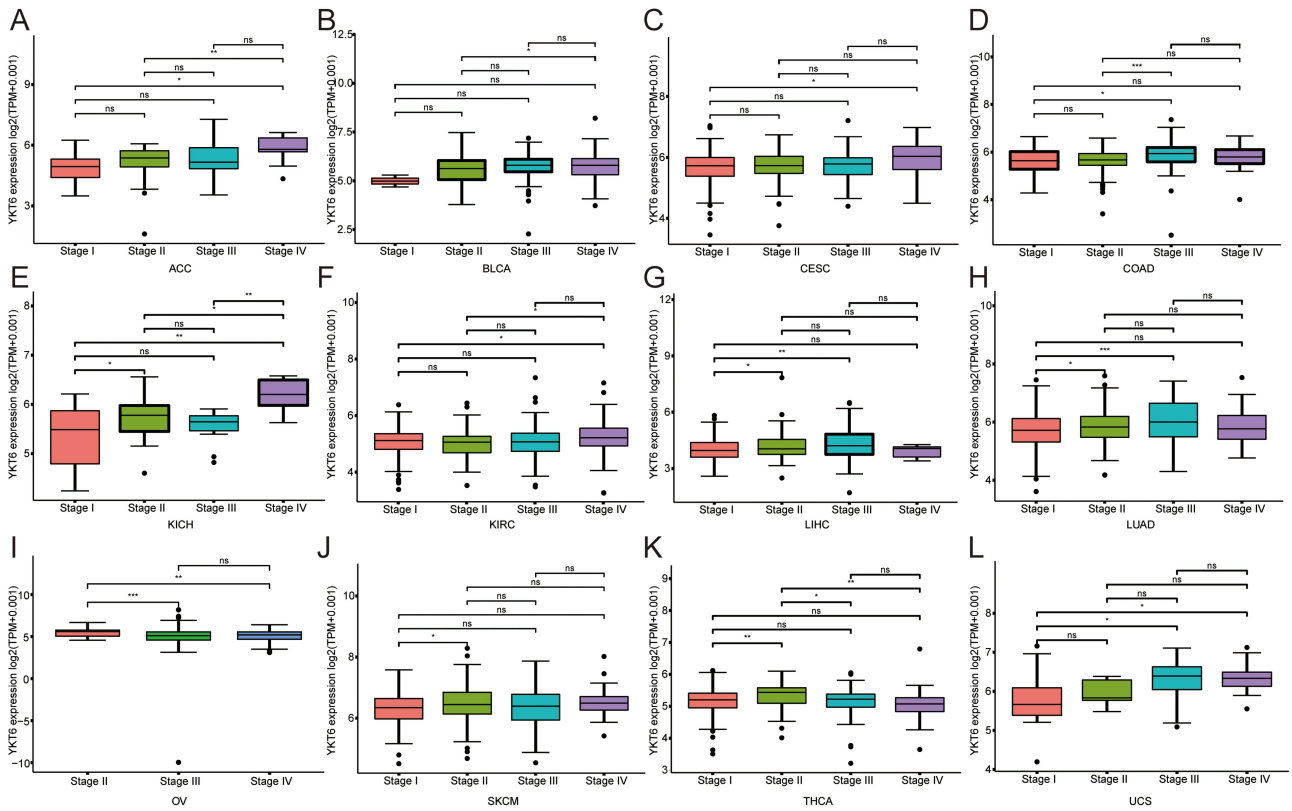

Figure 3 Assessment of the expression levels of YKT6 in various tumor stages. (**A**–**L**) YKT6 expression in various tumor stages in the indicated tumor types. In adrenocortical carcinoma (ACC), bladder urothelial carcinoma (BLCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), kidney chromophobe (KICH), kidney clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), ovarian serous cystadenocarcinoma (OV), skin cutaneous melanoma (SKCM), thyroid carcinoma (THCA), and uterine carcinosarcoma (UCS), YKT6 expression was closely associated with the aggressive tumor stage. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns—not statistically significant.

and MESO patients (Figure 5C, p=0.031) was also associated with a poorer DFI in the TCGA cancer types. Moreover, univariate Cox regression analysis was performed to analyze the DSS data (Figure 5D), and the Cox regression analysis produced findings that were comparable to those for OS. We observed that YKT6 was a substantial risk factor for breast invasive carcinoma (BRCA), GBM, and KIRC. In addition, the independent prognostic value of YKT6 was analyzed via a K–M plot. Correlation analysis revealed that YKT6 expression across 33 different types of tumors was associated with a cancer prognosis, including adrenocortical carcinoma, breast cancer, head and neck cancer, glioma, liver cancer, lung cancer, mesothelioma, pancreatic adenocarcinoma, and ocular melanoma (Figure 5E–M). Furthermore, the correlations between YKT6 expression and the PFI are shown in Supplementary Figure 1. Afterward, we combined the findings of the survival study with those of the DFI and DSS analyses, and the findings, which were fundamentally identical to the results from the OS and DFI analyses, revealed that a high expression of YKT6 was strongly linked to a poorer prognosis in cancer patients.

Landscape of Genomic Alterations and Structural Variation in the YKT6 Gene

Genome mutation has been identified as a hallmark of cancer. Delineation of genetic alterations, including mutations, structural variants, amplifications or deep deletions, in the YKT6 gene across cancers was conducted via the cBioPortal database (Figure 6A). Amplification of the YKT6 gene was found to be the most frequent form of genetic alteration in adrenocortical carcinoma (>5%). Diffuse large B-cell lymphoma (>2%), uterine carcinosarcoma (>1.5%), glioblastoma multiforme (>1%), pheochromocytoma (>1%), paraganglioma (>1%), pancreatic adenocarcinoma (<1%), ovarian serous cystadenocarcinoma (<1%), and liver hepatocellular carcinoma (<1%) were found to have amplifications in only the YKT6 gene. Furthermore, mutations in the YKT6 gene were the most common type of genetic change in uterine corpus

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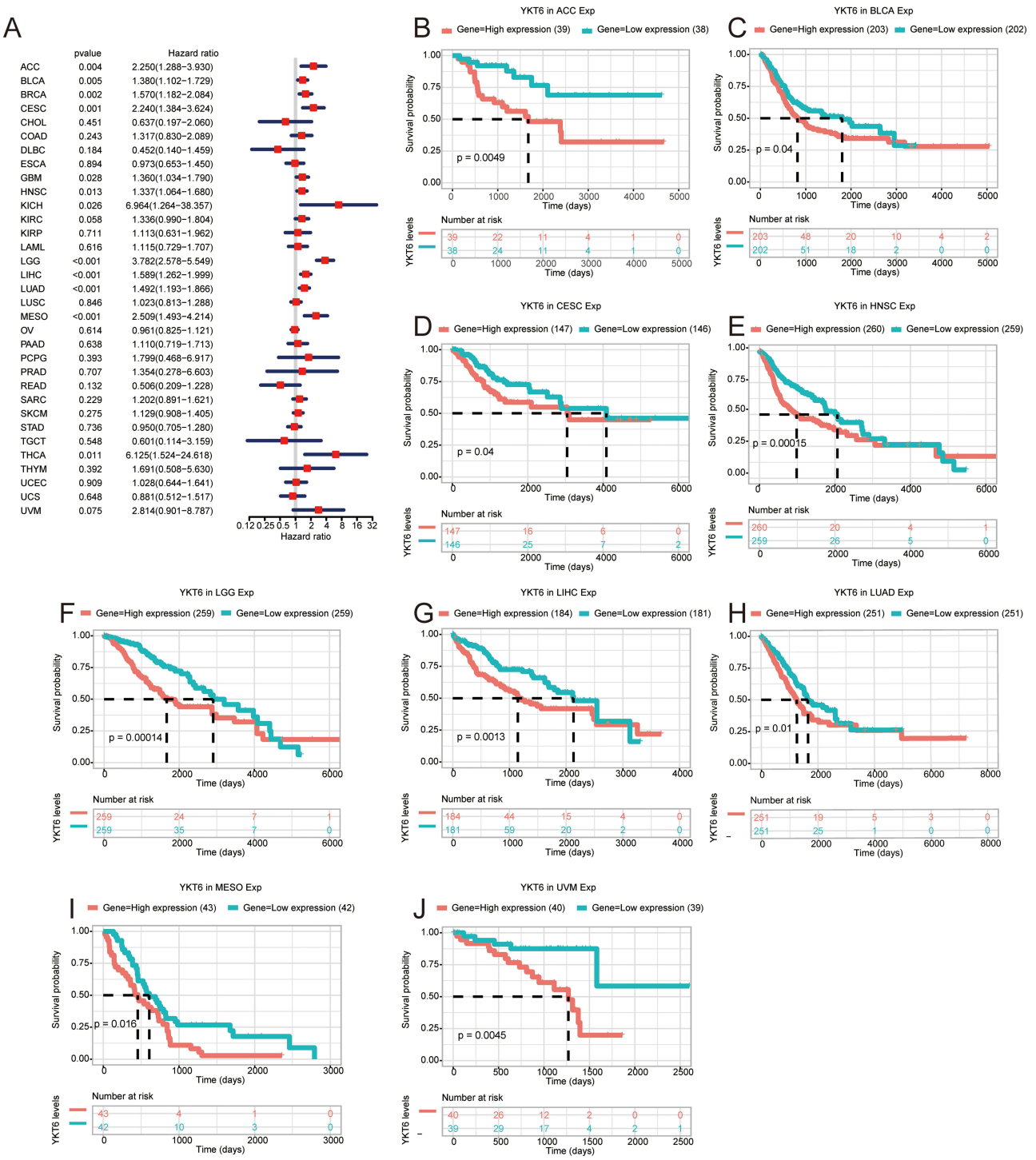

Figure 4 Prognostic value of YKT6 expression in multiple cancers: (**A**) the uniCox results of YKT6 across cancers. (**B**–**J**) K–M OS plot demonstrating the correlation between YKT6 expression and prognosis in cancers, including ACC, BLCA, CESC, HNSC, LGG, LIHC, LUAD, MESO, and UVM.

endometrial carcinoma (>1%) and skin cutaneous melanoma (>1%), whereas substantial deletions were observed in esophageal adenocarcinoma. Interestingly, a structural variant in the YKT6 gene was found only in sarcomas (Figure 6A). According to the cBioPortal database, YKT6 amplification was shown to be the most common type of YKT6 gene mutation across cancers. In the next step, we calculated the correlation between YKT6 gene expression and copy number in each tumor via copy number data from the cBioPortal database (Figure 6B). DLBC had the highest

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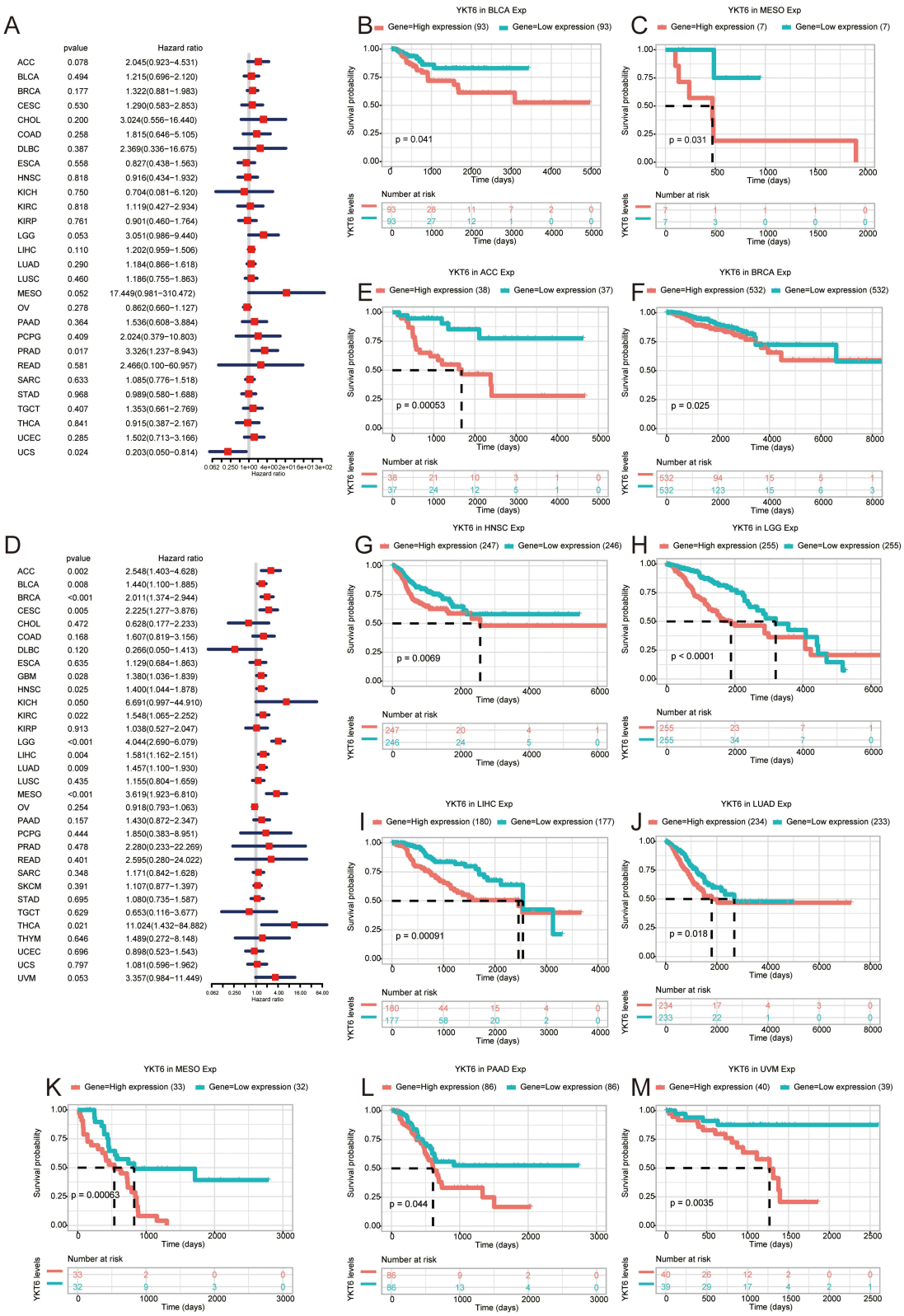

Figure 5 Pan-cancer prognostic significance of YKT6 for DFI and DSS. (**A**) The uniCox results show YKT6 expression and DFI across cancers; Kaplan–Meier DFI results of YKT6 in BLCA (**B**) and MESO patients (**C**); (**D**) the uniCox results show YKT6 expression and DSS across cancers; (**E**–**M**) Kaplan–Meier DSS results of YKT6 in ACC, BRCA, HNSC, LGG, LIHC, LUAD, MESO, PAAD, and UVM patients.

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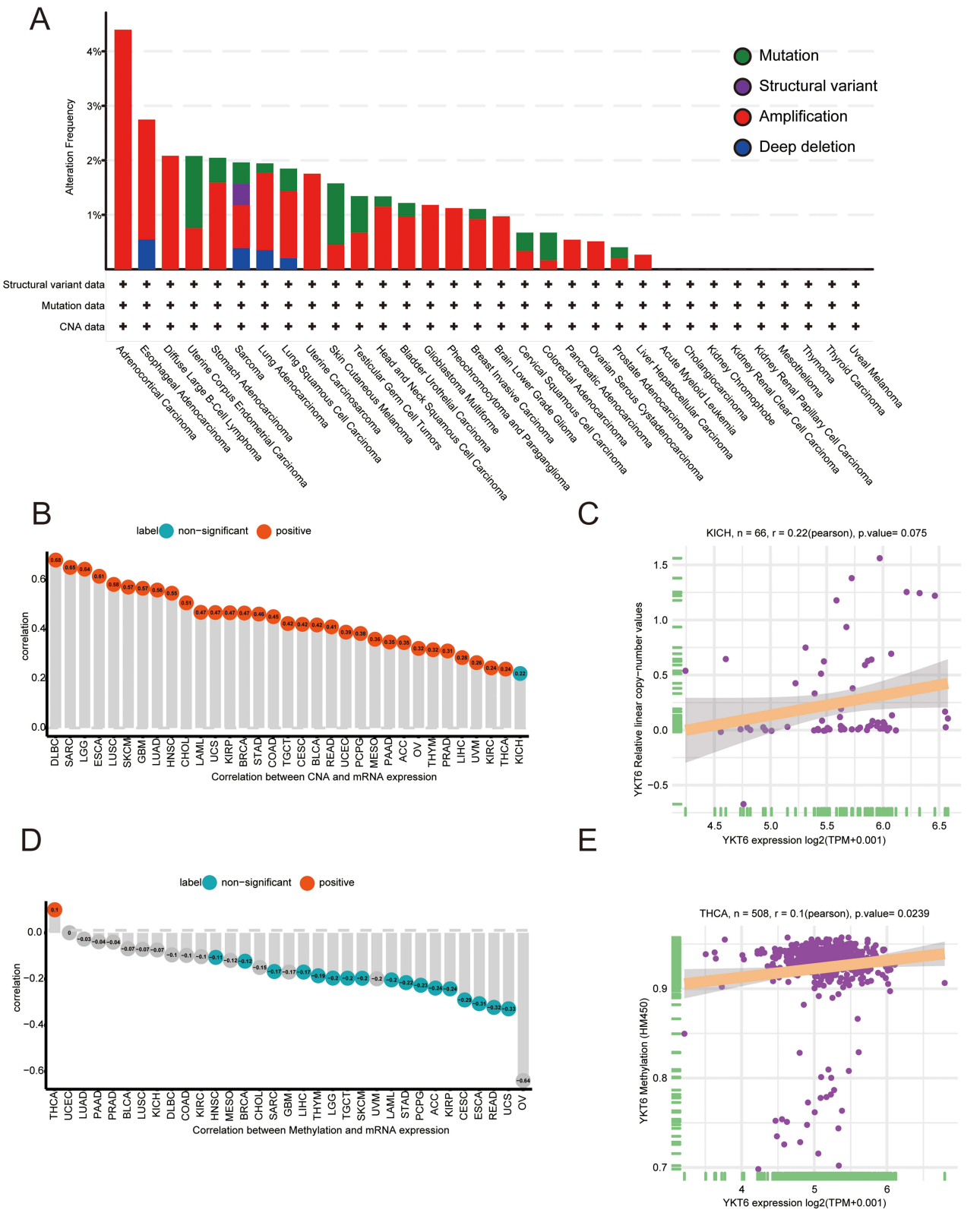

Figure 6 Gene alteration and structural variation of YKT6: (**A**) landscape of genomic alterations of YKT6 across cancers via the cBioPortal database; (**B**) correlation between YKT6 gene expression and copy number across cancers; (**C**) correlation between YKT6 mRNA expression and copy number alterations was not significant only in KICH; (**D**) Pearson's correlation between YKT6 expression and methylation of the YKT6 promoter in each tumor; (**E**) correlation between YKT6 mRNA expression and methylation was significant only in THCA.

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correlation coefficient for the association between somatic copy number alterations (CNAs) and YKT6 expression. Furthermore, we found that only in KICH was the correlation between YKT6 mRNA expression and copy number alterations not significant (Figure 6C). Furthermore, we calculated the Pearson correlation coefficient between YKT6 expression and the methylation of the YKT6 promoter in each tumor type (Figure 6D). The methylation data were obtained from the HM450 dataset of the cBioPortal database. In general, hypermethylation of the promoter leads to the downregulation of mRNA expression. As shown in Figure 6E, among the 33 cancers, only THCA had significant correlation between YKT6 mRNA expression and methylation.

Relationship Between YKT6 Expression and the Tumor Microenvironment

Previous studies have repeatedly revealed the essential roles of the tumor microenvironment (TME) and tumor immune microenvironment (TIME) during tumor progression and their roles in predicting the response to immunotherapy. Hence, we provide a comprehensive overview of the role of YKT6 in the pan-cancer tumor microenvironment. The ESTIMATE algorithm was applied to calculate the stromal cell scores, immune cell scores, and ESTIMATE scores for 33 types of cancer. The results demonstrated that YKT6 had the greatest positive correlations with the immune scores in BLCA, LAML, LGG, and UVM (Figure 7A). In ACC, CESC, ESCA, GBM, HNSC, LUAD, LUSC, SKCM, STAD, TGCT, THCA, THYM, UCEC, and UCS, YKT6 expression was inversely correlated with the immune score (Figure 7B). With respect to the stromal score, YKT6 had the greatest positive correlations with the stromal scores in LAML, LGG, READ, and UVM (Figure 7C). In BLCA, READ, HNSC, LAML, LGG, PCPG, SARC, and UVM, YKT6 expression was inversely correlated with the stromal score, but it was positively correlated with the stromal scores in GBM, LUSC STAD, TGCT, THCA, and UCEC (Figure 7D). Overall, we summarized the features of the tumor microenvironments of 33 cancers in Figure 7E.

YKT6 Regulates Interferon Signaling, Lymphocyte Activation, and Activation of Antigen-Presenting Cells Across Cancers

Furthermore, an analysis of the coexpression of YKT6 and immune-related genes was conducted. An investigation of the correlation between YKT6 expression and the infiltration of 25 immune cells into 32 tumors (LAML excluded) through the ImmuCellAI database was conducted. A negative correlation was observed between the expression of YKT6 and the infiltration of numerous types of immune cells in ESCA, HNSC, STAD, THCA, and UCS. UVM, SARC, PRAD, KIRP, STAD, and LGG were associated with immune cell types in a positive manner (Figure 8A). Numerous immune cell types, including activated and naive CD4+ and CD8+ T cells, macrophages, NKT cells, natural killer cells, and regulatory T cells, were examined.

YKT6 is Correlated with Immunotherapeutic Responses in Multiple Tumors

Accumulating evidence has further demonstrated that antitumor immunity is a predictive indicator of the efficacy of immunotherapy. Microsatellite instability (MSI) and the tumor mutation burden (TMB) have been reported to serve as strong predictors of the response to immune checkpoint inhibitors. Immune checkpoint inhibitors are effective in treating tumors with high MSI (MSI-H) and TMB. Hence, to determine whether YKT6 expression levels are a predictor of immunotherapeutic responses across multiple cancer types, we analyzed the relationships between YKT6 expression levels and MSI and TMB. YKT6 expression was positively correlated with MSI in CESC (P=0.002), GBM (P=0.022), KIRC (P=0.015), LIHC (P=0.012), LUAD (P=0.043), LUSC (P=0.008), SARC (P=0.001), and TGCT (P=0.036). There was a negative association with MSI in COAD (P=0.001), DLBC (P=0.016), and THCA (P=0.003) (Figure 8B). Furthermore, YKT6 expression was positively related to TMB in ACC (P=0.048), LUAD (P=8.67e-8), and SARC (P=0.0001) and negatively related to TMB in HNSC (P=0.039) and THCA (P=0.038) (Figure 8C).

Moreover, further research was conducted to determine the correlation between YKT6 and immune-stimulating (Figure 8D)/immune-inhibitory genes (Figure 8E). Previous studies have shown that genetic variations in immune-stimulating genes can predict outcomes in patients with metastatic tumors treated with immunotherapy. The expression of immune-stimulating genes was positively correlated with YKT6 in the majority of the tumors, especially in OV, UVM,

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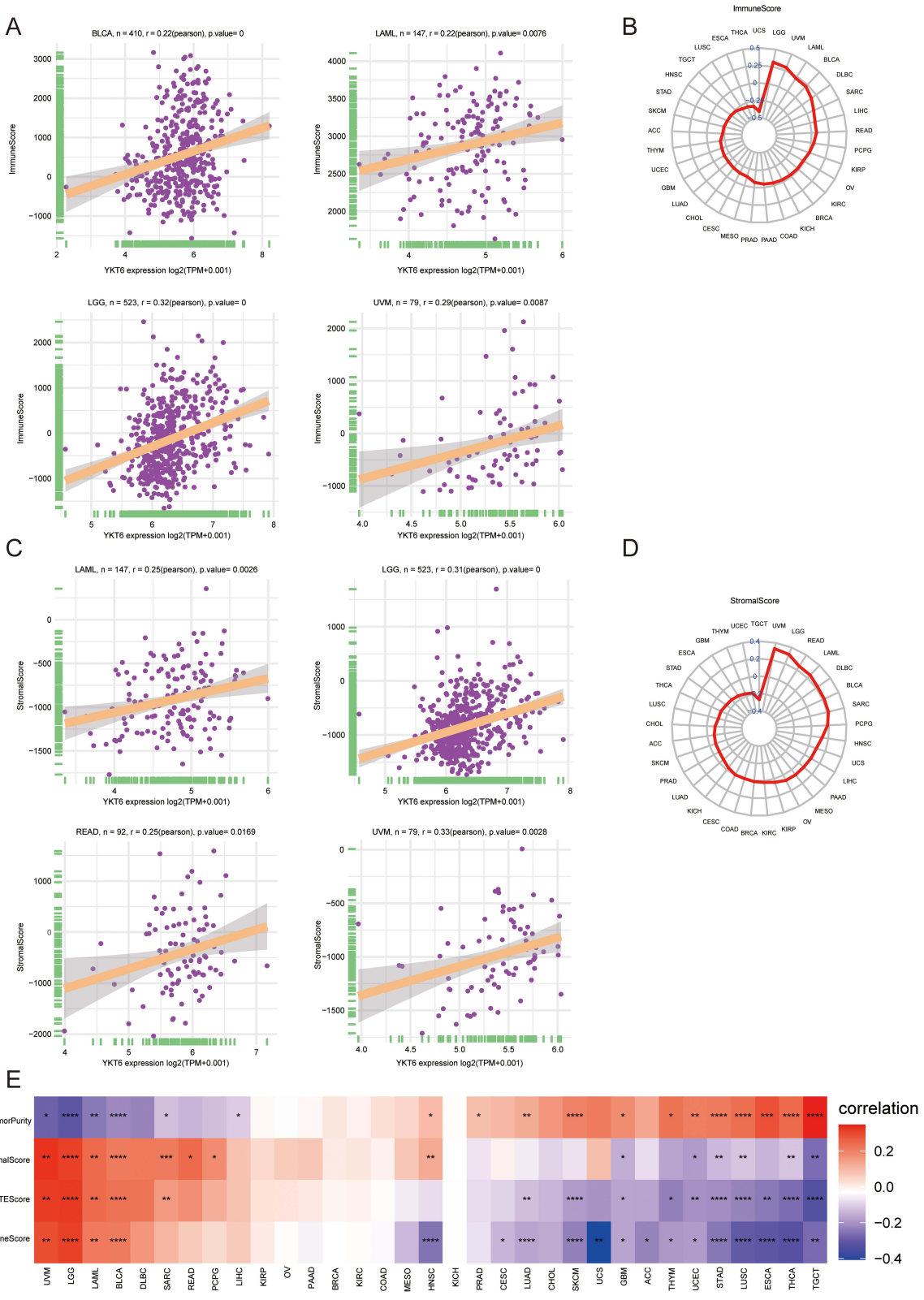

Figure 7 Tumor microenvironment analysis of YKT6 across cancers: (**A**) the top 4 cancers most positively correlated with immune scores based on the ESTIMATE algorithm; (**B**) immune score radar plot depicting the correlation with YKT6 expression across cancers; (**C**) the top 4 cancers most positively correlated with the stromal score based on the ESTIMATE algorithm; (**D**) Stromal score radar plot depicting its correlation with YKT6 expression across cancers; (**E**) heatmap representing the correlation between YKT6 expression and TME scores across cancers. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

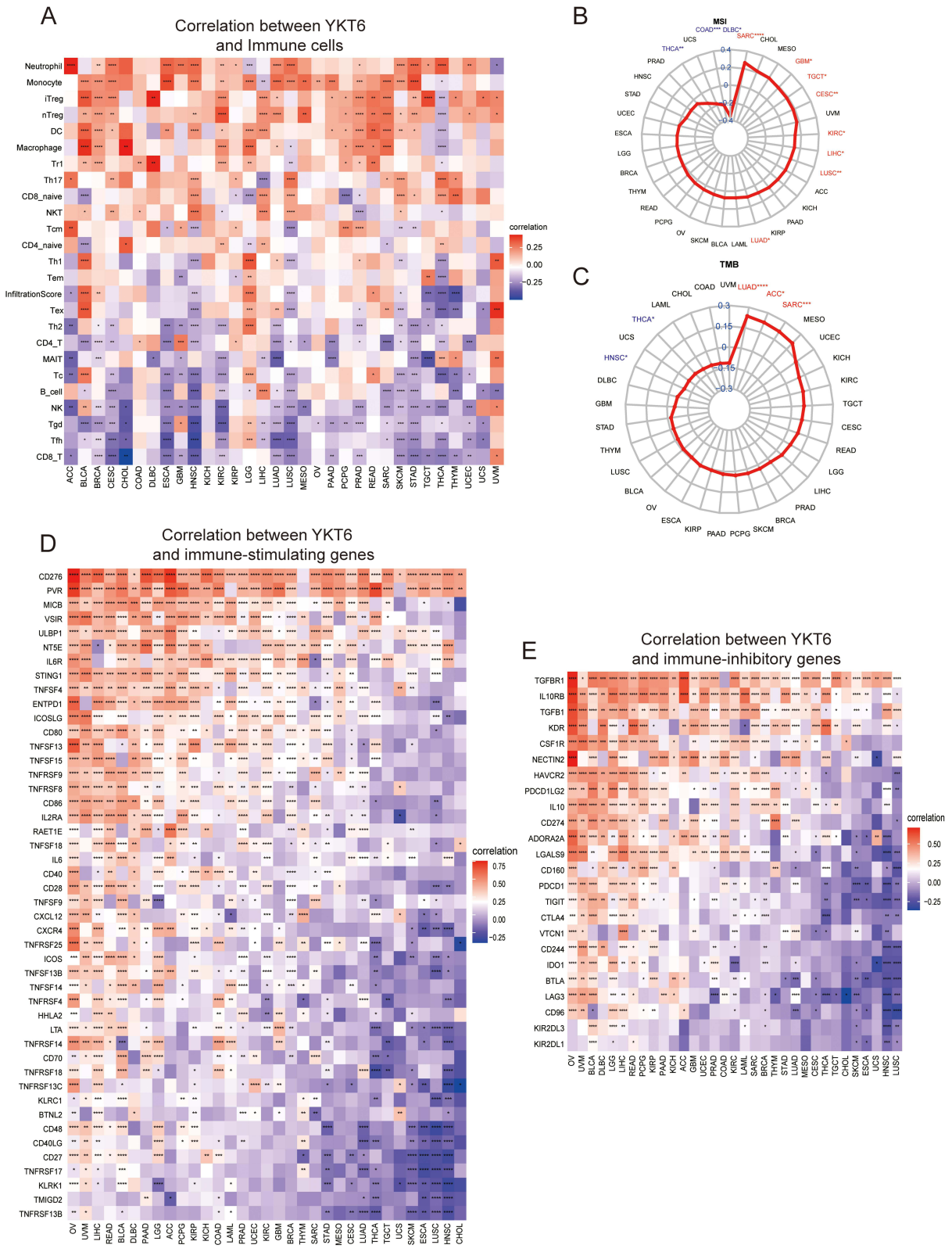

Figure 8 Pan-cancer analysis of immune cell infiltration and immune checkpoints. (**A**) The correlation between YKT6 expression and the infiltration of 25 immune cell types in 32 tumors. Relationships between YKT6 expression levels and MSI (**B**) or TMB (**C**). Correlations between the levels of YKT6 and immune-stimulating (**D**) or immune-inhibitory genes (**E**). **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

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LIHC, READ, and BLCA. In SKCM, ESCA, LUSC, HNSC, and CHOL, YKT6 expression was negatively related to the expression of immune-stimulating genes. For immune-inhibitory genes, the expression of YKT6 was positively correlated with the expression of OV, UVM, BLCA, and DLBC. YKT6 expression was negatively correlated with these genes in CHOL, SKCM, ESCA, UCS, HNSC, and LUSC.

Drug Response Analysis and GSVA Across Cancers

In patients treated with linsitinib, leflunomide, BMS-345541, KRAS inhibitor-12, LGK974, oxaliplatin, ABT737, ML323, and venetoclax, YKT6 expression was positively related to the response to those drugs (Figure 9A). Additionally, to further investigate the biological significance of YKT6 expression in different tumor tissues, we conducted GSVA. The results of the enrichment pathway analysis are shown in Figure 9B. GSVA revealed enrichment of 50 pathways in 33 tumor types. Among these differentially enriched pathways, the 3 pathways most closely related to the 33 tumor types were the mRORC1 signaling pathway, the PI3K/AKT/mTOR pathway, and the unfolded protein response pathway. The 3 pathways associated with the most negative correlations were the bile acid metabolism, xenobiotic metabolism, and KRAS signaling pathways.

Further Analysis of YKT6 in CESCs

The results of the GO functional annotation, KEGG pathway analysis, and GSEA enrichment analysis are shown in Figure 10A. An integrated analysis of the 300 genes most related to YKT6 revealed that the biological processes (BPs) enriched in this dataset were related primarily to Golgi vesicle transport, polar organelle localization and the endoplasmic reticulum-to-Golgi vesicle. KEGG analysis indicated that YKT6 positively regulates pathways associated with neurodegeneration, amyotrophic lateral sclerosis, and amyotrophic lateral sclerosis. The results of GSEA-Reactome analysis of CESCs revealed that YKT6 functions in the endoplasmic reticulum–Golgi transport step and vesicular transport. The detailed pathway involved antigen processing, asparagine N-linked glycosylation, and membrane trafficking.

To further establish a link between YKT6 and mutations in CESC patients, the mutation profiles of patients with high/low YKT6 expression levels were assessed. The top 10 differentially mutated genes are shown in the waterfall plot in Figure 10B. Mutations in KMT2D, LRP1B, SYNE1, and USH2A were specific to the high-risk group, whereas mutations in DMD, FLG, EP300, and ADGRV1 were specific to the low-risk group. The mutation type varied from gene to gene. Moreover, dual immune checkpoint inhibitor (ICI) combination therapy, which involves coinhibition of PD-1/PD-L1 in cancer immunotherapy, has become a promising therapy for enhancing the benefits gained from ICI treatment. Therefore, we investigated the correlation between YKT6 and major immune checkpoint genes (Figure 10C). The results indicated that the CD274 gene was coexpressed with YKT6. Furthermore, TGFB1 and TGFBR1 have been proven to be involved in processes regulating the immunosuppressive microenvironment. Therefore, we determined the TGFB1/TGFBR1 ratios for various cancer types. In this section, we explored specific molecules of the TGF-β signaling pathway and found that YKT6 was positively associated with TGFB1 (Figure 10D)/TGFBR1 (Figure 10E) expression via pan-cancer analysis. YKT6 may mediate autophagy flux changes in CESCs through targeting the TGFB1/TGFBR1 signaling pathways.

qPCR Validation and Immunohistochemical Staining

The expression of YKT6 is relatively high in CESCs and thus displays a tissue-specific expression pattern. Quantitative real-time PCR assays were performed to detect YKT6 expression in cervical cancer cell lines and normal HEK cells to confirm the success of the bioinformatic analysis. As shown in Figure 11A, consistent with previous results, YKT6 expression levels were higher in the cervical cancer cell lines than in the normal HEK cell lines. Furthermore, we determined the relationships between the YKT6 expression level and clinicopathological characteristics, including age, pathology, lymph node metastasis, tumor grade, and stage (Table 1). Examples of mild, moderate, and strong IHC staining of YKT6 are shown in Figure 11B. As shown in Table 1, in the analysis of YKT6 expression and clinicopathological characteristics, high YKT6 expression was correlated with a high tumor grade.

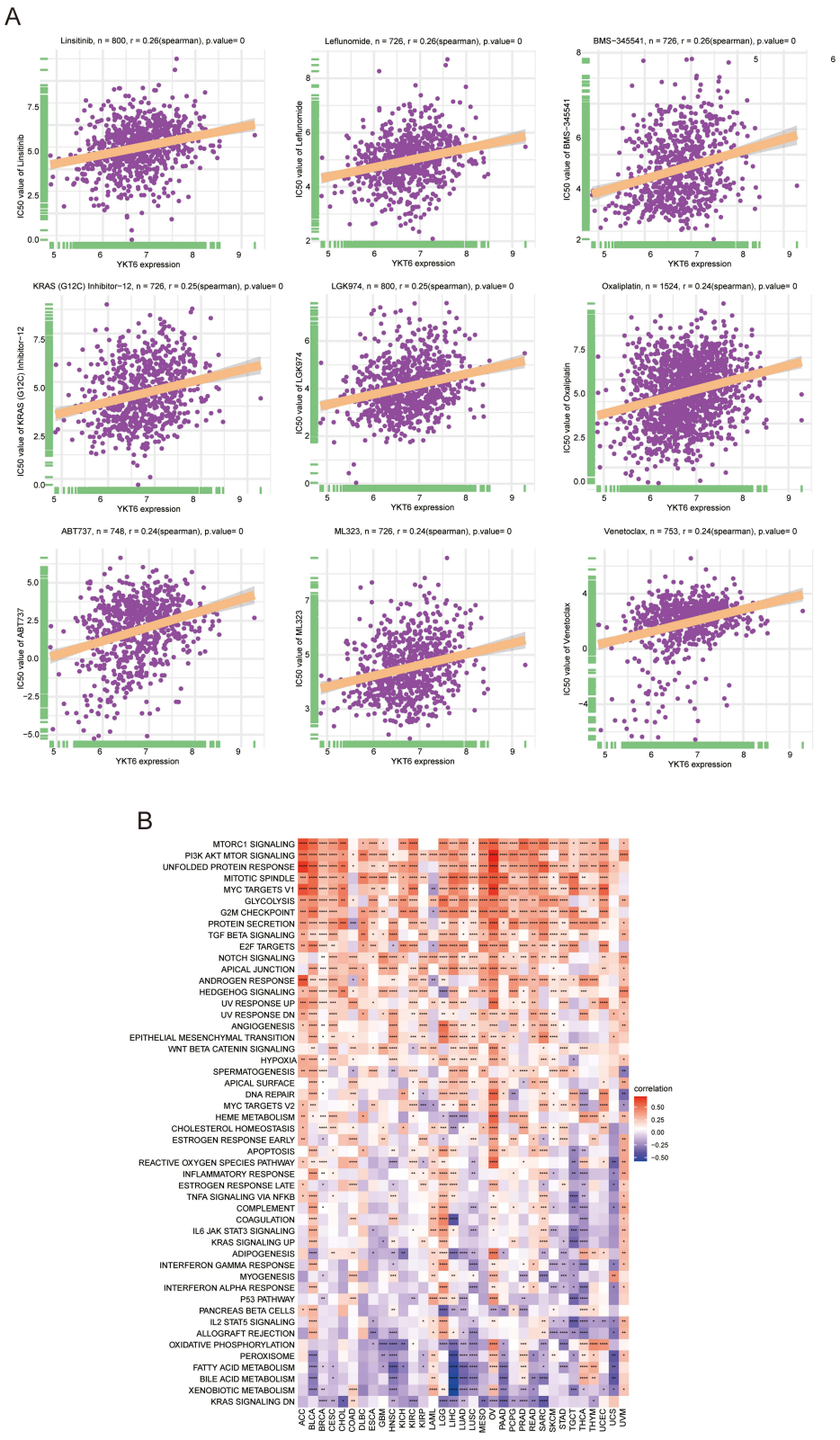

Figure 9 Drug response analysis and GSVA: (**A**) YKT6 expression was positively correlated with drug response in patients treated with specific inhibitors; (**B**) GSVA analysis revealed the enrichment of 50 pathways in 33 tumor types. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001.

https://doi.org/10.2147/OTT.S491310

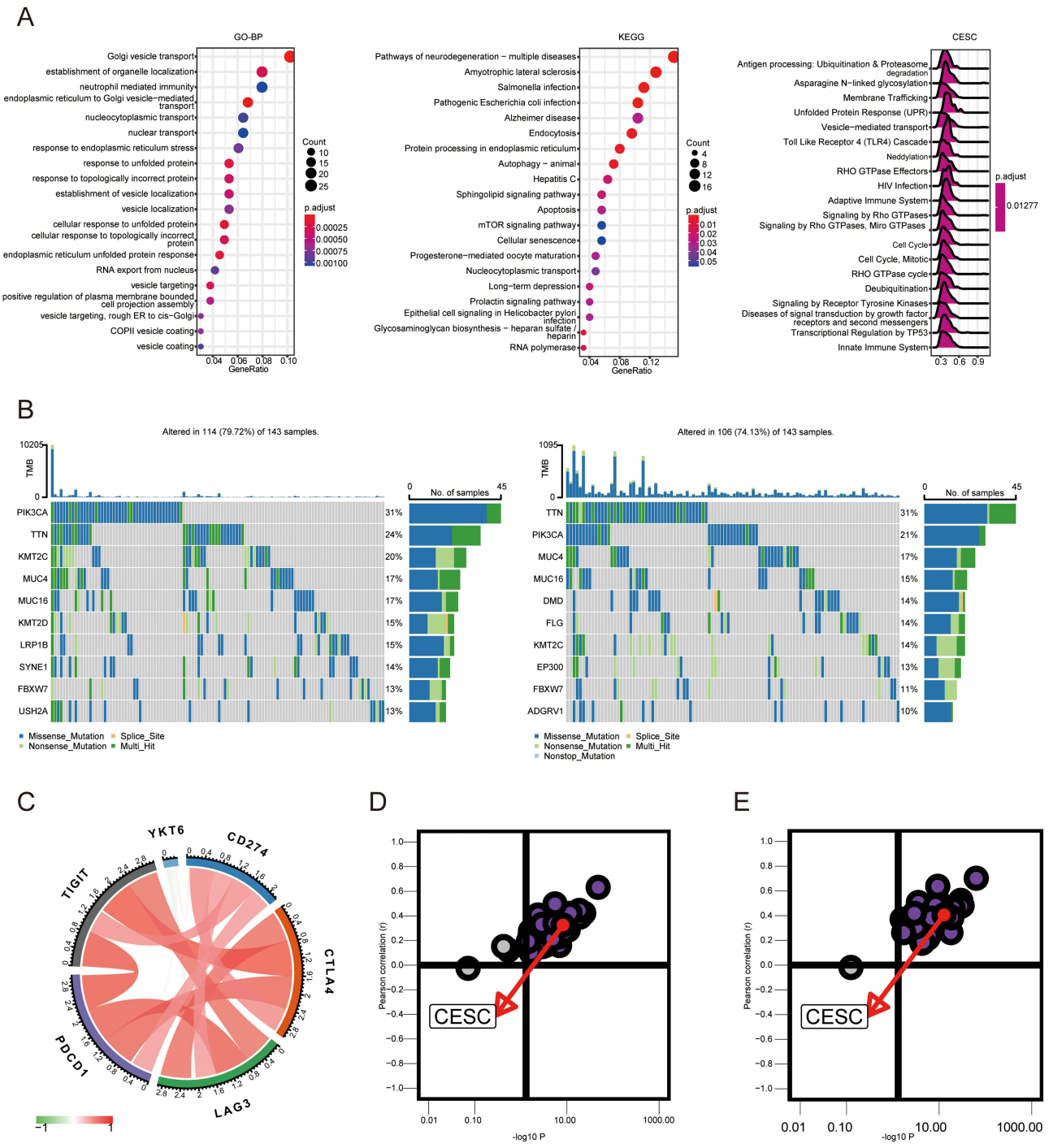

Figure 10 Further analysis of YKT6 expression in CESCs: (**A**) GO and KEGG analyses and GSEA of YKT6-related genes in CESCs; (**B**) mutational profiles of CESC patients with high and low YKT6 expression; (**C**) correlation between YKT6 and major immune checkpoint genes in CESCs; YKT6 was positively associated with TGFB1 (**D**) and TGFBR1 (**E**).

Depletion of YKT6 Inhibits HeLa Cell Proliferation

An investigation was undertaken to ascertain the function of YKT6 in the human cervical cancer cell line HeLa via in vitro experiments. First, we transfected HeLa cells with YKT6 siRNA, specifically YKT6-siRNA1 and YKT6-siRNA3, which resulted in a considerable decrease in YKT6 mRNA expression (Figure 11C). Moreover, we performed colony formation assays with HeLa cells. As shown in Figure 11D, knockdown of YKT6 impaired the proliferation of HeLa cells, suggesting that knockdown of YKT6 leads to a reduction in proliferation.

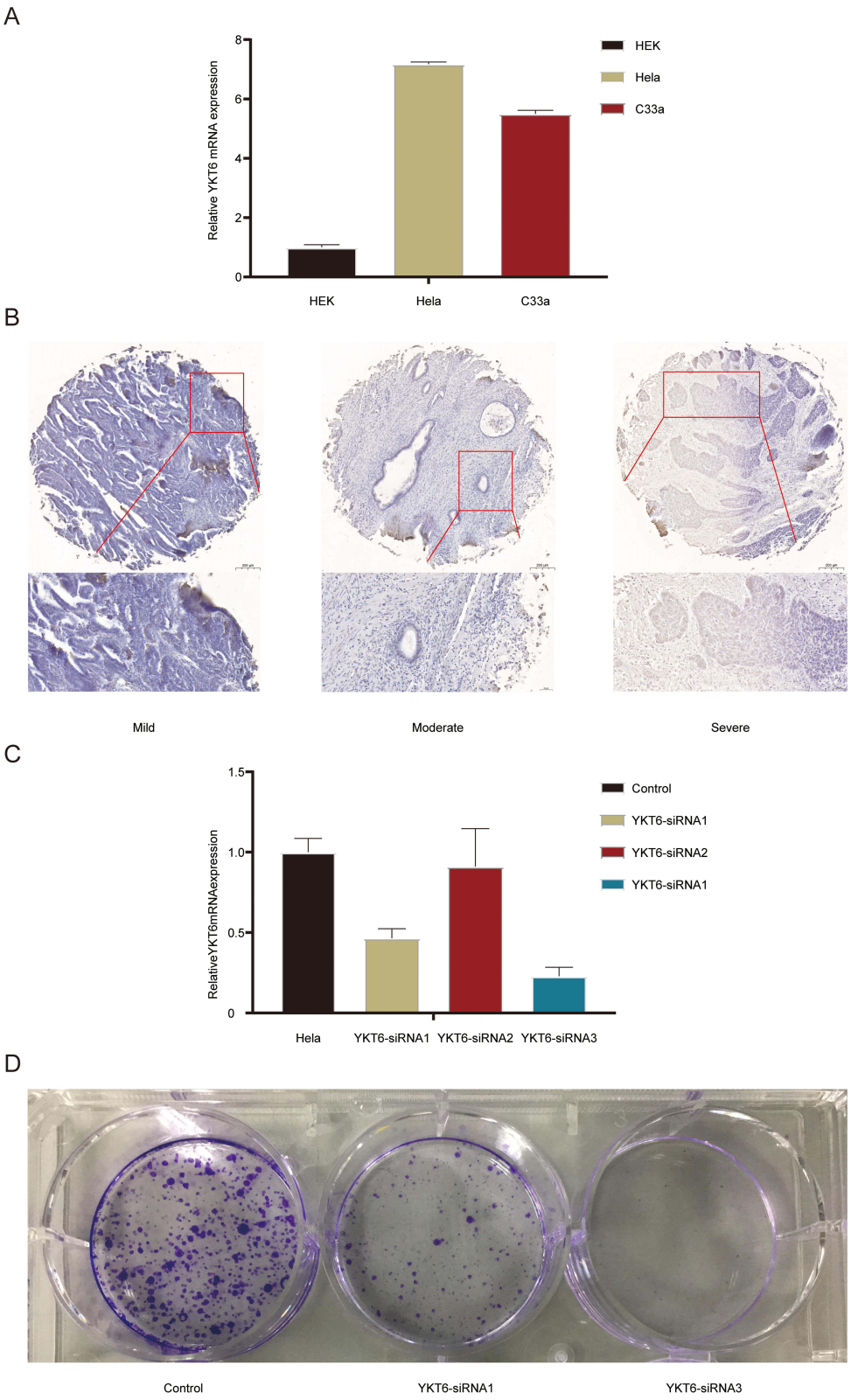

Figure 11 Further validation of YKT6 expression in CESCs: (**A**) qPCR results for the expression of YKT6 in the human cervical cancer cell line and human HEK cells; (**B**) immunohistochemistry showing varying degrees of YKT6 staining, scale bar: 200 μm (upper) and 50 μm (lower); (**C**) qPCR results confirming the knockdown of YKT6 in the human cervical cancer HeLa cell line by siRNA; (**D**) knockdown YKT6 hampered the growth of HeLa cells.

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Table 1 Correlation Analysis Between YKT6 Expression and Clinicopathological Characteristics in 26 Cervical Cancer Patients

Characteristics	Cases, n	YKT6 Expression Level		χ^2	P-Value
		High, n	Low, n		
Patients (N)	26				
Age				0.001	0.97
≤50 years	19	8	11		
>50 years	7	3	4		
Pathology				0.15	0.69
Squamous cell carcinoma	13	5	8		
Adenocarcinoma	13	6	7		
Lymph node metastasis				0.82	0.36
None	23	9	14		
Yes	3	2	1		
Grade				5.11	<0.05*
Low grade	16	4	12		
High grade	10	7	3		
Stage				3.35	0.067
(Tx/T0/T1)	17	5	12		
(T2-T4)	9	6	3		

Notes: Comparisons were considered statistically significant when *p < 0.05.

Discussion

In recent years, convincing data have revealed the vital role of cancer immunotherapy in preventing primary tumor development and progression.[18,19] Consistent with this idea, the killing efficiency and potential of immune cells, such as cytotoxic T cells (CTLs) and natural killer (NK) cells, are often influenced by the TME.[20] The advent of these new immunotherapy approaches has improved survival in patients with advanced malignancy. However, a considerable proportion of patients are nonresponders.[21,22] Autophagy has been demonstrated to be one of the key factors involved in TME construction and the regulation of tumor resistance to immunotherapy.[23] SNARE proteins have been shown to participate in the membrane fusion involved in most steps of autophagy.[24–26] Thus, since SNARE proteins have been identified as key components of autophagy, we explored the role of SNARE proteins in human cancer. Unlike other SNARE proteins implicated in autophagy, YKT6 is highly evolutionarily conserved, and it is the only SNARE protein that is still evolutionarily conserved in species ranging from yeast to mammals.[17,27,28]

Ykt6 is an essential R-SNARE that contains an N-terminal longin domain.[29] As YKT6 lacks a transmembrane domain, it is transiently associated with the membrane via a lipid anchor. This unique feature indicates that YKT6 serves as a nonconventional, regulatory SNARE protein in autophagosome–lysosome fusion.[30,31] YKT6 plays a significant role in the fusion of autophagosomes and lysosomes,[32] but the immunomodulatory-related prognostic biomarkers of YKT6 that can function as immunomodulators in CESCs and pan-cancer still need to be explored in detail.

First, we assessed YKT6 expression via multilevel analysis. We found that YKT6 was highly expressed in 30 of the 33 tumor types. YKT6 was highly expressed in all tumors except for KIRC, LAML, and THCA. Furthermore, increased YKT6 expression was closely associated with the aggressive tumor stage in 12 of the 33 tumor types. In the next step, we analyzed the prognostic significance of YKT6 across cancers via Kaplan–Meier survival analysis by mining the TCGA database. In terms of OS and DFI, according to our findings, high YKT6 expression is associated with longer OS and DFI.

To further determine the substantial differences in high YKT6 and low YKT6 expression, we characterized the landscape of genomic alterations and structural variation in the YKT6 gene across cancers. According to the cBioPortal database, YKT6 amplification was shown to be the most common type of YKT6 gene mutation across cancers. Furthermore, we focused on the immunomodulatory properties of YKT6 across cancers. A negative correlation was observed between the YKT6 expression level and multiple immune cell types in ESCA, HNSC, STAD, THCA, and UCS.

In addition, positive associations between the YKT6 expression level and UVM, SARC, PRAD, KIRP, STAD, and LGG with immune cell types has been observed. Moreover, the correlations between the YKT6 expression level and MSI and TMB indicate that this gene can be regarded as an indicator of immunotherapeutic responses across a variety of cancer types. YKT6 expression was positively related to the expression of immune-stimulating genes, especially in OV, UVM, LIHC, READ, and BLCA. In SKCM, ESCA, LUSC, HNSC and CHOL, YKT6 expression was negatively related to the expression of immune-stimulating genes. Among the immune-inhibitory genes, YKT6 expression was positively correlated with OV, UVM, BLCA, and DLBC. YKT6 expression was negatively correlated with CHOL, SKCM, ESCA, UCS, HNSC, and LUSC.

To further verify the potential role of YKT6 in the treatment of CESCs, further research was conducted on the high/low YKT6 expression mutational profile in CESC patients. Different mutation patterns and signatures reflect specific genetic backgrounds. Moreover, we found that YKT6 is involved in the TGFB1 and TGFBR1 signaling pathways in CESC patients and may mediate the fusion of autophagosomes and lysosomes, affecting the response of CESC patients to immunotherapy.

Furthermore, on the basis of these results, we validated the high YKT6 expression level in cervical cancer cell lines. YKT6 expression was higher in cervical cancer cell lines than in HEK cells. Moreover, an exploratory study of YKT6 expression and clinicopathological characteristics in 26 cervical cancer patients was conducted. We demonstrated that YKT6 was highly expressed in advanced CESCs. YKT6, a key SNARE protein involved in membrane fusion and autophagy, has emerged as a promising cancer immune biomarker, particularly in CESCs. Elevated expression of YKT6 in CESC tissues is correlated with a poor prognosis, indicating its potential as a prognostic marker. In addition to its role in tumor progression, the involvement of YKT6 in modulating the TME and immune cell infiltration further underscores its utility as an immune biomarker. Specifically, high YKT6 expression has been linked to altered immune responses, including the infiltration of tumor-associated immune cells such as T cells and macrophages, suggesting that it plays an immune evasion mechanism role. This dual function—serving both as a marker of immune modulation and a predictor of poor clinical outcomes—positions YKT6 as a valuable target for therapeutic strategies aimed at enhancing immune responses in CESC patients, particularly in the context of immunotherapy. Thus, the potential of YKT6 as an immune biomarker extends beyond its diagnostic value, as it offers a promising avenue for personalized treatment strategies in cervical cancer patients.

This study focused on cervical cancer using HeLa and C33A cell lines, and the experimental design provides credible preliminary evidence for the role of YKT6 in cancer diagnosis. This study provides guidance for subsequent research. This investigation is not without its limitations. Expanding the validation to additional cancer types is an important next step to enhance the generalizability of our findings. Further experiments could expand the novelty of the study. However, we believe that our findings contribute significantly by identifying YKT6 as a potential prognostic biomarker across multiple cancers and providing information its association with immune infiltration and autophagy. Our study also provides a unique focus on cervical cancer, which remains underexplored in terms of autophagy and immune regulation. These results establish a strong foundation for future research into YKT6 as a therapeutic target.

Our study focused primarily on establishing YKT6 as a prognostic marker and its role in autophagy and immune modulation. Future research should aim to validate these findings in additional cancer types and in vivo models. Preclinical studies targeting YKT6 in combination with ICIs or chemotherapies will be critical to translate these insights into clinical applications.[33] Furthermore, structural studies designed with specific inhibitors or modulators of YKT6 could accelerate its development as a therapeutic target. By integrating these therapeutic perspectives, we extend the translational relevance of YKT6 from a prognostic biomarker to a potential target for precision medicine in oncology.

Conclusion

In summary, the results of the pan-cancer analysis provided new insight into the SNARE protein YKT6 as a target gene and candidate oncogene with actionable mutations. Moreover, this study validated the high expression of YKT6 in CESC cell lines and tissues. These results confirm that YKT6 has the potential to be a prognostic indicator in CESCs.

Abbreviations

CESC, cell carcinoma and endocervical adenocarcinoma; TCGA, The Cancer Genome Atlas; CCLE, Cancer Cell Line Encyclopedia; GTEx, Genotype-Tissue Expression; SNARE, Soluble N-ethylmaleimide-sensitive factor attachment protein receptor; CAN, copy number alteration; HPV, human papillomavirus; ICB, immune checkpoint blockade; HEK, human embryonic kidney; KIRC, kidney renal clear cell carcinoma; AML, acute myeloid leukemia; THCA, thyroid carcinoma; GBM, glioblastoma multiforme; CML, chronic myelogenous leukemia; ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; COAD, colon adenocarcinoma; KICH, kidney chromophobe; KIRC, kidney clear cell carcinoma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; SKCM, skin cutaneous melanoma; UCS, uterine carcinosarcoma; OS, overall survival; DFI, disease-free interval;

DSS, disease-free survival; PFI, progression-free interval; CNAs, copy number alterations; TME, tumor microenvironment; TIME, tumor immune microenvironment; MSI, microsatellite instability; TMB, tumor mutation burden; CTLs, cytotoxic T cells; NK, natural killer.

Ethics Approval

The institutional review board of Hospital of Chengdu University of Traditional Chinese Medicine approved the study protocol.

Author Contributions

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

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Disclosure

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

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