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Pan-cancer analysis of ACHE as a biomarker for cancer prognosis and immunotherapy

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ABSTRACT. Background: ACHE belongs to the carboxylesterase family and is also classified as the acylhydrolase superfamily.It plays an important role in many cancers. **Methods:** Using TCGA multi-omics data, we employed R software and various online tools to analyze the differential expression of ACHE in 33 tumor tissues versus normal tissues, survival prognosis, immune infiltration, and potential biological functions. **Results:** ACHE affects the prognosis of multiple cancers and is closely associated with the tumor immune microenvironment and tumor immune cell infiltration. Thus, ACHE may serve as a prognostic biomarker for cancer. **Conclusion:** Comprehensive pan-cancer analysis identifies ACHE as correlated with immune infiltration and cancer prognosis in various tumors, suggesting its potential as a novel immune modulation target, and providing new insights for cancer treatment.
Keywords: ACHE; Pan-cancer; Prognosis; Immune Infiltration; Bioinformatics.

INTRODUCTION

Cancer is a major cause of death worldwide and one of the most common diseases (Bray F et al., 2024). The onset of cancer is occurring at younger ages. Rates are rising among people under 50 in many countries (Teng Y et al., 2024). Scientists have developed several treatments. These include immunotherapy (Emens LA et al., 2024), gene therapy (Cesur-Ergün B, 2023), and epigenetic therapy (Alagheband Y et al., 2022). However, most cancer types lack effective early-detection biomarkers (Qian C et al., 2023). Early detection and screening are essential. They can significantly improve survival rates for nearly all cancer types (Xia C et al., 2023).

Acetylcholine (ACh) is a key neurotransmitter in the central and peripheral nervous systems. It plays a significant role in many biological processes. Researchers have identified the acetylcholine signaling pathway as a critical target in cancer detection and treatment (Friedman JR et al., 2019). Beyond its role in transmitting signals between neurons, ACh functions as a growth factor. It promotes cell growth in various types of cancer. Tumor cells produce large amounts of ACh. This helps them grow and spread rapidly (Song P, 2008).

Acetylcholinesterase protein (AChE) is a serine protease that breaks down acetylcholine (ACh). It is found mainly at the neuromuscular junction and in cholinergic systems (Arikawa-Hirasawa et al., 2002). It has strong hydrolytic activity (Sperling LE et al., 2008; Dvir H et al., 2010). The Acetylcholinesterase gene (ACHE) is located on human chromosome 7q22 (Boberg DR et al., 2013). ACHE belongs to the carboxylesterase family and is also classified as the acylhydrolase superfamily. This region is often altered in cancers such as leukemia, head and neck cancer, lymphoma, lung cancer, and breast cancer (Villacis RA et al., 2017). The gene has six exons and five introns. Alternative splicing of the gene produces three isoforms: AChE-S, AChE-R, and AChE-H. AChE-S is a tetrameric protein. It connects exons 4 and 6 and is highly expressed in apoptotic cells and tissues. AChE-H is a dimeric protein. It links exon 4 and exon 5 and anchors to the erythrocyte membrane. AChE-R is a monomeric, soluble protein. It helps regulate stress and inflammatory responses (Meshorer E, 2006; Jiang H, 2008; Zimmermann M, 2013). AChE is not limited to cholinergic systems. It is expressed in many non-neuronal tissues and cells. Recent research has focused on its role in non-neuronal diseases, including cancer (Richbart SD et al., 2021).

Studies have found that AChE is closely linked to cancer development. Deleting the ACHE increases the amplification frequency of the proto-oncogene ERBB2 in breast cancer (Bernardi CC et al., 2010). AChE is also important in forming apoptosomes and regulating apoptosis (Park SE et al., 2007). Its potential as a biomarker or therapeutic target for cancer remains unclear. More research is needed to understand the specific roles of AChE in different cancer types and stages.

MATERIALS AND METHODS

Data Collection

The TCGA database (https://portal.gdc.cancer.gov) is the largest repository of cancer genomic information. In this study, we used the raw mRNA expression dataset from TCGA. It includes data from 33 cancer types. The full names and abbreviations of these cancer types are listed

in (Supplementary Table S1). We also obtained the clinical data from the University of California, Santa Cruz (UCSC) Cancer Genome Browser (https://tcga.xenahubs.net).

Differential Expression Analysis of ACHE

We used R software (Version 4.3.2) along with the "limma" and "ggpubr" packages to visualize the differential expression of the ACHE in TCGA cancer tissues and adjacent normal tissues. The results are presented as box plots. To confirm these findings, we also used the scCancerExplorer database (https://bianlab.cn/scCancerExplorer). This database offers single-cell genomic, epigenomic (chromatin accessibility and DNA methylation), and transcriptomic data for 50 cancer types (Huang C et al., 2024). $P < 0.05$ was considered statistically significant.

Clinical Significance Analysis of ACHE in Pan-cancer

We analyzed the relationship between ACHE expression and prognosis, including overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI), across 33 cancer types. We used Kaplan-Meier curves for visualization. Box plots helped analyze the link between ACHE and various tumor clinical pathological stages. The clinical correlation results were shown using the "ggplot2" package.

Correlation of ACHE with TMB and MSI

Tumor mutational burden (TMB) is the total number of mutations in the coding regions of the tumor genome. It is an important biomarker for predicting the effectiveness of immunotherapy (Greillier L et al., 2018). Microsatellite instability (MSI) refers to changes in the length of microsatellite repeat sequences in the genome (Hause RJ et al., 2016). We examined the correlation between ACHE expression and TMB/MSI using Spearman's rank correlation coefficient. The results are presented using radar charts.

Correlation Analysis of ACHE and Immune Infiltration

We estimated tumor immune infiltration levels in various cancers using the TIMER tool (https://cistrome.shinyapps.io/timer/) and CIBERSORT (http://cibersort.stanford.edu/). ESTIMATE, a tool for analyzing tumor infiltration by stroma and immune cells (Newman AM et al., 2015), was used to calculate the stroma score and immune score for each case. We applied the CIBERSORT method to study the relationship between ACHE expression and the infiltration of 22 immune cell types. The "limma" package was used to analyze the common expression of ACHE and immune-related genes across different cancer types.

Gene Co-expression and Functional Enrichment Analysis

The "limma" package analyzed the co-expression of ACHE and immune-related genes across different cancers. Gene set enrichment analysis (GSEA) was conducted using the "ClusterProfiler" package. This helped predict the biological pathways and mechanisms linked to ACHE in various cancers.

RESULTS

Differential expression of ACHE in pan-cancer

We studied the expression of ACHE in various cancers. We found significant differences in ACHE levels between tumor tissues and normal tissues in 16 types of cancer. In BRCA, COAD, GBM, HNSC, KICH, LUSC, READ, THCA, and UCEC, AChE expression was lower in tumor tissues than in paired normal tissues ($P < 0.05$). In contrast, ACHE expression was higher in CHOL, ESCA, KIRC, KIRP, LIHC, LUAD, and PCPG compared to normal tissues ($P < 0.05$) (Figure 1A, B, C).

Prognostic value and clinicopathological staging correlation of ACHE in pan-cancer patients

We used univariate Cox regression and Kaplan-Meier survival analysis to examine the prognostic value of ACHE in pan-cancer, focusing on OS, DSS, DFI, and PFI. The univariate Cox regression showed a clear link between ACHE expression and survival in pan-cancer patients. The forest plot displayed that ACHE expression was significantly associated with survival in the following cancers: GBM (OS: HR=1.294, P=0.023; DSS: HR=1.310, P=0.028), KIRC (OS: HR=1.588, P<0.001; DSS: HR=1.682, P<0.001; PFI: HR=1.463, P<0.001), MESO (OS: HR=1.469, P=0.019), THCA (OS: HR=2.922, P=0.005; DSS: HR=4.704, P<0.001), UCEC (OS: HR=1.346, P=0.047; DSS: HR=1.545, P=0.004; PFI: HR=1.285, P=0.049), THYM (DSS: HR=8.506, P=0.030), ACC (DFI: HR=2.095, P=0.004; PFI: HR=1.564, P=0.002), and HNSC (DFI: HR=1.574, P=0.008). In these cancers, ACHE was a risk factor that negatively impacted prognosis. In contrast, high ACHE expression was linked to better survival in SKCM, LUAD, and DLBC. SKCM (OS: HR=0.753, P=0.009; DSS: HR=0.748, P=0.015), LUAD (DSS: HR=0.827, P=0.019), and DLBC (PFI: HR=0.407, P=0.019). $P < 0.05$ means that ACHE expression is significantly related to the prognosis of these types of cancers, HR>1 means that ACHE expression is a promoting factor of death (Figure 2A-D).

Kaplan-Meier survival curves further confirmed the association of ACHE expression with OS and DSS. High ACHE expression was associated with poor prognosis in cancers like GBM (OS: P=0.034; DSS: P=0.037), ACC (OS: P=0.044; DSS: P=0.033), UCEC (OS: P=0.023; DSS: P=0.029), UVM (OS: P=0.006; DSS: P=0.004), KIRC (OS: P<0.001; DSS: P<0.001), MESO (OS: P=0.004), THCA (DSS: P=0.010), and THYM (DSS: P=0.035). On the other hand, high ACHE expression in DLBC (OS: P=0.014; DSS: P=0.025), SKCM (OS: P=0.004; DSS: P=0.011), and LUAD (DSS: P=0.014) was associated with longer survival (Figure 3A-H, Figure 4A-J).

We also analyzed ACHE expression across different cancer stages. This analysis showed that ACHE expression was linked to the clinical stage in ACC, ESCA, HNSC, KIRC, KIRP, LUAD, MESO, and TGCT. (Figure 5A-H)

Correlation Between AChE Expression and TMB, MSI

TMB and MSI are important indicators of tumor immunity and immunotherapy efficacy. ACHE expression was positively correlated with TMB in BLCA, UCS, PRAD, LUSC, and LUAD ($P < 0.05$) and negatively correlated in READ ($P < 0.05$). ACHE expression was positively correlated with MSI in ACC, THYM, LAML, KIRP, KIRC, and ESCA ($P < 0.05$) but negatively correlated in BLCA, THCA, SKCM, SARC, LUAD, HNSC, and COAD ($P < 0.05$) (Figure 6A-B).

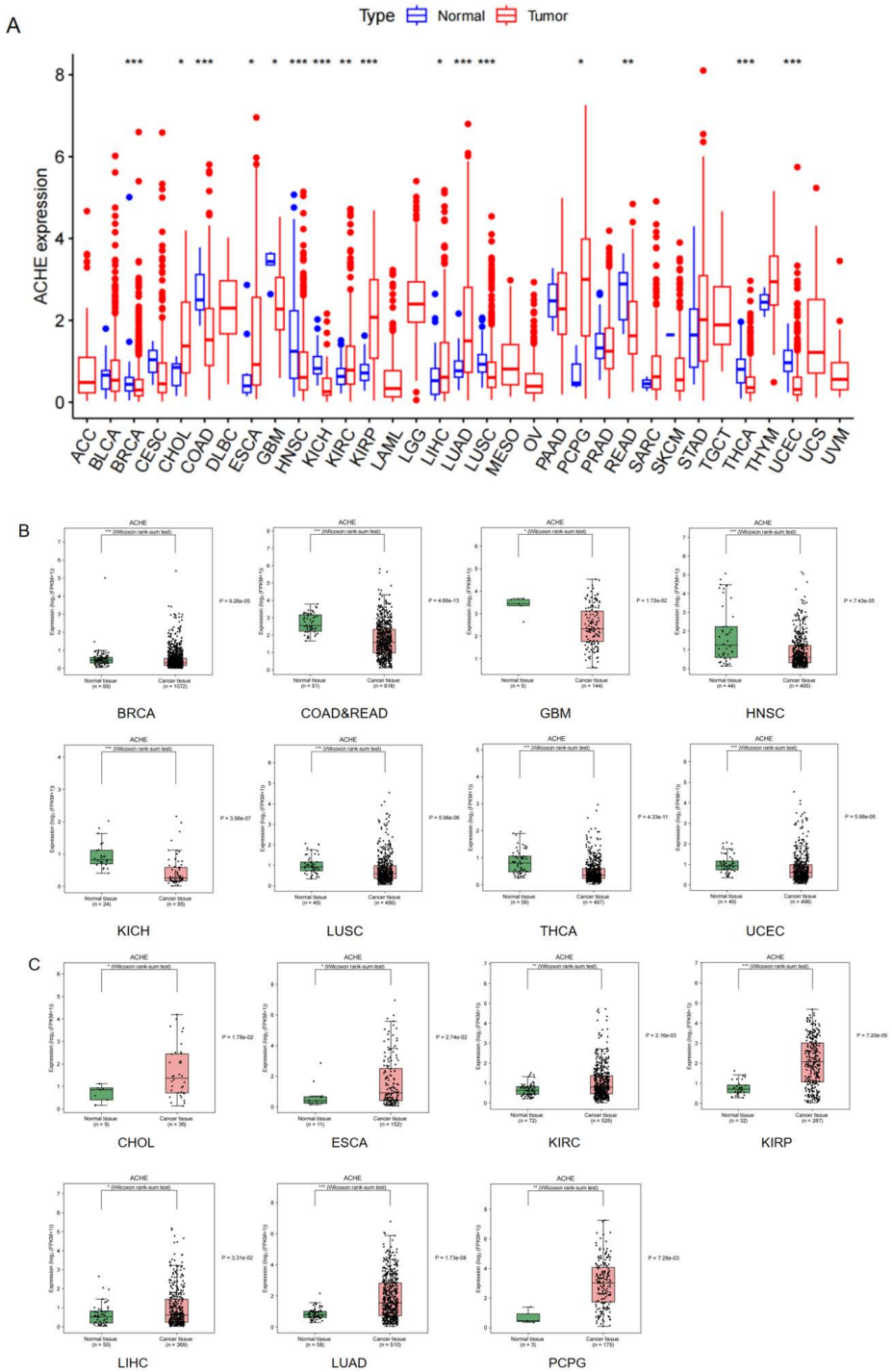


Figure 1. Differential expression of ACHE in pan-cancer (*P<0.05, **P<0.01, ***P<0.001).

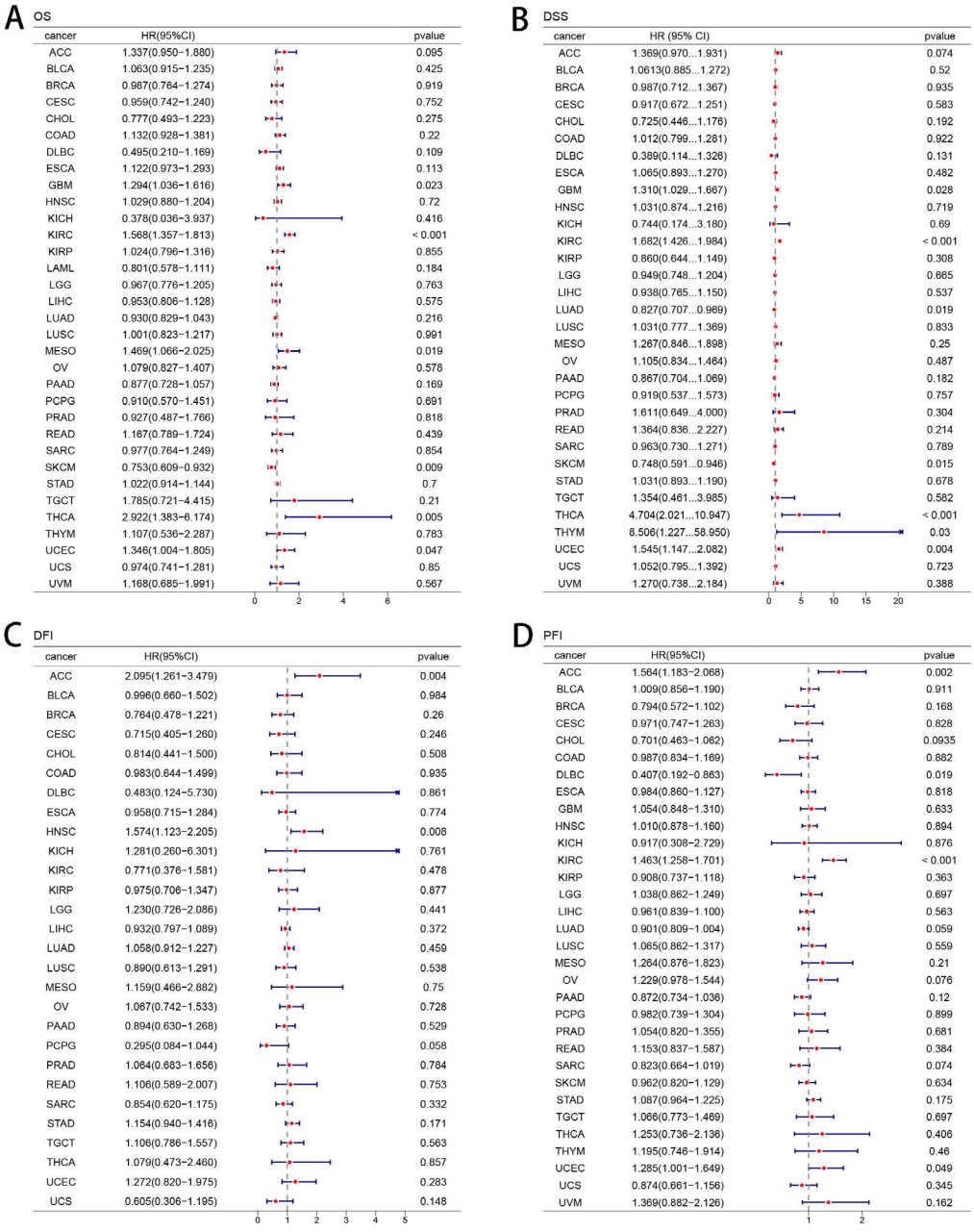


Figure 2. Forest plot of univariate COX regression analysis of OS, DSS, DFI and PFI of ACHE in pan-cancer

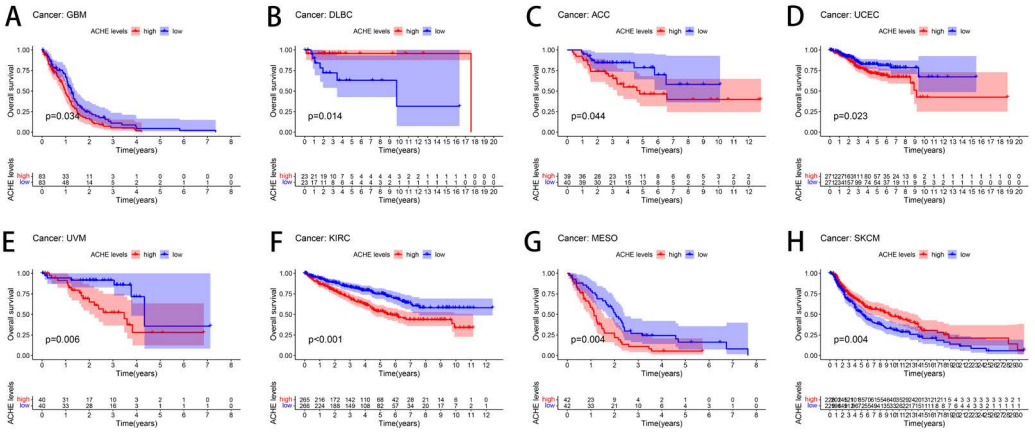


Figure 3. Kaplan-Meier survival curves of OS of ACHE in pan-cancer.

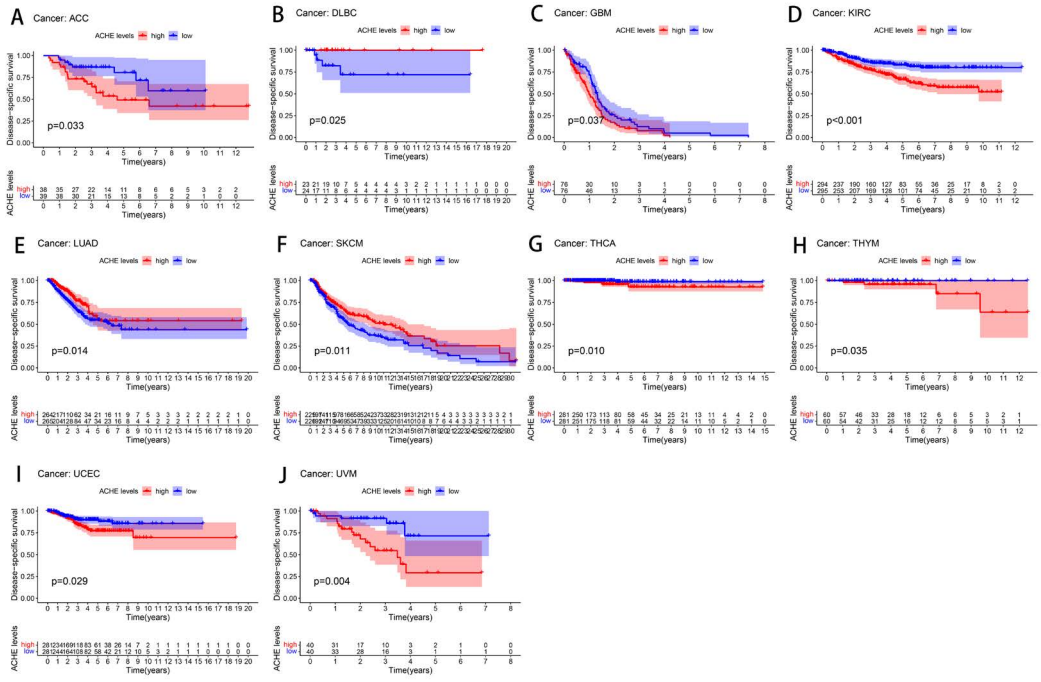


Figure 4. Kaplan-Meier survival curves of DSS of ACHE in pan-cancer.

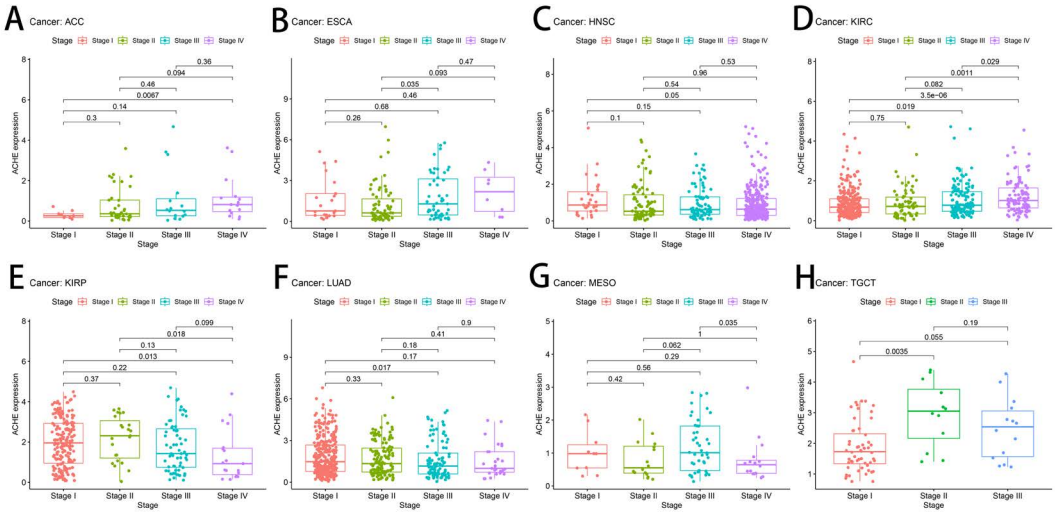


Figure 5. Relationship of ACHE expression to clinicopathologic stage.

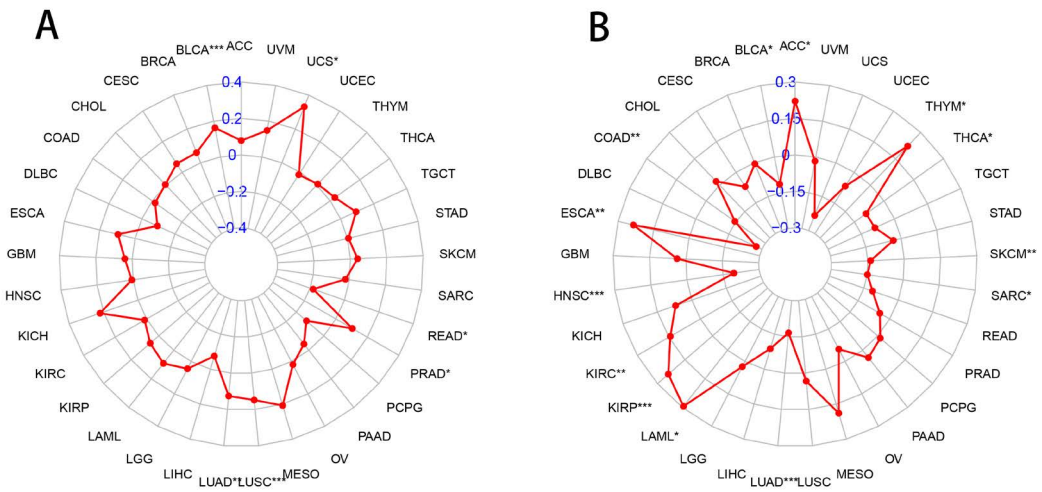


Figure 6. Relationship of ACHE expression to TMB and MSI (*P<0.05, **P<0.01, ***P<0.001).

AChE Expression and Immune Infiltration

We used the ESTIMATE algorithm to score immune and stromal components across 33 cancer types. We then examined the relationship between ACHE expression and these scores. We found a significant correlation between ACHE expression and immune/stromal scores. In cancers like BRCA, CESC, ESCA, HNSC, KIRC, LIHC, LUSC, PRAD, SKCM, UCEC, OV, THCA, and UVM, ACHE expression showed positive correlations with immune/stromal scores. In ACC, however, the immune score was negatively correlated (Figure 7A-U). This suggests that ACHE plays a role in regulating the tumor microenvironment, particularly in immune cell infiltration.

We performed CIBERSORT analysis and found that ACHE expression was strongly linked to the infiltration of immune cells. This includes B cells, CD4+ T cells, CD8+ T cells, regulatory T cells, NK cells, monocytes, macrophages, and neutrophils. The correlation was found in cancers such as BLCA, BRCA, ESCA, HNSC, KIRC, KIRP, LAML, LGG, LIHC, LUAD, LUSC, PRAD, SARC, SKCM, TGCT, THCA, THYM, and UCEC. Additionally, ACHE expression was negatively correlated with M2 macrophages in BRCA, KIRC, SARC, SKCM, and THCA (Figure 8).

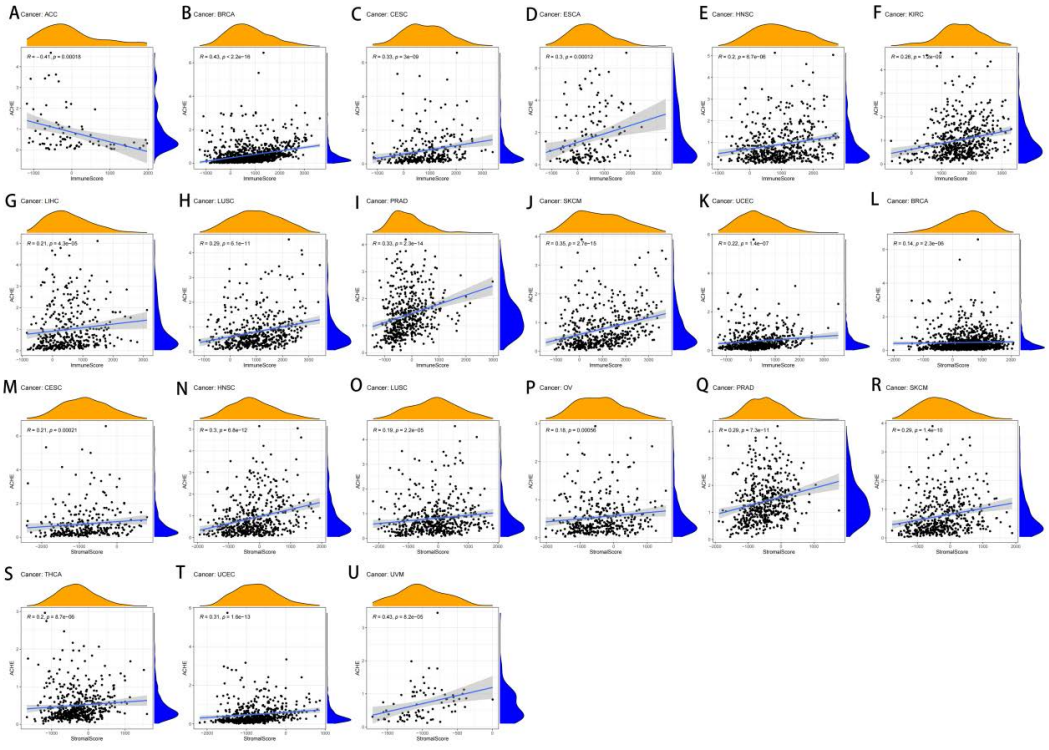
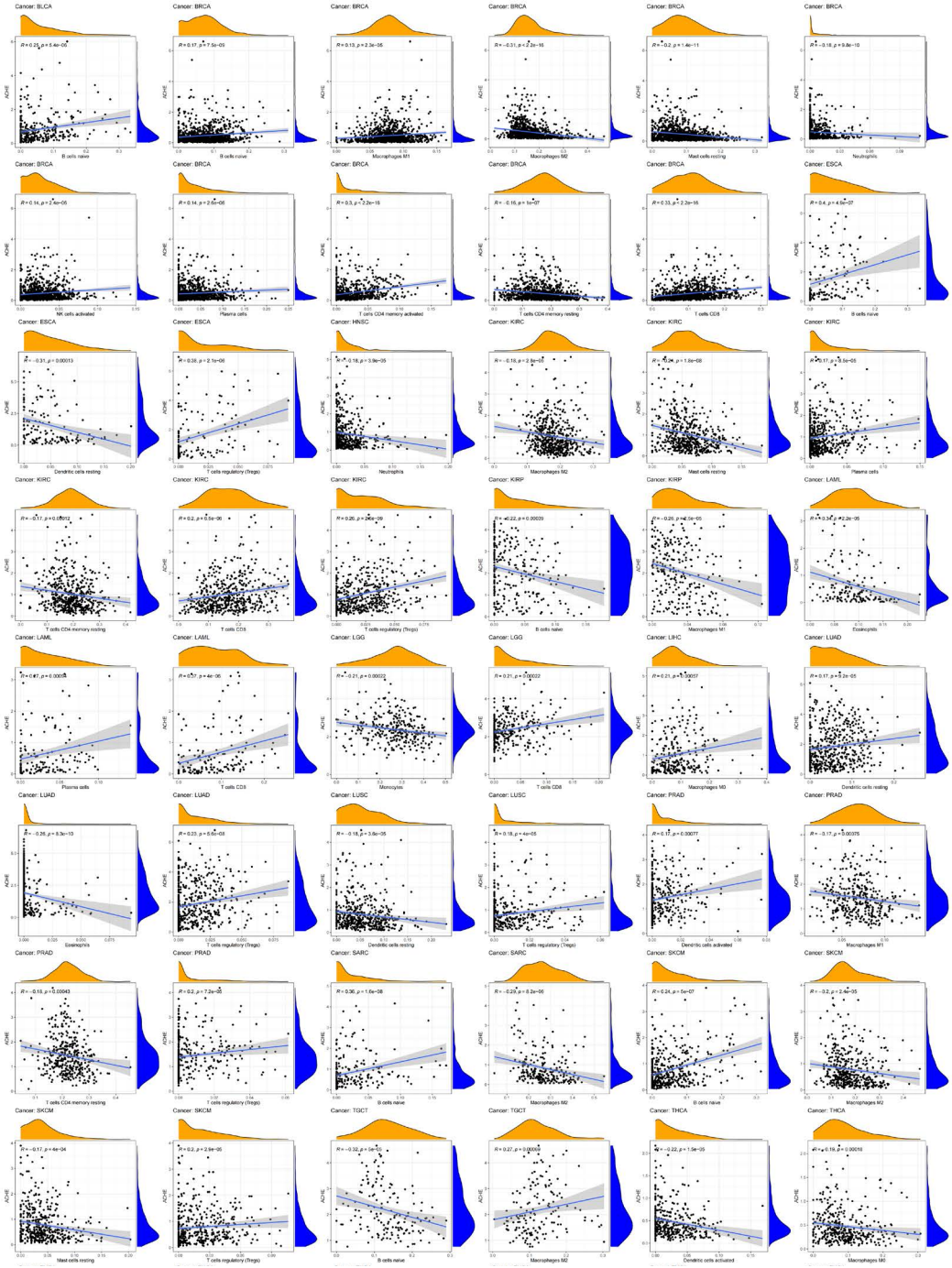


Figure 7. Correlation between ACHE expression and tumor immune microenvironment (A-K. Immune score, L-U.Stromal score).



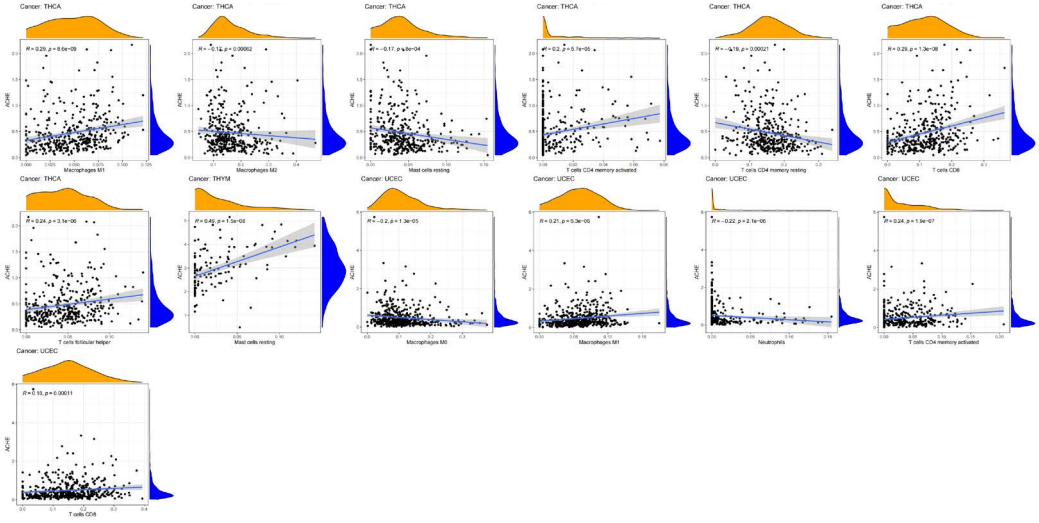


Figure 8. Correlation between ACHE expression and immune cell infiltration.

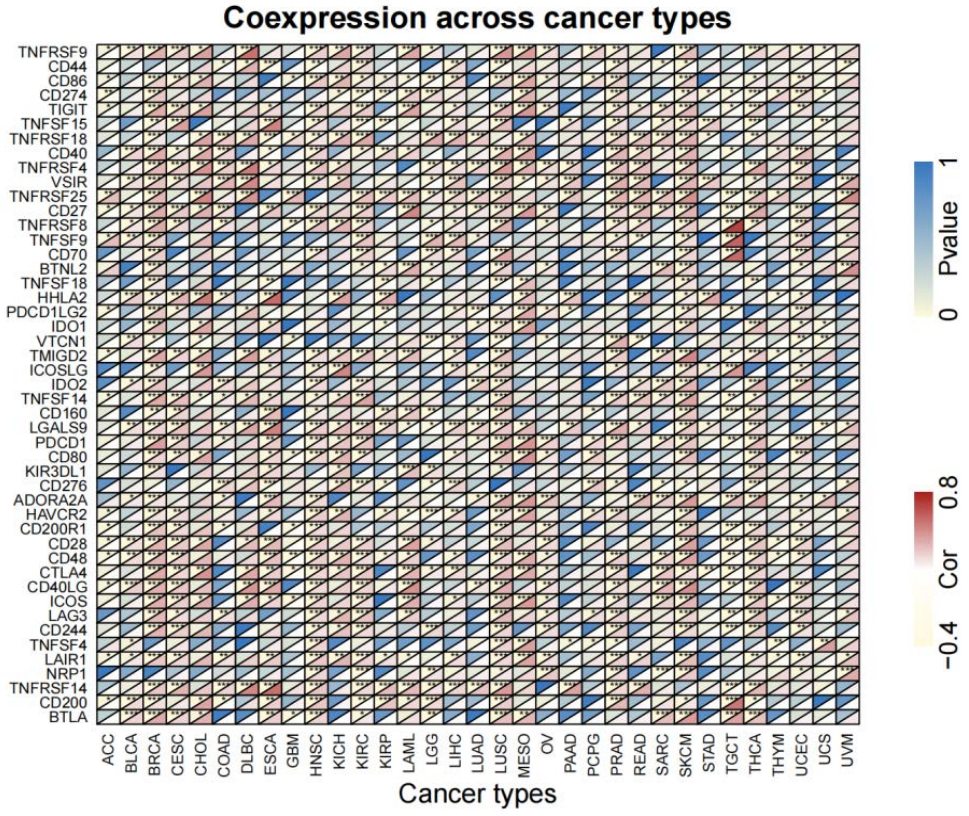


Figure 9. Heat map of co-expression of ACHE with immune-related genes.

We also conducted a gene co-expression analysis for 33 cancers. We looked at ACHE in relation to 47 immune-related genes, including BTLA, CD200, and TNFRSF14. Almost all immune-related genes showed co-expression with ACHE (Figure 9). This supports the idea that ACHE is key in regulating the immune gene network.

Pan-cancer AChE Enrichment Analysis

We performed gene set enrichment analysis (GSEA) to explore the biological functions of ACHE in tumors. In the gene enrichment analysis of ACC, KIRC, UVM, DLBC, and LUAD, we found that ACHE may play a role in immune regulation, gene expression, and antigen recognition in ACC. In KIRC, ACHE may influence angiogenesis, immune evasion, gene regulation, and cell movement. In UVM, ACHE is involved in immune evasion, signal transmission, and visual regulation. In DLBC, ACHE regulates interleukin-6 signaling, negatively affects vascular endothelial growth factor receptors, and positively regulates cellular senescence and the G1/S transition in the mitotic cell cycle. In LUAD, ACHE impacts mesenchymal stem cell differentiation, cardiomyocyte differentiation, protein localization to the cell surface, and complement activation (Figure 10).

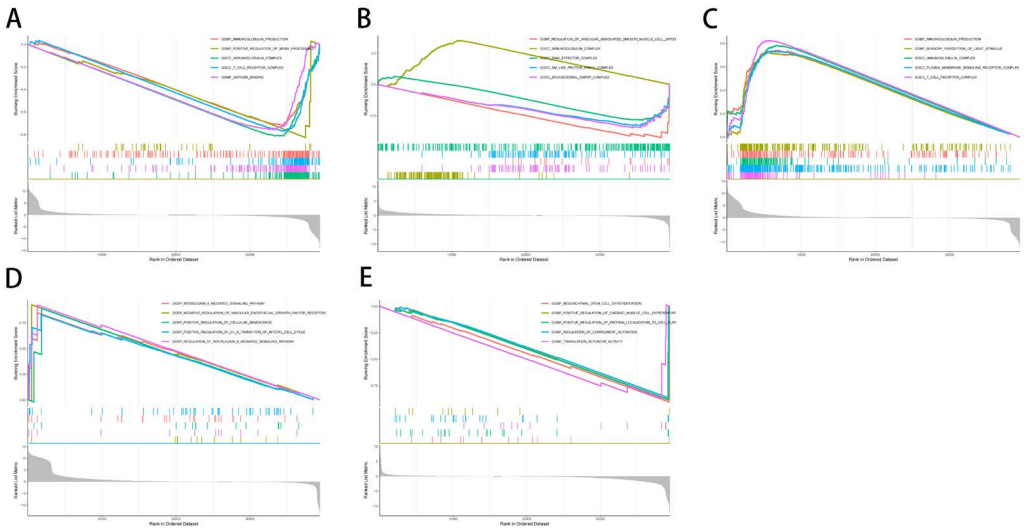


Figure 10. GSEA enrichment analysis of ACHE (A-E .KEGG analysis of ACC, KIRC, UVM, DLBC, LUAD).

DISCUSSION

Bioinformatics analysis of the TCGA cancer database revealed differences in AChE expression across various cancers. We also found variations in survival curves and clinical staging distributions. However, ACHE expression did not show a consistent pattern between normal and cancer tissues. In cancers such as BRCA, COAD, GBM, HNSC, KICH, LUSC, READ, THCA, and UCEC, ACHE may function as a tumor suppressor. Its expression is lower in tumor cells than in normal cells. In CHOL, ESCA, KIRC, KIRP, LIHC, LUAD, and PCPG, ACHE may act as an oncogene. Its expression is higher in tumor tissues compared to nearby normal tissues.

Studies have shown that in HNSC and laryngeal squamous cell carcinoma (LSCC), AChE enzyme activity is lower than in normal tissues. Patients with higher ACHE activity in LSCC tend to have longer survival. Low ACHE activity serves as an independent marker for clinical staging and lymph node status in HNSC and is also associated with poorer survival in HNSC patients (Castillo-Gonzalez AC et al., 2015). In COAD, AChE-H and AChE-S levels are much lower compared to normal colon tissues (Syed M et al., 2008). Our analysis corroborates these findings in HNSC and COAD. Immunohistochemistry shows that AChE expression in STAD tissues is lower than in adjacent normal tissues. Patients with higher AChE expression in STAD show improved survival (Xu H et al., 2014). In LIHC, immunohistochemical staining reveals that AChE expression is lower than in normal liver tissue. Higher AChE expression in LIHC is linked to better overall survival and disease-free survival (Zhao Y et al., 2011). These studies suggest that high ACHE expression may inhibit tumor growth. However, our study did not show differences in ACHE expression in STAD or its effect on prognosis. The bioinformatics analysis of LIHC also contradicted the immunohistochemical findings. Previous Research has shown that ACHE activity does not always match AChE protein levels. For example, in lung cancer cell lines, AChE expression is higher. However, much of it lacks catalytic activity, reducing overall AChE activity (Xi H et al., 2015). Despite these conflicting results, the overall pattern of ACHE expression and its relationship with cancer survival aligns with current research.

Some studies suggest that ACHE may stimulate tumor growth and progression. For instance, AChE activity is higher in human leukemia than in normal peripheral blood (Battisti V et al., 2009). The AChE-S isoform increased colony size in soft agar assays. It also promoted COAD cell adhesion to fibronectin (Syed M et al., 2008). The AChE-R isoform interacts with the protein RACK1 and PKCε. This interaction promotes glioma proliferation (Perry C et al., 2004). These findings suggest that ACHE could act as an oncogene. However, the role of acetylcholinesterase depends on factors like cell type, differentiation, biological half-life, and the binding partners of AChE isoforms (Richbart SD et al., 2021). Changes in ACHE levels in cancer tissues, compared to normal tissues, show its complex role in tumors. Previous studies have not detected ACHE expression in KICH, THCA, UCEC, CHOL, ESCA, KIRC, KIRP, or PCPG. This suggests that ACHE may serve as a diagnostic biomarker for these cancers.

Immunotherapy offers a promising approach to cancer treatment. However, only some patients experience positive results. This is due to immune resistance and immune-related side effects. Research indicates that the interaction between immune cells in tumors and cancer cells is crucial for cancer progression (Zhang Y, 2020). The tumor microenvironment (TME) is closely tied to patient prognosis (Chan T et al., 2019). It is also considered a potential biomarker for immunotherapy (Conway JR et al., 2018). AChE, traditionally known for its role in the nervous system, also influences tumor immune escape, cell growth, TME regulation, and protein production (Milanković V et al., 2025).

The relationship between ACHE and TMB/MSI provides valuable insights into its role in tumor immunity. TMB and MSI are critical biomarkers for predicting the efficacy of immune checkpoint inhibitors (ICIs) (Klempner SJ et al., 2020). ACHE showed a positive correlation with TMB in BLCA, UCS, PRAD, LUSC, and LUAD. This suggests that high ACHE expression may increase the mutational burden in these cancers. As a result, it could improve antigenicity and enhance the potential for immunotherapy. However, in READ, the correlation was negative. This may imply that ACHE plays a more complex role in different cancers. ACHE was also positively correlated with MSI in ACC, THYM, and LAML. In contrast, it was negatively correlated with BLCA, LUAD,

and other cancers. This two-way relationship highlights the complex effects of ACHE on immune responses. The immune microenvironment in the TME has two roles in tumor development. It can kill tumor cells through immune responses. On the other hand, immune escape mechanisms help tumor cells grow more quickly, which impacts the success of drug treatments. Immune checkpoint inhibitors enhance immune cells' ability to target and destroy tumor cells tumor cells. They show broad potential for treating various cancers (Wang Y et al., 2022).

The correlation between ACHE and immune infiltration underscores its critical role in modulating the tumor microenvironment (TME). Studies have demonstrated that patients with higher immune scores generally exhibit longer survival (Li X et al., 2019). Our analysis identified a strong association between ACHE expression and immune cell infiltration across various cancers. For instance, in cancers such as BRCA and KIRC, ACHE expression was positively correlated with immune and stromal scores, suggesting its potential role in recruiting and activating immune cells to support tumor immune surveillance. Conversely, in ACC, ACHE expression was negatively associated with immune scores, indicating its involvement in dampening immune responses and facilitating immune escape. Furthermore, CIBERSORT analysis revealed significant correlations between ACHE expression and levels of various immune cells, including CD8+ T cells, NK cells, and macrophages. Notably, a negative correlation with M2 macrophages suggests that ACHE may contribute to establishing an immunosuppressive TME.

ACHE is broadly co-expressed with immune-related genes, underscoring its pivotal role in regulating the immune gene network. Notably, it is co-expressed with immune suppressive molecules such as BTLA and CD200, suggesting that ACHE may modulate immune suppression and activation through diverse signaling pathways. Gene Set Enrichment Analysis (GSEA) indicates that ACHE is enriched in multiple cancer types, supporting its involvement in immune escape, antigen recognition, and gene regulation. Bioinformatics analysis further reveals that ACHE plays a multifaceted role in the tumor immune microenvironment (TME), with associations to tumor mutational burden (TMB), microsatellite instability (MSI), immune cell infiltration, and immune-related gene networks. These findings emphasize ACHE’s potential as a promising target for cancer immunotherapy.

ACHE expression is closely associated with prognosis, the tumor immune microenvironment, and immune cell infiltration across various cancers. ACHE plays a dual role in cancer, with its complex mechanisms suggesting potential utility in cancer immunotherapy. However, current findings remain largely theoretical and require further validation through cellular, tissue, and animal studies. Future research should prioritize elucidating how ACHE modulates immune pathways and assess its viability as a target for cancer immunotherapy. Such efforts could pave the way for innovative strategies and improved clinical guidance in cancer treatment.

AUTHOR CONTRIBUTIONS

The study was conceived and designed by TL, QH, and HT. TL and QH conducted the experiments and analyzed the data, while TL and HT prepared the initial draft of the manuscript. HX, KL, ZS, CB, ZC, XY, and YT provided critical revisions. All authors reviewed and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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DATA AVAILABILITY STATEMENT

All human data utilized in this study are publicly accessible. Additional data and materials are available upon reasonable request from the corresponding authors.

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Supplementary Table S1

Table S1. List of the full names and abbreviations of 33 human cancers.

Cancer Name (English)	Abbreviation
Adrenocortical Carcinoma	ACC
Bladder Urothelial Carcinoma	BLCA
Breast Cancer	BRCA
Cervical Squamous Cell Carcinoma and Endocervical Adenocarcinoma	CESC
Cholangiocarcinoma	CHOL
Colon Adenocarcinoma	COAD
Diffuse Large B-cell Lymphoma	DLBC
Esophageal Carcinoma	ESCA
Glioblastoma Multiforme	GBM
Head and Neck Squamous Cell Carcinoma	HNSC
Kidney Chromophobe	KICH
Kidney Renal Clear Cell Carcinoma	KIRC
Kidney Renal Papillary Cell Carcinoma	KIRP
Acute Myeloid Leukemia	LAML
Brain Lower Grade Glioma	LGG
Liver Hepatocellular Carcinoma	LIHC
Lung Adenocarcinoma	LUAD
Lung Squamous Cell Carcinoma	LUSC
Mesothelioma	MESO
Ovarian Serous Cystadenocarcinoma	OV
Pancreatic Adenocarcinoma	PAAD
Pheochromocytoma and Paraganglioma	PCPG
Prostate Adenocarcinoma	PRAD
Rectum Adenocarcinoma	READ
Sarcoma	SARC
Skin Cutaneous Melanoma	SKCM
Stomach Adenocarcinoma	STAD
Testicular Germ Cell Tumors	TGCT
Thyroid Carcinoma	THCA
Thymoma	THYM
Uterine Corpus Endometrial Carcinoma	UCEC
Uterine Carcinosarcoma	UCS
Uveal Melanoma	UVM