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Original Article

Pan-analysis reveals CACYBP to be a novel prognostic and predictive marker for multiple cancers

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Abstract: Objectives: Cancer has emerged as a global issue in terms of public health care and treatment. The significance of calcyclin binding protein (*CACYBP*) in various neoplasms suggests that it may serve as a novel biomarker for numerous types of human tumors. Methods: Our research investigated the differences in *CACYBP* expression between cancer tissues and normal tissues using a total of 18,787 samples from multiple centers. To explore the prognostic factor of *CACYBP* in cancers, we utilized Cox regression analysis and Kaplan-Meier curves. We also conducted Spearman's rank correlation analyses to determine the associations of *CACYBP* expression with the immune microenvironment, etc. Additionally, we applied gene set enrichment analysis to explore the underlying mechanisms of *CACYBP* in cancers. A partial validation of CacyBP expression in cancer tissues was performed through lung adenocarcinoma samples using Western blotting and paired *t*-test. Results: Compared to normal tissues, *CACYBP* exhibited high expression levels in 14 cancer types, including breast invasive carcinoma, and low expression levels in six cancers, including glioblastoma multiforme ($P < 0.05$). *CACYBP* expression was found to be significantly associated with the prognosis of 13 cancers, including adrenocortical carcinoma ($P < 0.05$). *CACYBP* demonstrated a robust ability to distinguish 15 cancers, including cholangiocarcinoma, from their control samples (area under the curve > 0.8). Furthermore, *CACYBP* expression was correlated with tumor mutational burden, microsatellite instability, and immune infiltration levels, indicating its potential as an exciting target for cancer treatment. *CACYBP* may exert its effects on several signaling pathways, including cytokine-cytokine receptor interaction, in various cancers. Compared with paired adjacent specimens, the expression level of CacyBP protein was up-regulated in lung adenocarcinoma specimens ($P < 0.05$), partially validating the increased expression of *CACYBP* in cancers. Conclusions: *CACYBP* has the potential to serve as a novel prognostic and predictive marker for multiple human cancers.

Keywords: Tumor, prognosis, prediction, immunology, biomarker

Introduction

Cancer has emerged as a global issue in public health care and treatment. According to estimated data in 2020, the annual number of newly diagnosed cancer cases exceeded 18.1 million, while the number of cancer-related deaths reached 9.6 million around the world [1]. There exist various conventional approaches to treating cancer, such as surgical intervention, radiation therapy, and chemotherapy. Molecular targeted therapy and immunotherapy have been gradually attracting the attention of clinical practitioners [2, 3]. However, for most tumors, there is still a lack of biomarkers that can accurately predict patient prognosis and cancer status. Therefore, exploring such novel biomarkers suitable for multiple cancers is likely to benefit cancer patients.

The human calcyclin binding protein (CacyBP) was encoded by the gene *CACYBP*. CacyBP is a highly conserved protein with distinct biological functions, and thus plays a significant role in regulating calcium ion signal transduction, cell proliferation, and apoptosis in cells [4, 5]. Based on this, the relationship between CacyBP and the onset and progression of diverse diseases, particularly tumors, has been documented. In rectum adenocarcinoma (READ),

CacyBP levels are elevated in cancer cells but remain undetected in the normal colonic epithelium; the protein also promotes the proliferation of colorectal cancer cells [6]. In the context of non-small-cell lung cancer (NSCLC), the expression of CacyBP in cancerous tissue is notably elevated, compared to healthy tissue. Furthermore, this protein has been shown to stimulate the proliferation and invasion of NSCLC cells through the regulation of Akt signaling pathway [7]. Therefore, *CACYBP* has been identified as a crucial factor in the progression and growth of diverse malignancies, underscoring its potential as a promising therapeutic target for effective cancer management. However, a comprehensive evaluation of *CACYBP* across multiple types of cancer has yet to be conducted, thereby necessitating further investigation.

Using a large sample size, this study comprehensively investigates *CACYBP* expression and its clinical value in human cancers. In addition, it explores the correlation between *CACYBP* and immune filtration levels as well as the underlying mechanisms of *CACYBP* in tumors, thereby enhancing the understanding of *CACYBP* as a novel prognostic and predictive marker for human neoplasms.

Materials and methods

Collection of public CACYBP mRNA expression, CACYBP protein level, and clinical characteristics data

Transcriptome data to evaluate *CACYBP* mRNA expression in normal tissues (including 8671 samples) were obtained from the Genotype-Tissue Expression database [8], which contains a multitude of samples from *Homo sapiens*. The Xena database provided access to *CACYBP* mRNA expression and clinical information for 33 types of cancer from the Cancer Genome Atlas, and the data from 10,080 samples (n = 9358 cancer samples, n = 722 control samples) were collected for this study. The mRNA expression levels were subjected to $\log_2 (x + 1)$ transformation using R (v4.2.2). Data on immunohistochemical staining were obtained from The Human Protein Atlas [9] to detect CacyBP protein levels in cancer and normal tissues. A total of 30 specimens, consisting of 15 cancer tissue specimens and 15 normal tissue specimens, were collected from this database for further analysis. The Xena database was utilized to retrieve the American Joint Committee on Cancer (AJCC) stage, age, gender, overall survival (OS), disease-specific survival (DSS), disease-free interval (DFI), and progression-free interval (PFI) of individuals with cancer.

Collection of tumor mutational burden, microsatellite instability, neoantigen count, and immune microenvironment data

Data on tumor mutational burden (TMB), microsatellite instability (MSI), and neoantigen count for multiple cancers were applied in this research. The TIMER [10, 11] algorithm can predict the immune abundance of B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells for cancer patients. The ESTIMATE [12] algorithm is recognized for its ability to detect immune abundance through the following three score categories: stromal (on stromal cells), immune (on immune cells), and ESTIMATE scores (on tumor purity). The TIMER algorithm data were accessible through the official TIMER website, while the ESTIMATE algorithm data were obtained from Sanger Box (version 3.0) [13].

Signaling pathways that may be affected by CACYBP

The Kyoto Encyclopedia of Genes and Genomes (KEGG) database [14, 15] provides information about multiple signaling pathways. The study utilized the "clusterProfiler" package [16] to investigate the potential KEGG signaling pathways of *CACYBP* in 33 cancers through gene set enrichment analysis. Those signaling pathways with a p-value of < 0.05 were selected in this study.

Collection of internal samples and use of Western blotting

The six samples with pathological confirmation collected in this study (three cases of lung adenocarcinoma [LUAD] and their corresponding adjacent non-cancerous tissues) were all from the 923 Hospital of Joint Logistics Support Force of Chinese People's Liberation Army. The basic clinical information of the LUAD and the adjacent non-cancerous tissue samples can be found in Supplementary Material 1.

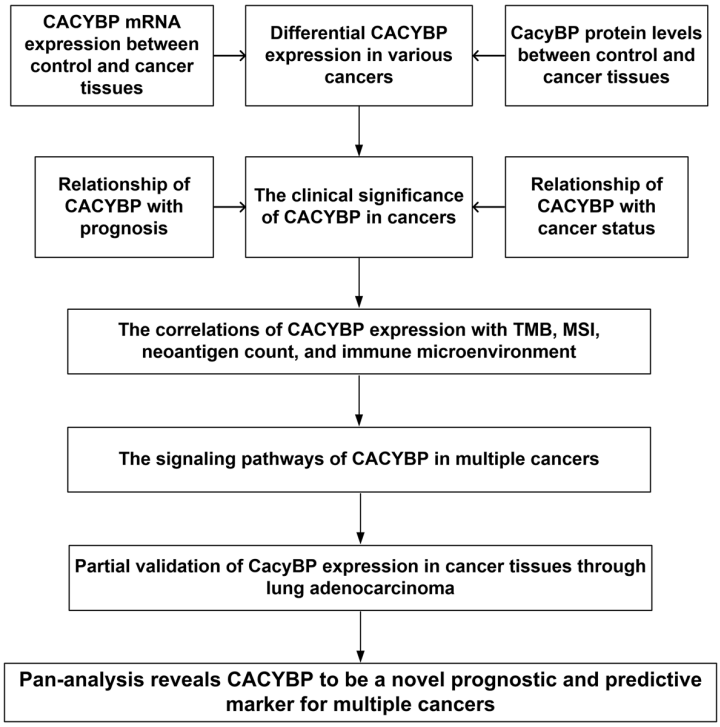

Figure 1. A detailed overview of the study. CACYBP, calcyclin binding protein. TMB, tumor mutational burden. MSI, microsatellite instability.

Western blotting was used to validate the protein expression levels of CacyBP in the LUAD tissues and the adjacent non-cancerous tissues. Samples were treated with radio immunoprecipitation assay lysis buffer. After sodium dodecyl sulfate polyacrylamide gel electrophoresis, proteins were transferred onto a polyvinylidene fluoride membrane (Servicebio, Wuhan, China). The membrane was then blocked with 5% milk at room temperature (approximately 20°C) for 30 minutes, followed by overnight incubation at 4°C with a primary antibody. The primary antibody used was an anti-CacyBP antibody (11745-1-AP, Proteintech, Wuhan, China) diluted at a ratio of 1:3000. Afterward, the membrane was incubated with a secondary antibody (GB23303, Servicebio, Wuhan, China) diluted at a ratio of 1:5000 at room temperature for 30 minutes. Chemiluminescence assay was performed on the washed polyvinylidene fluoride membrane using an enhanced chemiluminescence kit (Servicebio, Wuhan, China). The exposed original images were analyzed and the grayscale values were outputted using AIWBwell™ software (Servicebio, Wuhan, China). The ratio of the grayscale value of CacyBP to the grayscale value of the internal control (α-tubulin, ab7291, ABCAM) represented the relative expression level of CacyBP protein.

Statistical analysis

To assess the disparity in *CACYBP* expression across distinct normal tissues, the Kruskal-Wallis test was employed. The Wilcoxon rank-sum test was utilized to investigate the relevance of *CACYBP* expression to the ages, genders, and AJCC stages of individuals with neoplasms. To determine the relevance of *CACYBP* expression to the prognosis of cancer patients, univariate Cox regression analysis and Kaplan-Meier plots were conducted utilizing the "survival" and "forestplot" packages. The optimal cutpoint for high- and low-*CACYBP* expression levels in each Kaplan-Meier curve was evaluated using the "maxstat" and "survminer" packages.

Using the "pROC" package [17] and Stata (v15.0), the area under the curve (AUC) of the receiver operating characteristics (ROC) curve and a summary ROC curve were calculated to assess the accuracy of *CACYBP* expression in distinguishing between cancers and controls. To assess the associations between *CACYBP* expression and TMB, MSI, neoantigen, and the immune environment, Spearman's rank correlation analyses were performed. Paired *t*-test was used to compare the CacyBP protein levels between the LUAD tissues and the adjacent non-cancerous tissues.

Results

A detailed overview of the study is provided in **Figure 1**.

Dysregulated expression of CACYBP in human neoplasms

Based on the analysis of Genotype Tissue Expression data, the expression of *CACYBP* exhibited significant variation across different tissues. Specifically, normal tissues of the brain, nerve, and ovary demonstrated high expression of *CACYBP*, while expression in some tissue types (e.g., the heart, liver, kidney,

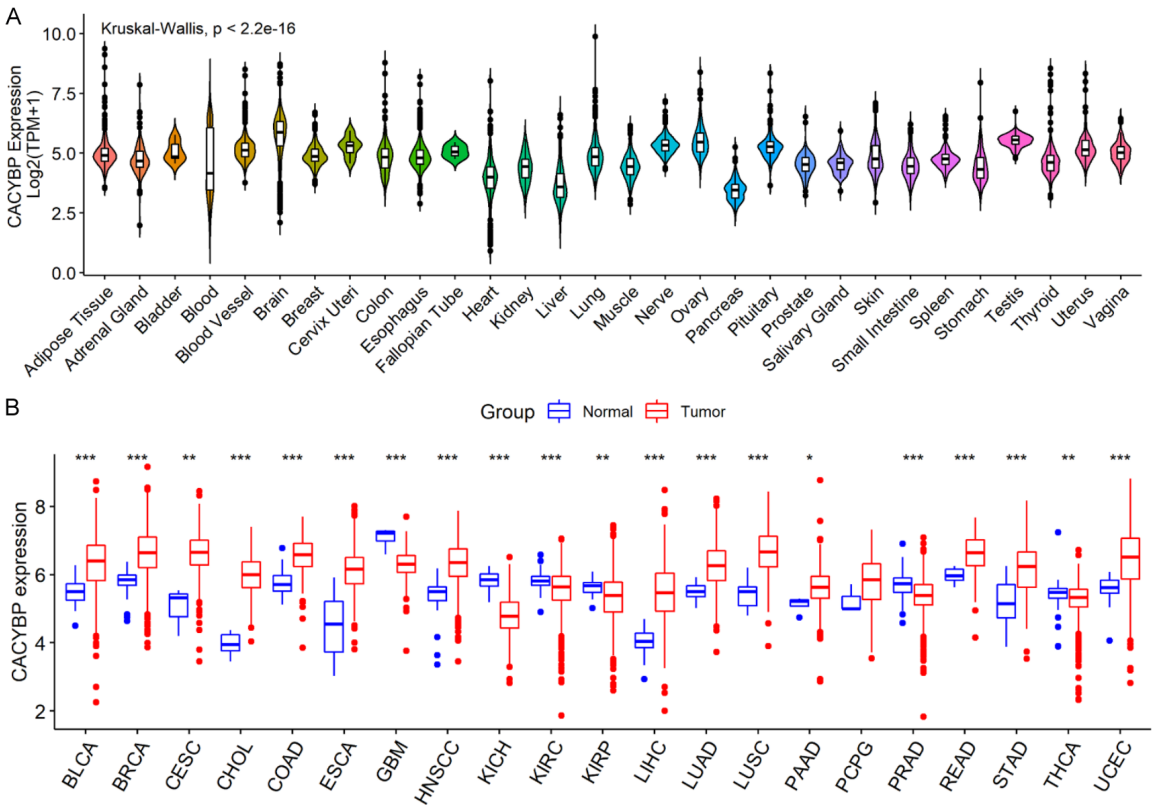

Figure 2. *CACYBP* expression in normal and pan-cancer tissues at mRNA levels. (A) Distinct *CACYBP* expression in various normal tissues of humans. (B) Different *CACYBP* expressions between cancer tissues with their normal tissues. For (B), each *p*-value is based on the Wilcoxon rank-sum test; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

muscle, and pancreas) was significantly lower ($P < 0.05$; **Figure 2A**). Among 20 of the 21 observed cancer types (except for pheochromocytoma and paraganglioma), the distribution of *CACYBP* in cancer tissues was significantly different from that in control tissues ($P < 0.05$; **Figure 2B**). Upregulation of *CACYBP* expression was observed in 14 cancers, including bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), head and neck squamous cell carcinoma (HNSCC), liver hepatocellular carcinoma (LIHC), LUAD, lung squamous cell carcinoma (LUSC), pancreatic adenocarcinoma (PAAD), READ, stomach adenocarcinoma (STAD), and uterine corpus endometrioid carcinoma (UCEC), while downregulation was observed in glioblastoma multiforme (GBM), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), prostate adenocarcinoma (PRAD), and thyroid carcinoma (THCA) ($P < 0.05$; **Figure 2B**).

For the 20 cancers listed above, the CacyBP protein-level data of 15 were available from the Human Protein Atlas. As shown in **Figure 3**, there was no difference in CacyBP protein levels in the BLCA and READ tissues compared to their control tissues. For other cancers, CacyBP expression at the protein level was consistent with that at the mRNA level; CacyBP protein levels were increased in BRCA, CESC, COAD, HNSCC, LIHC, LUAD, LUSC, PAAD, STAD, and UCEC, while they were decreased in kidney cancer (relevant to KICH, KIRC, and KIRP), PRAD, and THCA (**Figure 3**).

Correlation between expression of CACYBP and clinical parameters

Variations in clinical parameters can lead to divergent prognoses among cancer patients

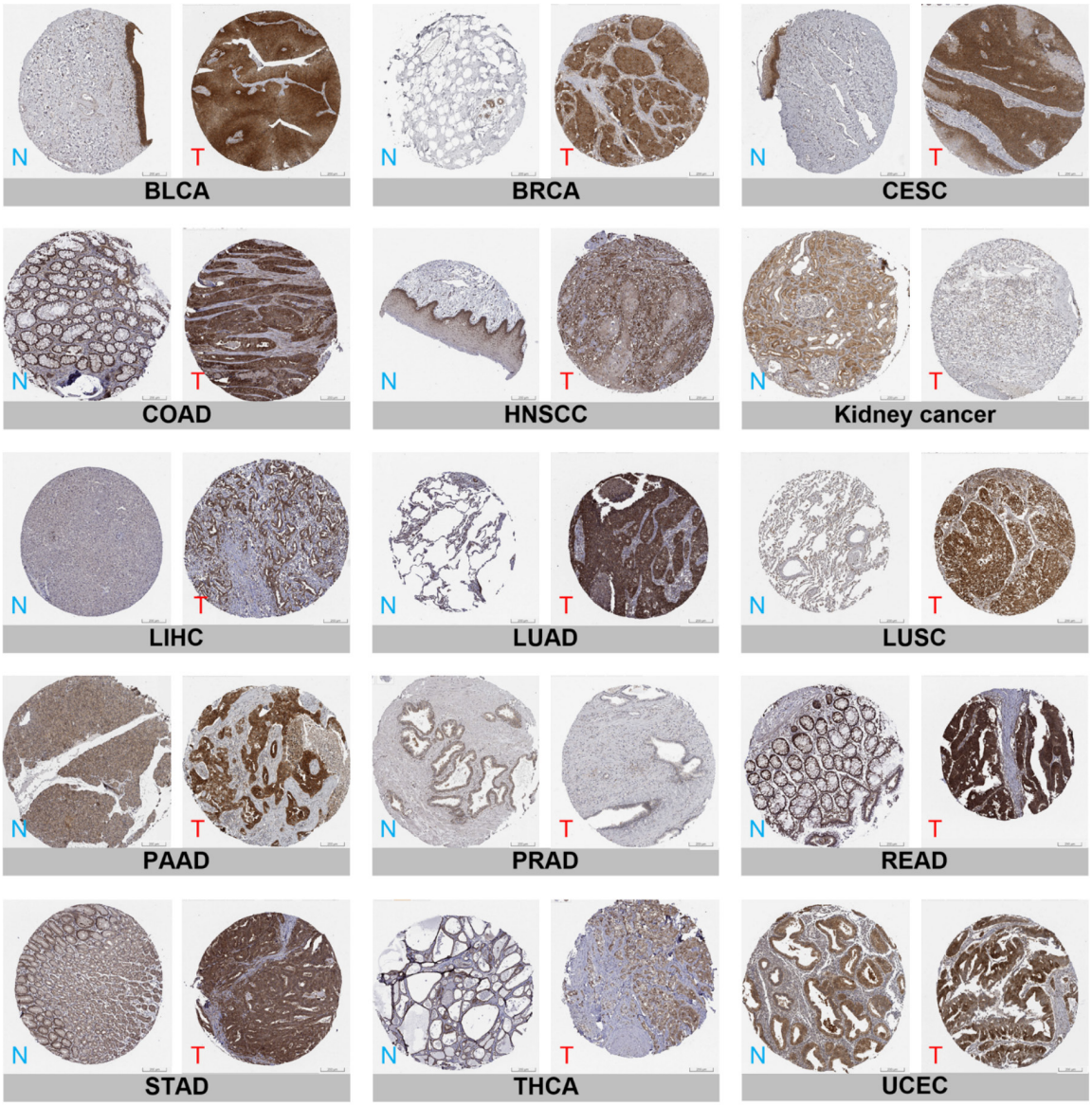

Figure 3. The staining intensity of the anti-CacyBP antibodies in 15 cancers. Under the microscope, the staining intensity of the anti-CacyBP antibodies in 12 cancer tissues (except for the bladder urothelial carcinoma [BLCA] and rectum adenocarcinoma [READ]) is stronger than that in normal tissues, while a weaker staining intensity for anti-CacyBP is observed in PRAD tissues compared to normal prostate tissues. Images are available from v21.0.proteinatlas.org. "N" refers to normal tissues, while "T" indicates tumor tissues.

[18]. A significant correlation ($P < 0.05$) between *CACYBP* expression and AJCC stages was observed in nine cancers, including adrenocortical carcinoma (ACC), BLCA, BRCA, HNSCC, KICH, KIRP, LIHC, LUAD, and STAD (Supplementary Material 2). In these tumor types, advanced AJCC stages tended to represent higher *CACYBP* expression ($P < 0.05$, Supplementary Material 2). Elevated *CACYBP* levels were found in young patients (< 65 years old) in ESCA, LIHC, LUSC, and PAAD ($P < 0.05$, Supplementary Material 3). Male patients were observed with higher *CACYBP* expression in four cancers, namely HNSCC, LUAD, READ, and skin cutaneous melanoma (SKCM) ($P < 0.05$, Supplementary Material 4). The opposite phenomenon was found in four other cancers, namely KIRP, brain lower grade glioma (LGG), sarcoma (SARC), and STAD ($P < 0.05$, Supplementary Material 4).

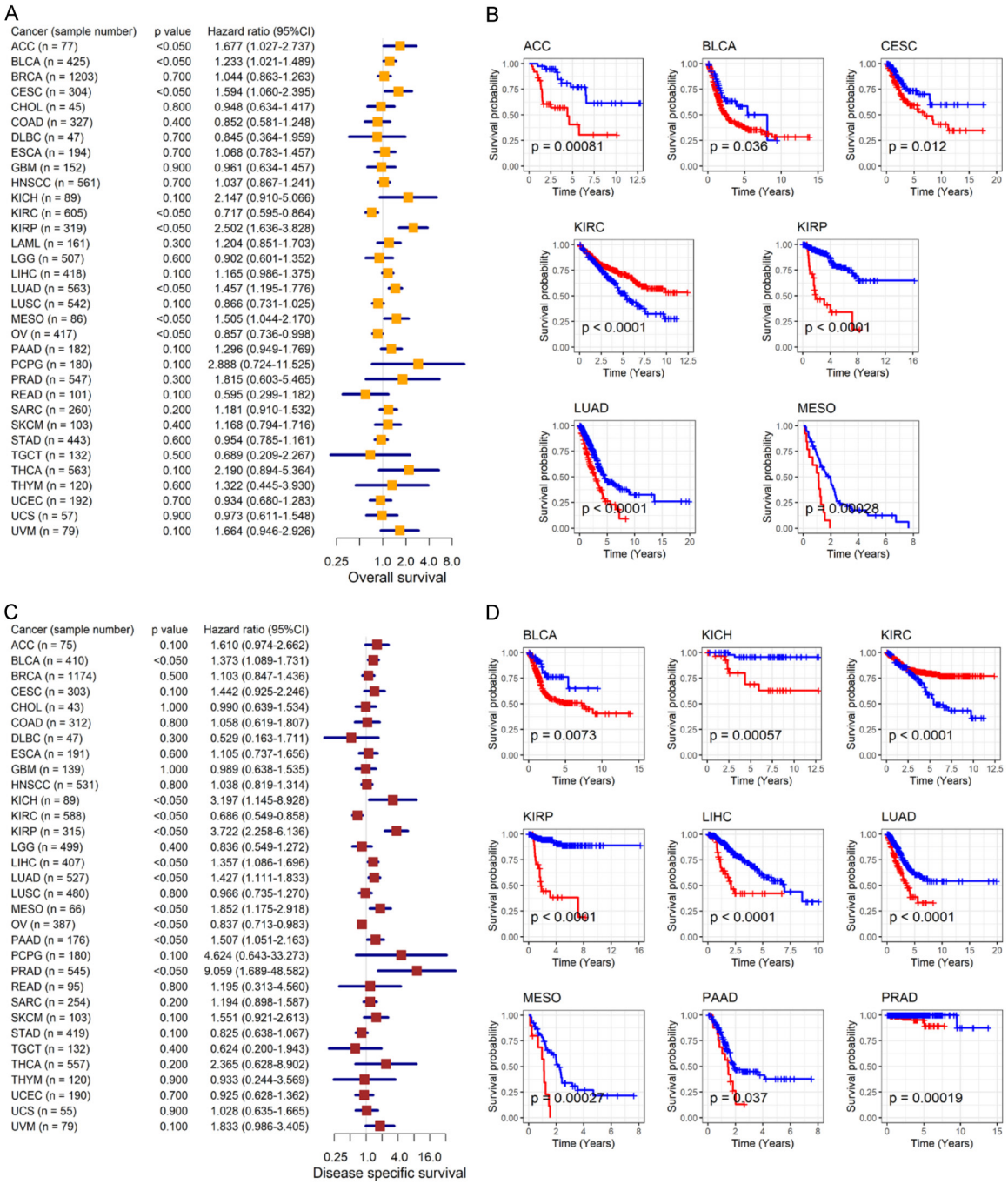

Figure 4. Relation of *CACYBP* expression with overall survival (A and B) and disease-specific survival (C and D) of cancer patients. (A and C) Univariate Cox regression analysis. (B and D) Kaplan-Meier curves with *p*-values based on log-rank tests.

The prognostic significance of CACYBP expression

For OS and/or DSS, high *CACYBP* expression was significantly correlated with shortened survival time in patients suffering from ACC, BLCA, CESC, KICH, KIRP, LIHC, LUAD, MESO, PAAD, and PRAD and associated with better prognosis in patients with KIRC ($P < 0.05$, **Figure 4**). With regard to DFI and PFI, increased *CACYBP* expression was relevant to the poor prognosis in patients with ACC, BLCA, CESC, KIRP, LIHC, LUAD, MESO, SKCM, and uveal melanoma (UVM), while it demonstrated a favorable prog-

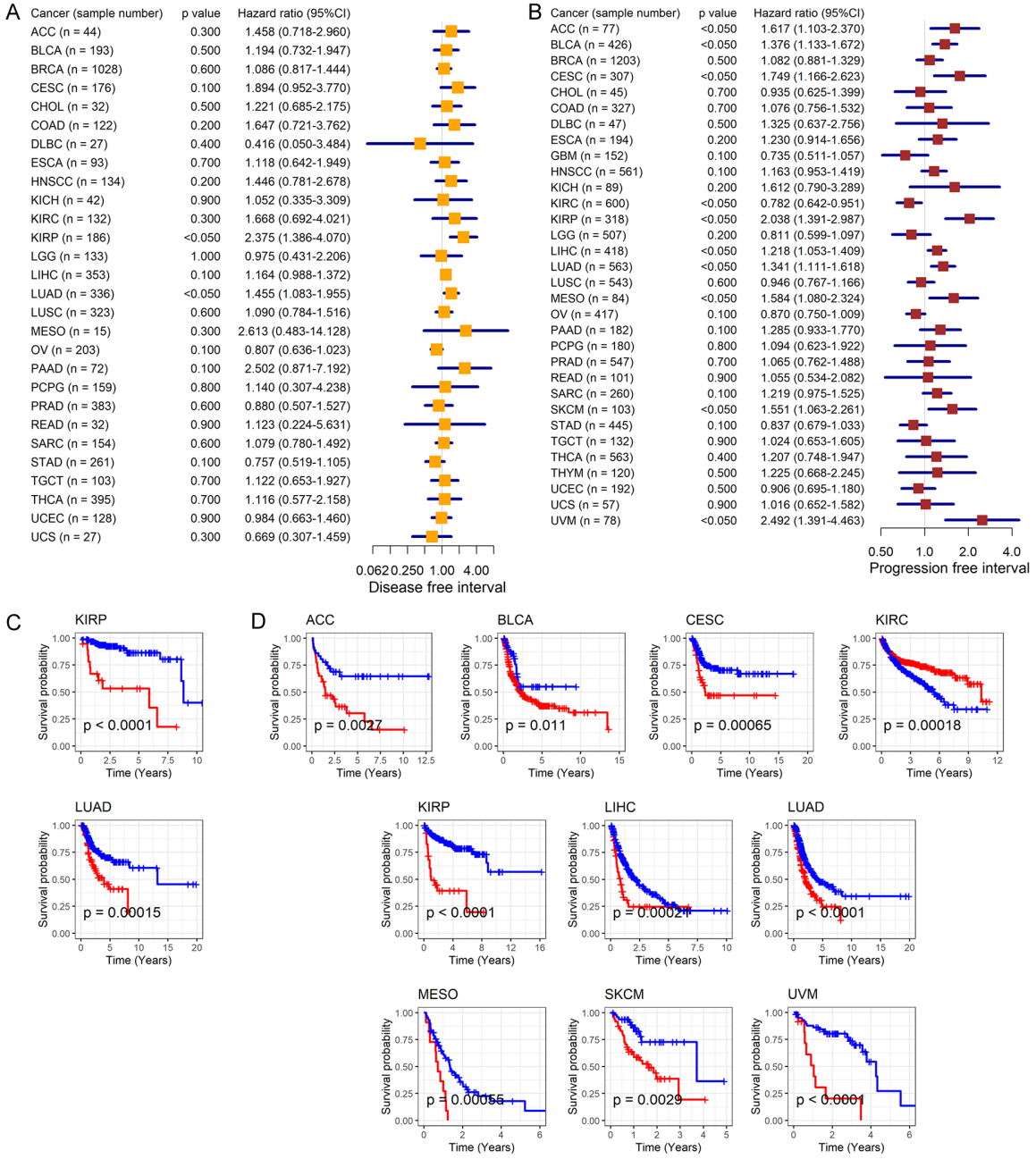

Figure 5. Relation of *CACYBP* expression with disease-free interval (A and C) and progression-free interval of cancer patients (B and D). (A and B) Univariate Cox regression analysis. (C and D) Kaplan-Meier curves with *p*-values based on log-rank tests.

nosis in patients with KIRC ($P < 0.05$, **Figure 5**). Thus, *CACYBP* represented a risk prognosis factor for most cancers.

Predictive significance of CACYBP expression

The prediction of cancer status in patients is of great clinical importance in cancer management. This study highlights the efficacy of *CACYBP* in distinguishing between cancer tissues and control tissues in 15 out of 21 cancers (AUC > 0.80, **Figure 6A**). A comprehensive analysis of the 21 cancers also reveals that *CACYBP* expression can distinguish between cancer patients and healthy individuals (AUC = 0.95, 95% CI: 0.92-0.96; **Figure 6B**). Remarkably, *CACYBP* expression demonstrated exceptional diagnostic accuracy in eight cancers, namely CESC, CHOL, ESCA, GBM, HNSCC,

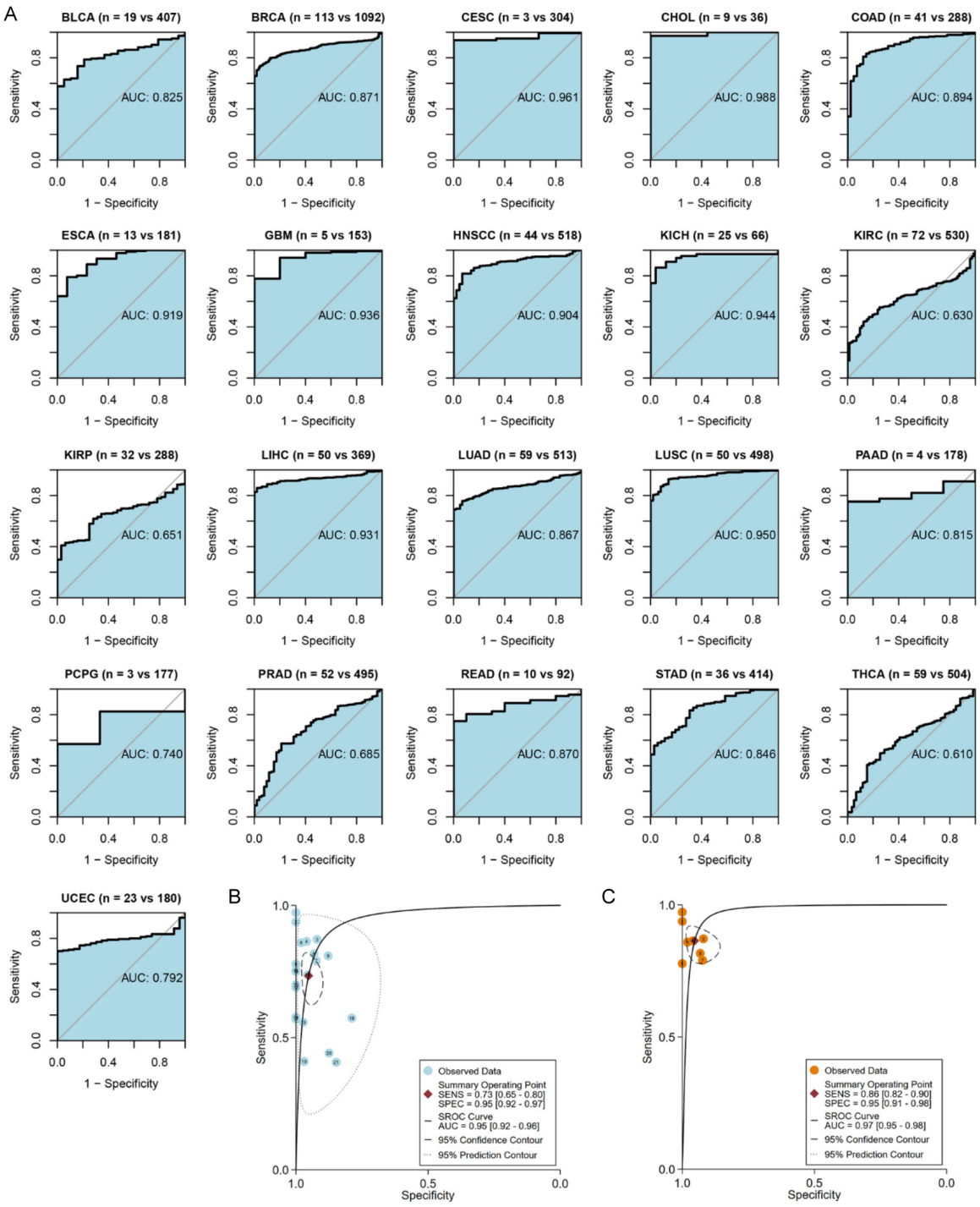

Figure 6. The ability of *CACYBP* expression to distinguish these cancer tissues from their normal tissues in pan-cancer. A. In receiver operating characteristic curves, *CACYBP* demonstrated pronounced effects in identifying cancer tissues from their control counterparts based on the area under the receiver curve (AUC). B. In summary receiver operating characteristic curves, the *CACYBP* expression can accurately identify 21 types of cancer samples from their controls. C. In summary receiver operating characteristic curves, the *CACYBP* expression can accurately identify eight types of cancer samples from their controls.

KICH, LIHC, and LUSC (AUC > 0.90, **Figure 6A**), indicating the significant potential of *CACYBP* as a predictor for those cancers, with an AUC value of 0.97 (**Figure 6C**). Therefore, *CACYBP* may be a valuable marker for predicting the cancer status of specific neoplasm types.

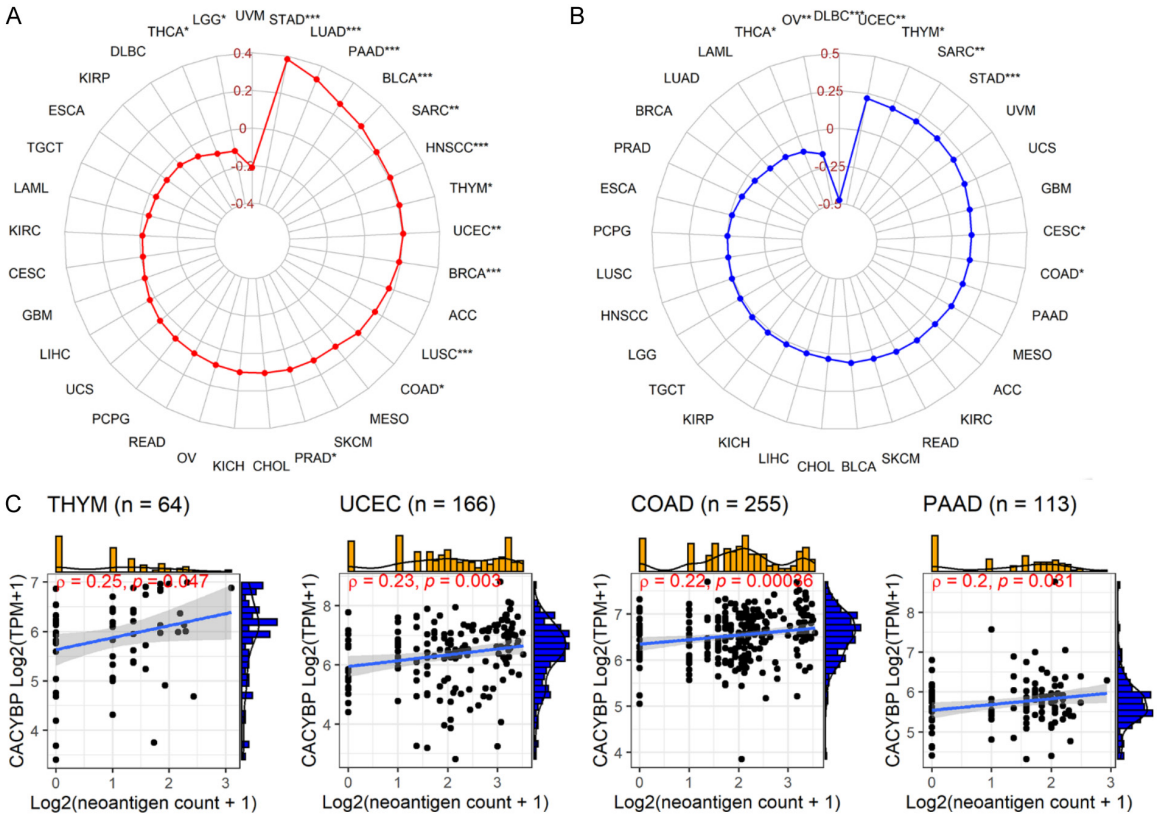

Figure 7. Correlations of CACYBP expression with TMB, MSI, and neoantigen count. A. CACYBP expression was positively correlated with TMB. B. CACYBP expression was positively correlated with MSI. C. CACYBP expression is positively associated with neoantigen count in four cancers.

Association between CACYBP expression and TMB, MSI, neoantigen, and the immune microenvironment

A significant positive correlation was noted between the levels of *CACYBP* expression and TMB in BLCA, HNSCC, LUAD, PAAD, SARC, STAD, THYM, and UCEC ($\rho > 0.2$, $P < 0.05$, **Figure 7A**). The expression levels of *CACYBP* were positively relevant to MSI in UCEC ($\rho > 0.2$, $P < 0.05$) and negatively associated with MSI in DLBC ($\rho < -0.2$, $P < 0.05$, **Figure 7B**). *CACYBP* expression had a mild positive relationship with neoantigen number in THYM, UCEC, COAD, and PAAD ($\rho > 0.2$, $P < 0.05$, **Figure 7C**).

The TIMER and ESTIMATE data were utilized to evaluate the association between *CACYBP* expression and the tumor immune microenvironment. There was a positive association between the expression levels of *CACYBP* and almost all of B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells in ACC, KICH, KIRC, PRAD, and THCA ($P < 0.05$, **Figure 8**). However, decreased *CACYBP* expression exhibited a significant negative correlation with all immune, stromal, and estimate scores in some cancers-particularly LGG, LUSC, SARC, SKCM, TGCT, and UCEC ($P < 0.05$, **Figure 9**). These results demonstrate that *CACYBP* may affect the immune microenvironment through distinct aspects in different cancers.

Underlying signaling pathways of CACYBP

KEGG signaling pathways were utilized to exploit the underlying mechanisms of *CACYBP* in 32 types of human cancer. As shown in **Figure 10A**, *CACYBP* plays a crucial role in the onset and development of ESCA, LGG, and LUSC through complex mechanisms, since it was found to affect multiple signaling pathways. The analysis identified 11 KEGG signaling pathways associated with *CACYBP*, such as "olfactory transduction", "neuroactive ligand

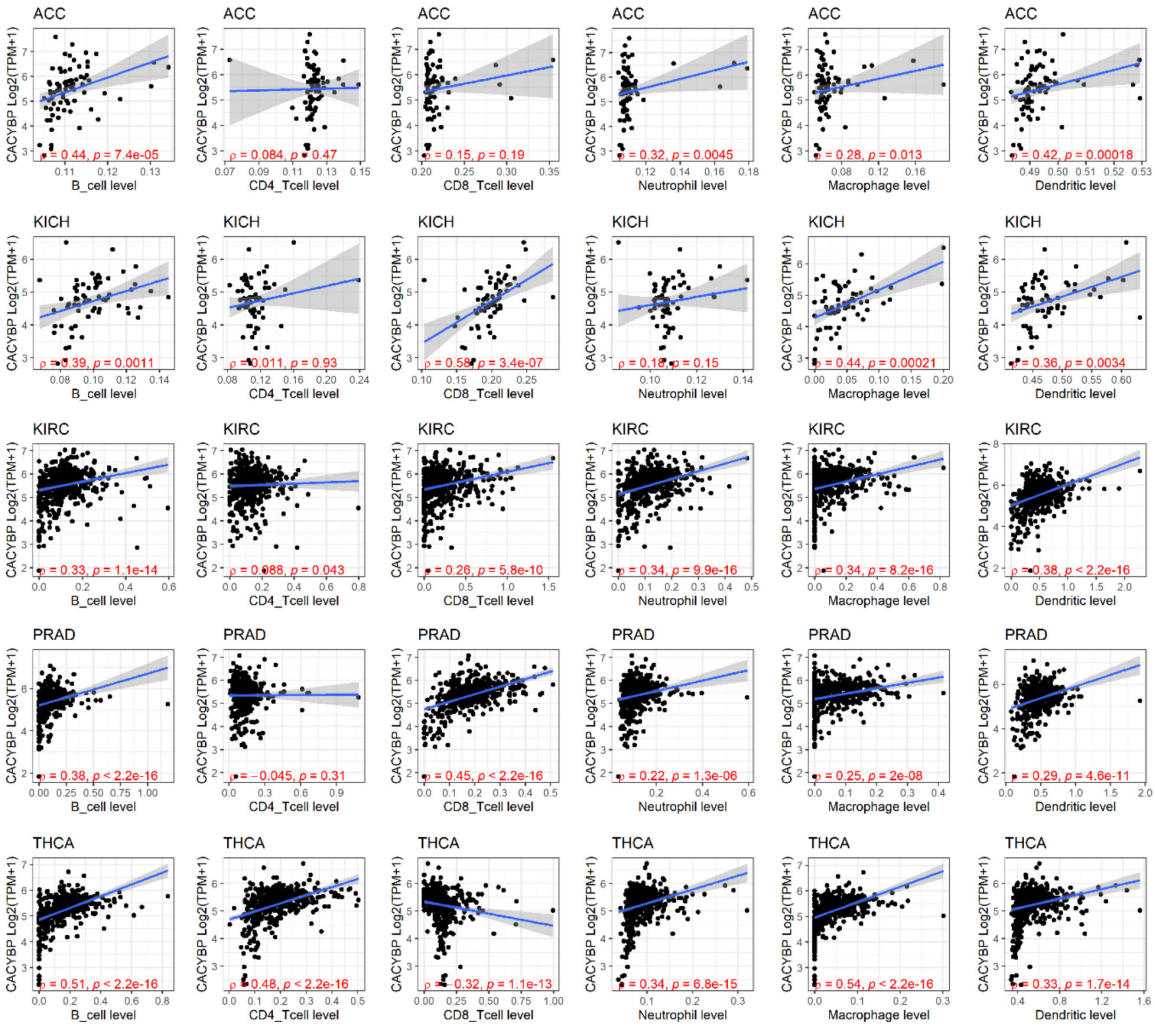

Figure 8. Relevance of CACYBP expression with immune infiltration levels. The Spearman correlation coefficient follows the letter "ρ".

receptor interaction", "cytokine-cytokine receptor interaction", and "calcium signaling pathway", in various cancers (**Figure 10B**).

Partial validation of CacyBP expression in cancer tissues through LUAD samples

As shown above, *CACYBP* is highly expressed in various tumors. Considering that lung cancer is the leading cause of cancer-related death worldwide, this study explored the expression of the CacyBP protein in lung adenocarcinoma to verify its expression in cancer tissue using internal samples. Compared with paired adjacent specimens, the expression level of Cacybp protein was up-regulated in the LUAD specimens (**Figure 10C**). This result was statistically validated by a significance test ($P < 0.05$, **Figure 10D**).

Discussion

Cancer remains a pressing issue in the global public health landscape. The absence of reliable biomarkers presents a considerable hurdle in the care of cancer patients. While *CACYBP* has been identified as a biomarker for various cancers, a comprehensive investigation into its potential as a pan-cancer biomarker is lacking and warrants further study.

In the current study, we conducted a pan-cancer analysis using 10,080 samples to enhance the understanding of the clinical value of *CACYBP*. Our findings revealed both upregulated expression of *CACYBP* in 14 cancers (e.g., BLCA) and downregulated expression of *CACYBP* in six cancers (e.g., GBM); almost all of these expression trends were verified at the

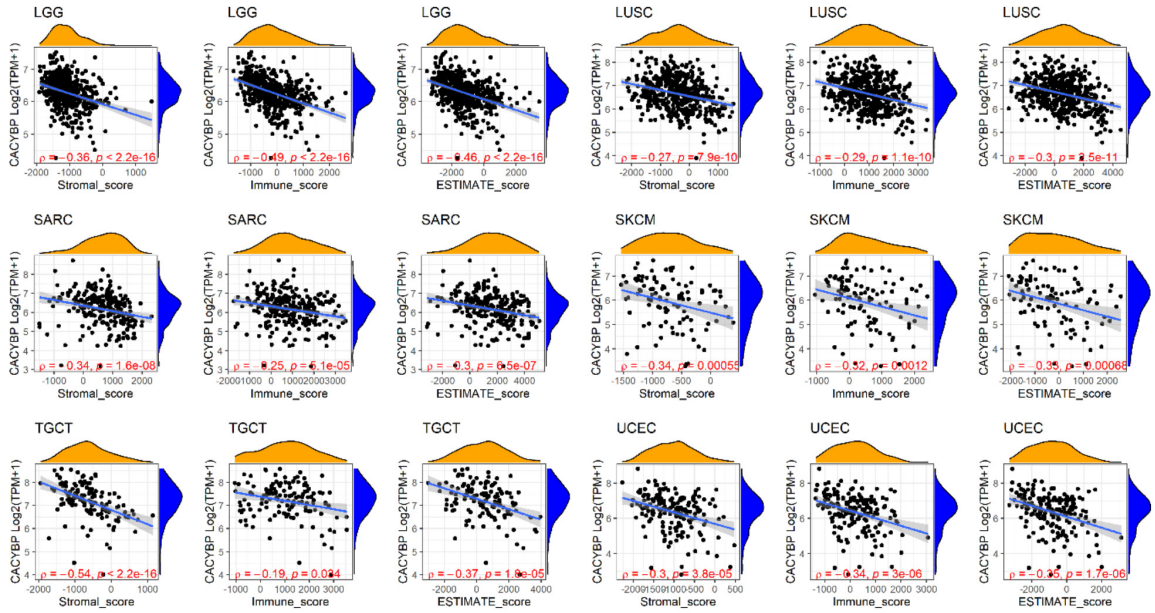

Figure 9. Relevance of CACYBP expression with immune microenvironment scores. The Spearman correlation coefficient follows the letter "ρ".

protein level. *CACYBP* was identified as a significant prognostic indicator for patients with 11 cancer types (ACC, and so on) in terms of OS and DSS. For DFI and PFI, increased *CACYBP* expression was associated with the prognosis of patients in 10 cancers (e.g., BLCA). *CACYBP* was also demonstrated as a predictive marker of cancer status for 15 cancers (e.g., CESC). Furthermore, the associations observed between *CACYBP* expression and TMB, MSI, neoantigen, and the immune microenvironment indicate that it may be an attractive target for multiple neoplasms.

For various types of cancers, the expression of *CACYBP* varies between cancer and control groups. Previous studies identified differentially expressed *CACYBP*/CacyBP in certain cancers, with increased expression in BLCA, BRCA, COAD, glioblastoma, LUAD, NSCLC, osteosarcoma, PAAD, prostate cancer, and STAD [6, 7, 19-25] and decreased expression in KIRC and chronic lymphocytic leukemia cells [26, 27]. Our study identified the results for BLCA, COAD, LUAD, PAAD, STAD, and KIRC. Additionally, it revealed overexpression of *CACYBP* in CESC, CHOL, ESCA, HNSCC, LIHC, LUSC, READ, and UCEC and low expression of *CACYBP* in GBM, KICH, KIRP, PRAD, and THCA. CacyBP protein levels were consistent with most of these cancers, based on our study. Among the listed cancers, the expression of *CACYBP*/CacyBP in BRCA remains disputed; one report identified low levels of *CACYBP*/CacyBP in BRCA [28], while another study [29] and our research displayed upregulated *CACYBP* mRNA and CacyBP protein expression levels in this disease. Unfortunately, the mechanisms of *CACYBP* expression in BRCA have not been explored, warranting further study. In summary, upregulation of *CACYBP*/CacyBP expression was observed in most cancers, while the opposite was found in several cancers.

CACYBP has been found to have varying prognostic implications and can serve as an excellent predictive marker for cancer status in certain types of cancers. Previous studies demonstrated a correlation between elevated *CACYBP* expression and unfavorable prognosis in individuals with BLCA, BRCA, glioblastoma, LUAD, or osteosarcoma [20, 22-24, 30]. In our study, we investigated the potential prognostic roles of *CACYBP* in multiple human cancers by analyzing survival data, including OS, DSS, DFI, and PFI. Our results showed overexpression of *CACYBP* to be associated with unfavorable OS and/or DSS in patients with ACC, BLCA, CESC, KICH, KIRP, LIHC, LUAD, MESO, PAAD, or PRAD, while it was linked to a favorable OS and/or DSS in patients with KIRC. Increased *CACYBP* expression was associated with a good prognosis in patients with KIRC, while it was also relat-

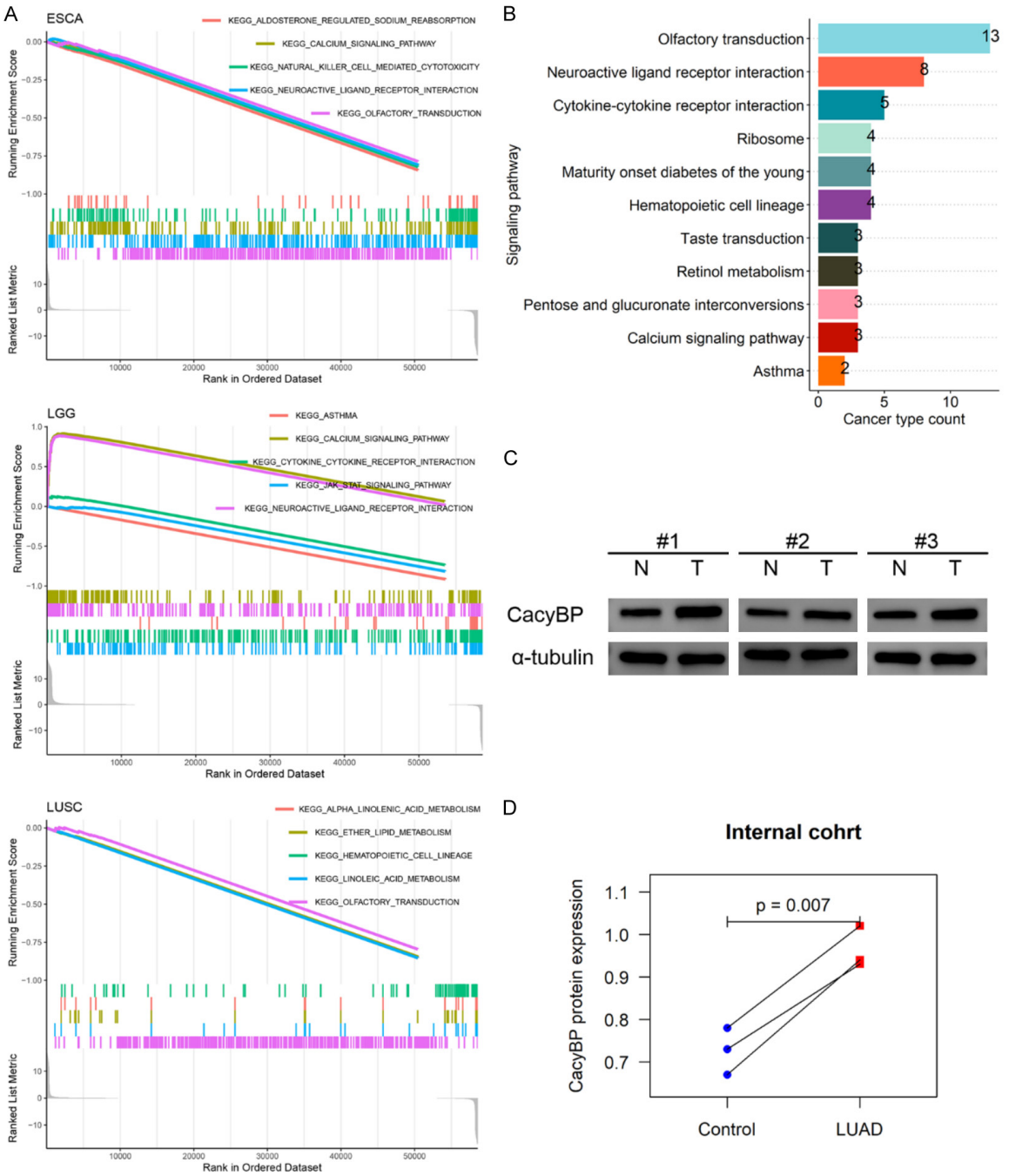

Figure 10. The potential mechanisms of CACYBP in pan-cancer and the protein expression of CacyBP in lung adenocarcinoma (LUAD). A. Gene set enrichment analysis suggests that *CACYBP* is identified to involve no fewer than five signaling pathways in some cancers. B. All signaling pathways that *CACYBP* may affect in cancers. C. The protein expression levels of CacyBP in LUAD detected by Western blot. D. Comparison of protein expression levels of CacyBP in LUAD and adjacent non-cancerous tissues. Paired *t*-test is used in this plot.

ed to a poor prognosis in patients in nine cancers, namely ACC, BLCA, CESC, KIRP, LIHC, LUAD, MESO, SKCM, and UVM. Additionally, our study revealed that *CACYBP* could distinguish between 15 types of cancer tissues and their normal tissues; this novel finding highlights the significant predictive value of *CACYBP* in cancers. Therefore, *CACYBP* may be an excellent prognostic and predictive biomarker for multiple cancers.

The mechanisms of *CACYBP* in cancers remain complex and unclear. TMB and MSI are considered to be effective biomarkers for various tumors because they aid in the diagnosis and treatment of cancers [31, 32]. *CACYBP* expression levels have been associated with TMB and/or MSI in BLCA, DLBC, HNSCC, LUAD, PAAD, SARC, STAD, THYM, and UCEC, suggesting that the gene may play a role in influencing TMB and MSI levels in these tumors. Additionally, *CACYBP* expression levels have been found to be correlated with neoantigen count in patients with COAD, PAAD, THYM, and UCEC, indicating that the gene may also impact the immune microenvironment of certain tumors. Notably, our study also revealed a significant positive association between *CACYBP* expression and the immune microenvironment. On the one hand, the expression of *CACYBP* showed a correlation with the infiltration levels of almost all six types of immune cells, namely B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells, in ACC, KICH, KIRC, PRAD, and THCA. This suggests that the gene may act as an oncogene contributing to the stimulation of immune response [20]. On the other hand, in LGG, LUSC, SARC, SKCM, TGCT, and UCEC, there was a negative correlation between *CACYBP* expression and immune, stromal, and estimate scores, which represents a prognosis risk factor for cancer patients and affects the immune response. These findings highlight that *CACYBP* may affect the immune microenvironment through distinct aspects in different cancers. Moreover, *CACYBP* may play a role in affecting up to 11 signaling pathways, such as “olfactory transduction”, “neuroactive ligand receptor interaction”, “cytokine-cytokine receptor interaction”, and “calcium signaling pathway”. Even in single cancer, *CACYBP* may play a crucial role in the occurrence and development of ESCA, LGG, and LUSC through several signaling pathways. These findings provide a clue for further experimental validation of the potential mechanisms of *CACYBP* in cancers.

In this study, some limitations should be noted. Initially, we failed to collect body fluid-related samples (e.g., blood specimens) to detect the ability of *CACYBP* expression in directly screening cancer patients from individuals without cancers. The sample size for investigating CacyBP protein levels was relatively small. It would be worthwhile to conduct experimental validation of the underlying mechanisms of *CACYBP*.

In summary, the expression of *CACYBP* varies across different types of human cancers, and this gene may be utilized as a prognostic and predictive marker for multiple cancer types.

Acknowledgements

The results shown in the study are based upon data generated by the TCGA, GTEx, The Human Protein Atlas, and Sanger Box (version 3.0). Data on the internal cohort can be obtained from the corresponding author.

Disclosure of conflict of interest

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

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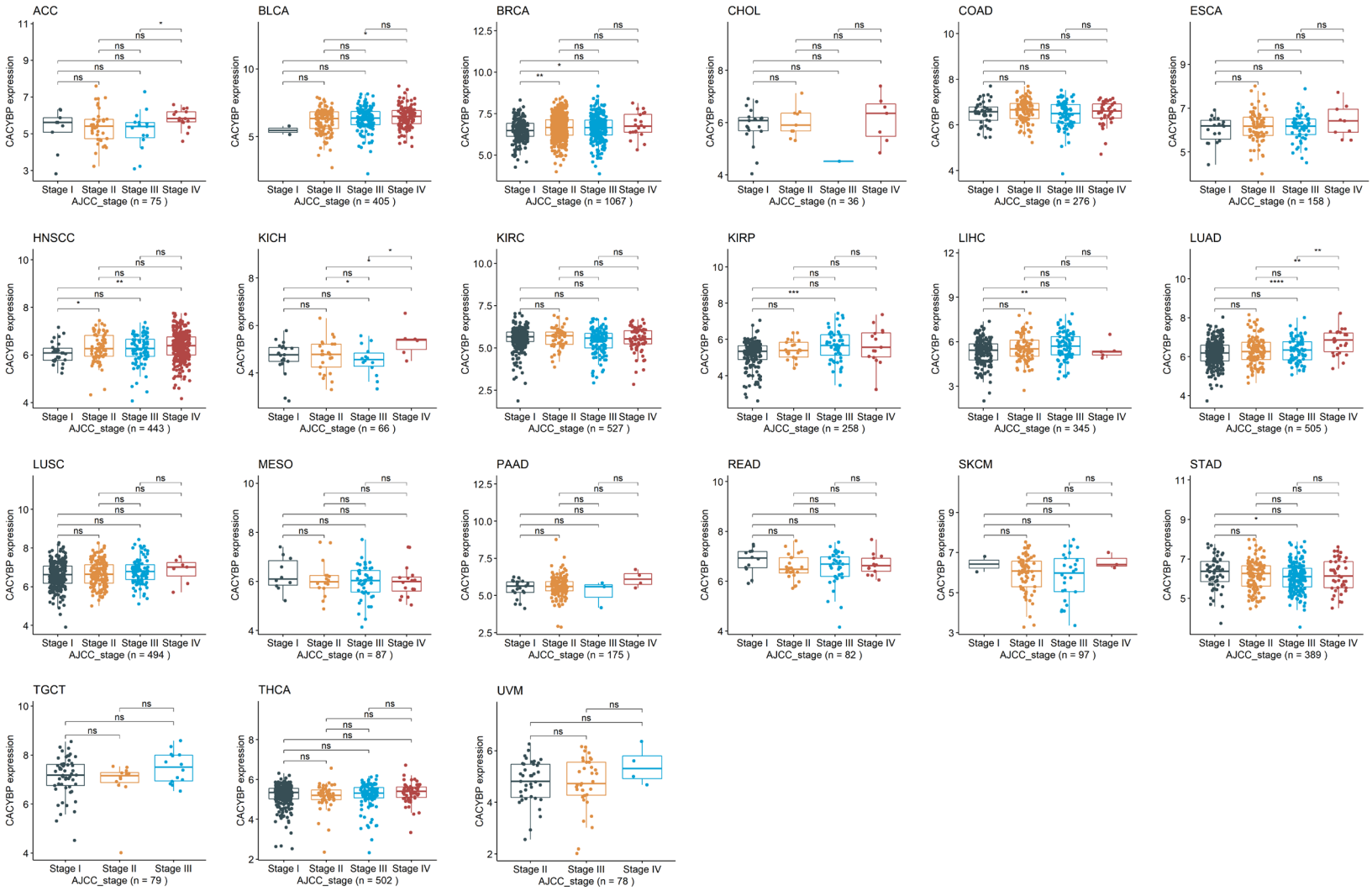
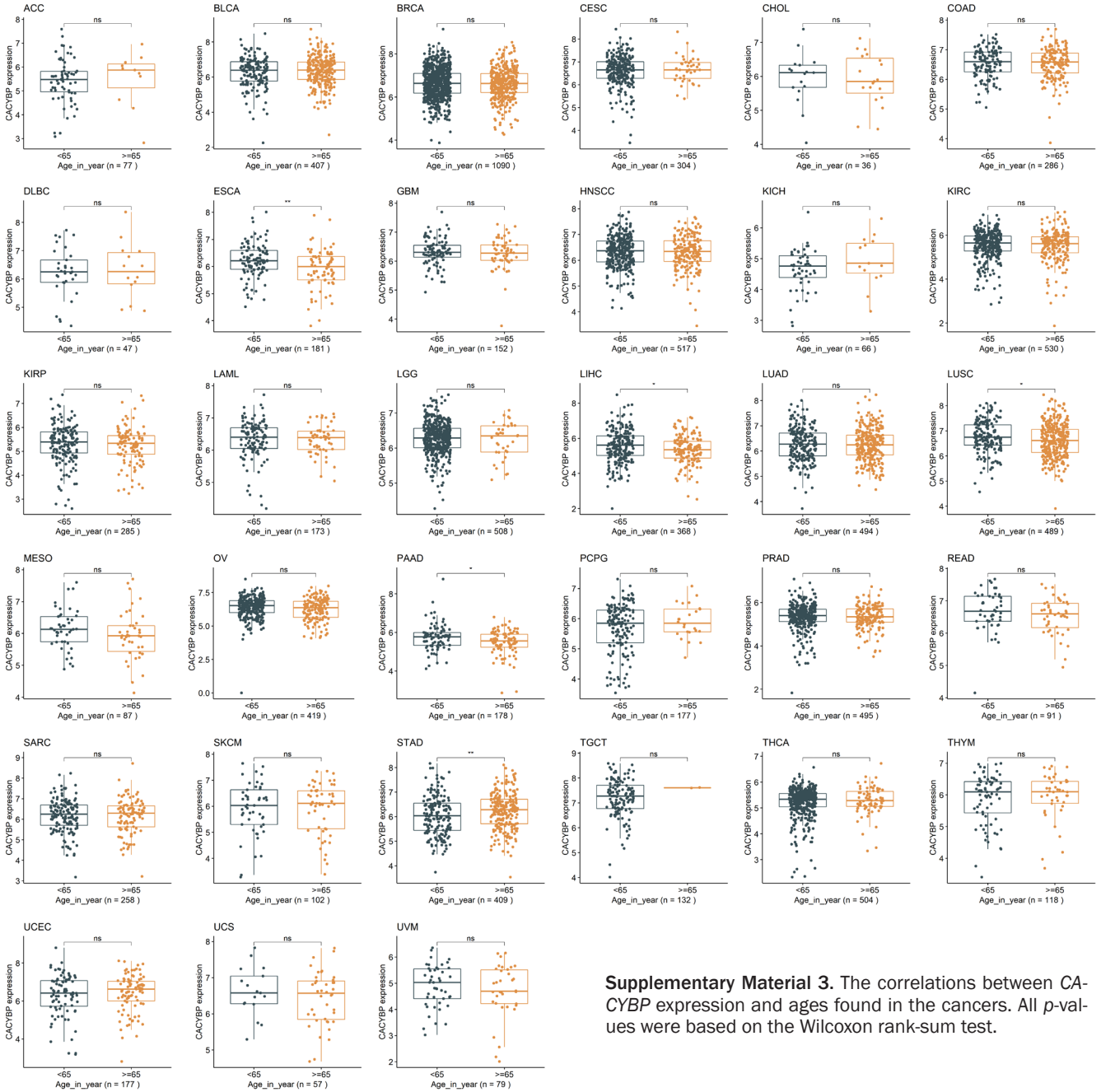
Supplementary Material 1. Basic clinical information for internal samples

Patient ID	Gender	Age (year)	Sample type
1	Male	59	LUAD & adjacent non-cancerous tissues
2	Male	67	LUAD & adjacent non-cancerous tissues
3	Female	59	LUAD & adjacent non-cancerous tissues



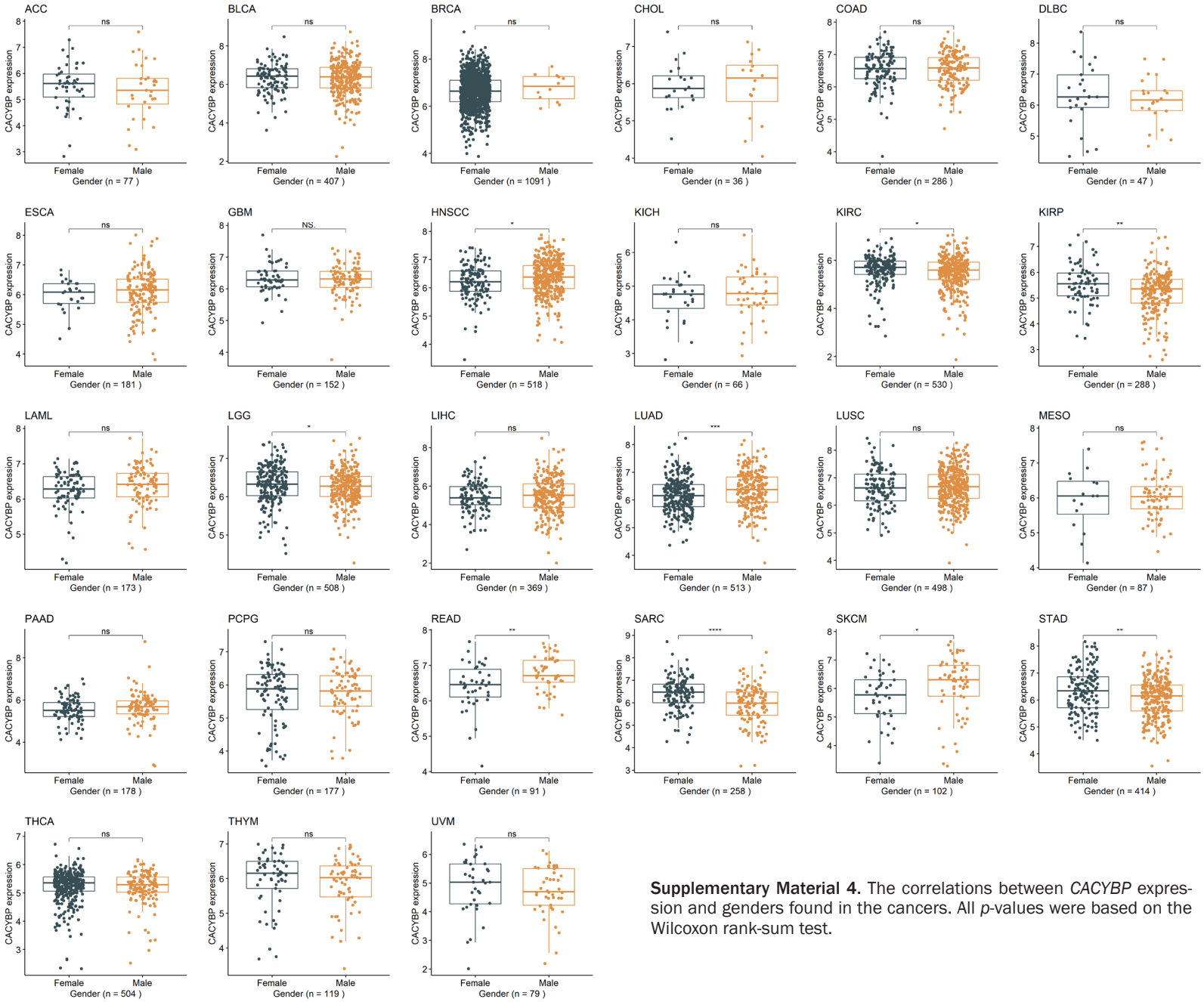
Supplementary Material 2. The correlations between *CACYBP* expression and AJCC stages found in the cancers. All *p*-values were based on the Wilcoxon rank-sum test.

CACYBP in multiple cancers



Supplementary Material 3. The correlations between *CACYBP* expression and ages found in the cancers. All *p*-values were based on the Wilcoxon rank-sum test.

CACYBP in multiple cancers



Supplementary Material 4. The correlations between *CACYBP* expression and genders found in the cancers. All *p*-values were based on the Wilcoxon rank-sum test.