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Analysis

Exploring the potential role of ADRB1 as a tumor suppressor gene and prognostic biomarker in pan-cancer analysis

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Abstract

Background The β_1-adrenergic receptor (β_1-AR) is an important membrane receptor belonging to the G protein coupled receptors, encoded by ADRB1. Norepinephrine released by the sympathetic nerve activates β-adrenergic receptors. Compared with β_2-adrenergic receptor (β_2-AR), β_1-AR has higher affinity for norepinephrine. However, the current research is mostly limited to the role of β_2-AR in cancer development, and the effect and mechanism of β_1-AR on cancer prognosis are still unclear.

Methods We analyzed ADRB1 expression in different types of cancer and corresponding normal tissues. The correlation between ADRB1 expression and pathological grade, stage and survival of cancers were analyzed. We explored the methylation level of ADRB1 in various cancers. The cBioPortal website was used to determine the mutation characteristics of ADRB1 in cancer tissues. Additionally, the CancerSEA website was employed to explore the correlation between ADRB1 expression and different functional states in cancers. We also analyzed the correlation between ADRB1 expression and immune checkpoint (ICP) genes, tumor mutation burden (TMB), microsatellite instability (MSI), neoantigens and cancer-infiltrating immune cells.

Results Our results showed that ADRB1 expression was downregulated in the majority of solid cancers. The ADRB1 expression was significantly associated with the prognosis of cancer. High expression of ADRB1 was found to be a protective factor for patients with several types of cancer. In some cancers, ADRB1 expression was associated with clinical pathological stages. Functional relevance analysis indicated the crucial role of ADRB1 in regulating multiple biological behaviors of cancer cells. The expression of ADRB1 was associated with TMB, MSI, neoantigens and immune cell infiltration in cancers.

Conclusions These comprehensive pan-cancer analysis suggested that ADRB1 plays a protective role in various cancer types, such as skin cutaneous melanoma and lung adenocarcinoma. This may provide a new idea for the clinical treatment of cancer patients.

Keywords ADRB1 · β_1-Adrenergic receptor · Pan-cancer analysis

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Abbreviations

NE	Norepinephrine
β-ARs	β-Adrenergic receptors
β_1-AR	β_1-Adrenergic receptor
β_2-AR	β_2-Adrenergic receptor
β_3-AR	β_3-Adrenergic receptor
ICP	Immune checkpoint
TMB	Tumor mutation burden
MSI	Microsatellite instability
OS	Overall survival
DFS	Disease-free survival
DSS	Disease-specific survival
PFS	Progression-free survival
CAN	Copy number amplifications
EMT	Epithelial–mesenchymal transition
ACC	Adrenocortical carcinoma
ALL	Acute lymphoblastic leukemia
BLCA	Bladder urothelial carcinoma
BRCA	Breast invasive carcinoma
CESC	Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL	Cholangiocarcinoma
COAD	Colon adenocarcinoma
COADREAD	Colon adenocarcinoma/Rectum adenocarcinoma Esophageal carcinoma
DLBC	Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
ESCA	Esophageal carcinoma
GBM	Glioblastoma multiforme
GBMLGG	Glioma
HNSC	Head and neck squamous cell carcinoma
KICH	Kidney chromophobe
KIPAN	Pan-kidney cohort (KICH + KIRC + KIRP)
KIRC	Kidney renal clear cell carcinoma
KIRP	Kidney renal papillary cell carcinoma
LAML	Acute myeloid leukemia
LGG	Brain lower grade glioma
LIHC	Liver hepatocellular carcinoma
LUAD	Lung adenocarcinoma
LUSC	Lung squamous cell carcinoma
MESO	Mesothelioma
NB	Neuroblastoma
OV	Ovarian serous cystadenocarcinoma
PAAD	Pancreatic adenocarcinoma
PCPG	Pheochromocytoma and paraganglioma
PRAD	Prostate adenocarcinoma
READ	Rectum adenocarcinoma
SARC	Sarcoma
SKCM	Skin cutaneous melanoma
STAD	Stomach adenocarcinoma
STES	Stomach and esophageal carcinoma
TGCT	Testicular germ cell tumors
THCA	Thyroid carcinoma
THYM	Thymoma
UCEC	Uterine corpus endometrial carcinoma
UCS	Uterine carcinosarcoma

UVM/UM	Uveal melanoma
WT	High-risk Wilms tumor

1 Introduction

Cancer, as one of the leading causes of death worldwide, not only brings significant suffering to individuals, but also imposes a heavy burden on society [1]. With population growth and an aging population, the incidence and mortality of cancer are continuously increasing, posing a serious threat to human health [1]. The occurrence of cancers is a complex multifaceted process involving multiple factors such as cancer cell proliferation, survival regulation, the cancer microenvironment and cancer immune infiltration [2].

The etiology of cancer is complex and diverse. Factors such as stress and anxiety can lead to sympathetic nervous system activation, thereby promoting cancer initiation and progression [3]. Previous research by Claire Magnon et al. clarified the importance of the sympathetic nervous system in development of prostate cancer [1]. Huan et al. analyzed the phenomenon and mechanism of the sympathetic nervous system to promote the occurrence of liver cancer by regulating inflammatory response [4]. Garramona et al. confirmed that enhanced sympathetic nervous system activity worsens the prognosis of lung cancer patients through clinical studies [5]. Sympathetic nervous activation can release norepinephrine (NE), thereby activating β-adrenergic receptors (β-ARs). There are three subtypes of β-ARs, including β_1-AR, β_2-adrenergic receptor (β_2-AR) and β_3-adrenergic receptor (β_3-AR). β_1-AR is mainly distributed in the cardiovascular system. β_2-AR is primarily expressed in the kidneys and lungs. While β_3-AR is mainly found in adipose tissue [6]. The activation of stimulatory G (Gs) protein coupled β-ARs leads to the increase of adenylate cyclase activity and subsequent production of cyclic adenosine monophosphate (cAMP) [7]. The increase of cAMP level activates protein kinase A (PKA), which in turn phosphorylates target proteins, such as cAMP response element binding protein (CREB) [8]. Phosphorylation of CREB can promote cell gene expression and cell proliferation [9]. Current research on the relationship between adrenergic receptors and cancer has focused mainly on β_2-AR, which has been found to affect the occurrence and development of gastric cancer [10], lung cancer [11] and pancreatic cancer [12]. In malignant melanoma, activation of β_2-AR inhibits apoptosis of tumor cells and promotes tumor growth and metastasis by down-regulating pro-apoptotic proteins (such as Bax) and up-regulating anti-apoptotic proteins (such as Bcl-2) [13]. In recent years, studies have shown that β_3-AR can promote the proliferation of melanoma cells by interacting with cell cycle and apoptosis related signaling pathways [14]

In cancer treatment, patients with non-small cell lung cancer (NSCLC), the use of β-AR antagonists for β_2-AR has shown better clinical outcomes [15]. In addition, β_2-AR blockers inhibit the invasion and proliferation of pancreatic cancer cells by suppressing the cAMP/PKA and Ras pathways [16]. β-AR antagonists have also been found to enhance the anti-angiogenic effect of chemotherapy regimens and prevent breast cancer metastasis [17]. However, there are few reports on the effect of specific blocking of β_1-AR on cancer therapy. Therefore, whether β_1-AR can be a cancer therapeutic target still needs clinical validation.

It is intriguing to note that norepinephrine does not exhibit the highest affinity for β_2-AR. In vivo, β_1-AR has a 20-fold higher affinity for NE compared to β_2-AR [18]. This suggests that NE released from the sympathetic nervous system may preferentially bind to β_1-AR in cancers. However, the impact of β_1-AR on cancers has been largely overlooked. Therefore, this study aims to analyze the expression levels of ADRB1 in cancers and its impact on patient prognosis, the effect of ADRB1 on cancer development in terms of genetic mutations, immune checkpoint (ICP) genes, tumor mutation burden (TMB), microsatellite instability (MSI), neoantigens and immune cell cancer infiltration.

2 Materials and methods

2.1 Data collection

To verify the prognostic impact of ADRB1 expression in LUAD, PAAD, SKCM and BRCA patients. The raw data of GSE31210 (LUAD), GSE85916 (PAAD), GSE19234 (SKCM), GSE88770 (BRCA) and GSE1456 (BRCA) were obtained from the Gene Expression Omnibus database (GEO https://www.ncbi.nlm.nih.gov/geo/). And the raw data of TCGA-LUAD, TCGA -PAAD, TCGA -SKCM were downloaded from Xena (https://xena.ucsc.edu).

2.2 Analysis of ADRB1 gene expression

HPA website was used to analyze the expression of ADRB1 in normal human tissues. The differential expression of the ADRB1 in different cancer tissues and their normal tissues was examined using the TIMER2.0 website (https://cistrome.shinyapps.io/timer/). Since there are some types of cancers in the TIMER2.0 database without paired normal tissue, the "Expression analysis Box Plots" module of the GEPIA2 website (http://gepia2.cancer-pku.cn/#analysis) was used to further explore the expression of ADRB1 in cancerous and corresponding normal tissues. Data was obtained from TCGA and the GTEx datasets.

2.3 The analysis of clinical phenotype and prognosis

The association of ADRB1 expression with clinicopathological parameters such as clinical stage was analyzed using the Sangerbox website. To explore the association between ADRB1 expression and patient prognosis, we used overall survival (OS), disease-free survival (DFS), disease-specific survival (DSS) and progression-free survival (PFS) as indicators. Cox regression was performed using the Sangerbox website (http://Sangerbox.com/Tool) to evaluate the prognostic significance of ADRB1 in patients. Kaplan–Meier survival curves were plotted, and survival difference between different groups were evaluated using the log-rank test.

2.4 External independent validation

GSE31210 (LUAD), GSE85916 (PAAD), GSE19234 (SKCM), GSE88770 (BRCA) and GSE1456 (BRCA) were processed consistently using R 4.4.1. All data were retained in the patient cohort. Subsequently, the R packages "survival" and "survminer" were used for survival analysis.

2.5 Analysis of DNA methylation

The UALCAN website (https://ualcan.path.uab.edu/analysis-prot.html) was used to analyze the methylation status of ADRB1 in cancers and normal tissues.

2.6 Genetic mutation analysis

The cBioPortal website (https://www.cbioportal.org/) was used to analyze the mutation frequency, type and copy number amplifications (CNA) of ADRB1 in different types of cancer. The "Mutation" module in the cBioPortal website was used to display mutation site information for ADRB1. The correlation between ADRB1 mutations and clinical prognosis was derived from the "Comparison/Survival" module.

2.7 Association of ADRB1 and cancer functional status

On the CancerSEA website (http://biocc.hrbmu.edu.cn/CancerSEA/home.jsp), we obtained data on the correlation between ADRB1 and 14 functional states across 10 different cancers and visualized it using the "pheatmap" package in R language.

2.8 Correlation analysis between ADRB1 expression and cancer immune markers

The Sangerbox website was used to analyze the correlation between ADRB1 expression and cancer immune markers, such as ICP, TMB, MSI and neoantigens.

2.9 Analysis of the immune infiltration of the cancers

We first used the TIMER, EPIC, MCPCOUNTER, CIBERSORT, QUANTISEQ, XCELL and TIDE algorithms in the TIMER2.0 website to evaluate the correlation of ADRB1 expression and immune infiltration levels in different TCGA cancers. Subsequently, we used the "Immune Infiltration Analysis" module of the Sangerbox website to analyze the impact of ADRB1 on cancer immune infiltration using the TCGA and GTEx datasets.

2.10 Gene enrichment analysis

The gene expression profile data for LUAD, SKCM, and PAAD were downloaded from the Xena database. Using R language, a Pearson correlation analysis was conducted between all genes in the three types of cancer and ADRB1. The top 300 genes with the highest correlation coefficients were selected as related genes, and similar genes to ADRB1 were obtained from the GEPIA database. Venn diagrams was used to illustrate the genes that were common to both the related genes and the similar genes of ADRB1 across the three types of cancer. Subsequently, the DAVID database was employed to perform enrichment analysis on the commonly occurring genes, identifying the KEGG pathways.

2.11 Statistical analysis

External validation of prognosis analysis was performed using the "survival" and "survminer" packages in R language. Cox regression analysis was used to evaluate the correlation between gene expression levels and prognosis. Kaplan–Meier analysis was used to calculate patient survival rates. The log-rank test was used for comparisons between different groups. An unpaired t-test was used to compare differences between two groups. These results were considered as statistically significant at $P < 0.05$.

3 Results

3.1 Expression analysis of ADRB1 in cancer and normal tissues

We analyzed the expression of ADRB1 in normal tissues using the HPA website. The results showed that ADRB1 is highly expressed in tissues such as the placenta, heart muscle and lung. While it is not expressed in cervix, ovary and tongue (Fig. 1A). Analysis using the TIMER2.0 database indicated that the expression of ADRB1 was significantly increased in cancers such as cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), kidney chromophobe (KICH) and prostate adenocarcinoma (PRAD). In contrast, its expression was significantly reduced in cancers such as breast invasive carcinoma (BRCA), cholangiocarcinoma (CHOL), colon adenocarcinoma (COAD), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), rectum adenocarcinoma (READ) and thyroid carcinoma (THCA) (Fig. 1B). Subsequently, we used the GEPIA2 website to analyze differences of expression of ADRB1 between cancer and normal tissues such as brain lower grade glioma (LGG), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), adrenocortical carcinoma (ACC), thymoma (THYM), sarcoma (SARC), acute myeloid leukemia (LAML), skin cutaneous melanoma (SKCM), uterine carcinosarcoma (UCS), testicular germ cell tumors (TGCT) and ovarian serous cystadenocarcinoma (OV), which data sourced from TCGA and GTEx. The analysis indicated that ADRB1 expression was significantly reduced in LGG (Fig. 1C).

3.2 Association between ADRB1 expression and clinical pathological characteristics

To elucidate the relationship between ADRB1 and cancer clinical features, we analyzed the association between ADRB1 expression and cancer stage. The results indicated that expression of ADRB1 varied significantly with cancer

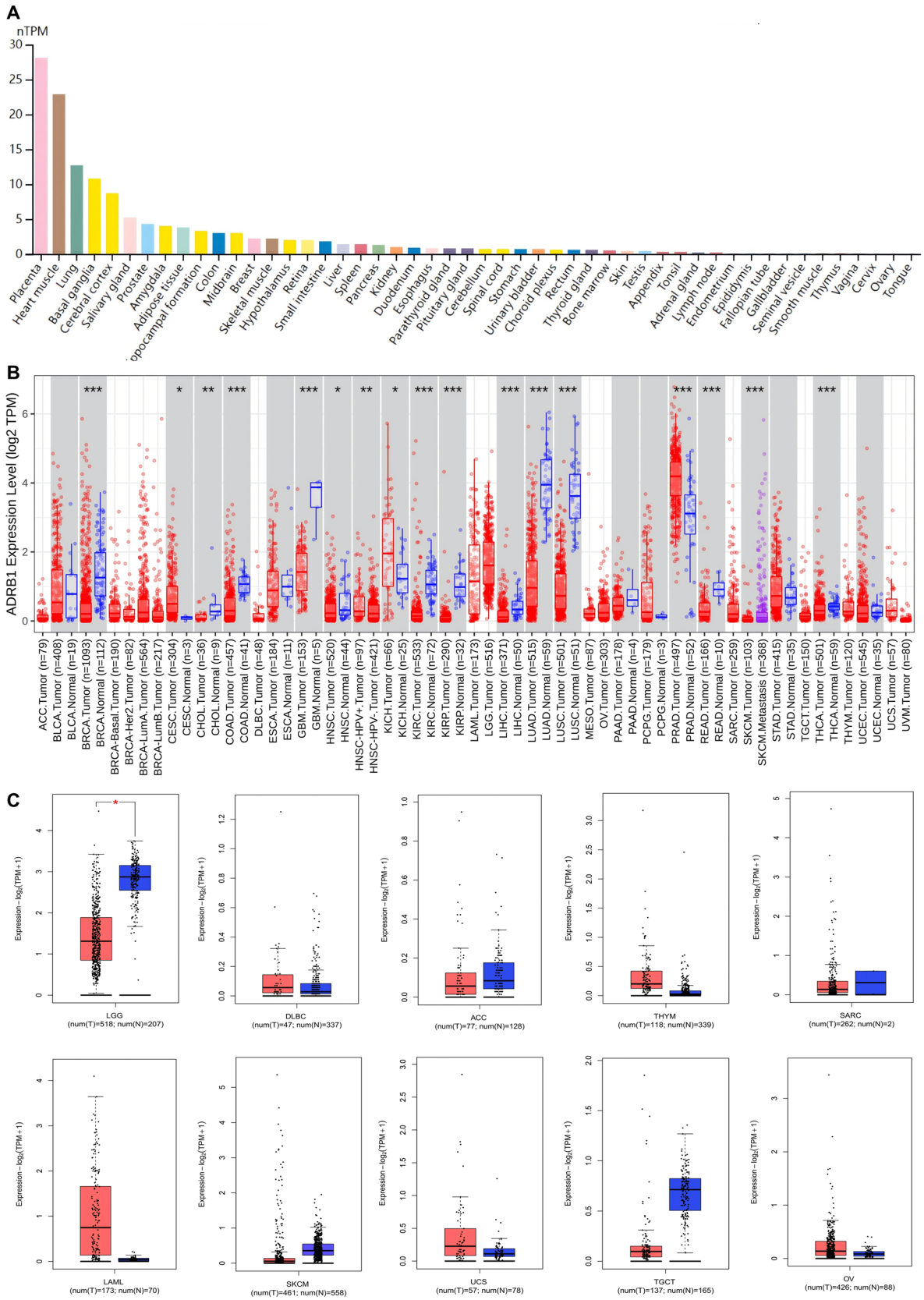

Fig. 1 Expression of ADRB1 in cancer and normal tissues. **A** The expression of ADRB1 in diverse normal tissues. **B** The expression of ADRB1 in cancer and normal tissues from the TCGA pan-cancer dataset of TIMER2.0. **C** The expression of ADRB1 in cancer and normal samples in the TCGA and GTEx combined datasets. Differences between the cancers and normal groups were analyzed using unpaired t-test. *P < 0.05, **P < 0.01, ***P < 0.001

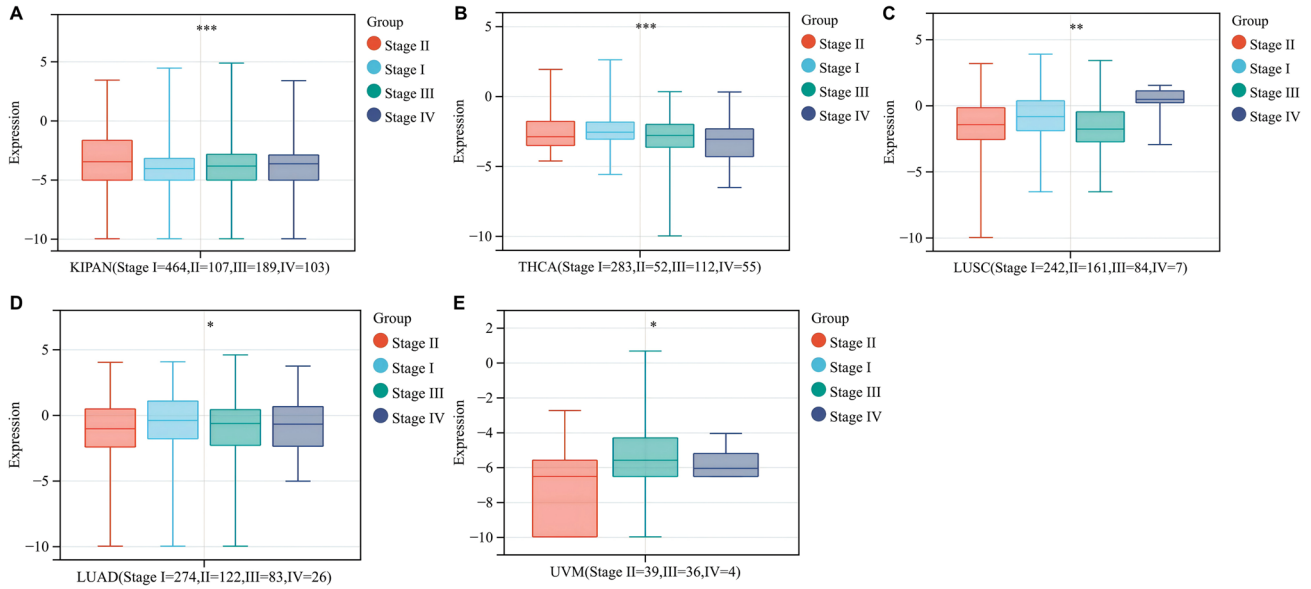

Fig. 2 The relationship between ADRB1 expression and tumor clinical stage, grade. **A**–**E** The expression of ADRB1 was significantly correlated with different clinical stages in KIPAN, THCA, LUSC, LUAD and UVM. Differences between the stage and grade were analyzed using the unpaired t-test. *P < 0.05, **P < 0.01, ***P < 0.001

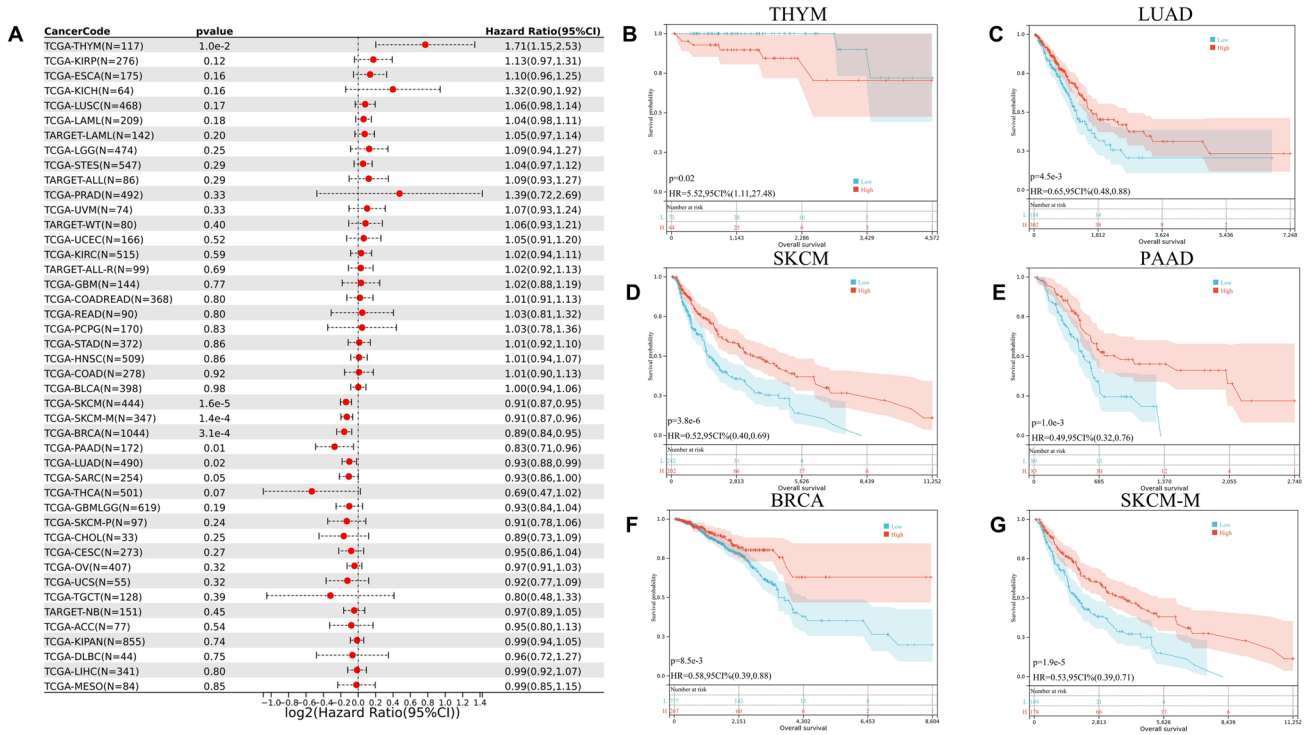

Fig. 3 Relationship between ADRB1 expression and overall survival. **A** Forest plot of the correlation between ADRB1 expression and OS in 33 cancers. **B**–**G** Kaplan–Meier analysis curve of the relationship between ADRB1 expression and OS. The log-rank test was used for comparisons between different groups

staging in cancers such as the pan-kidney cohort (KIPAN), THCA, LUSC, LUAD, and uveal melanoma (UVM). The highest expression levels were observed in stage II of cancers such as THCA, LUAD, and UVM (Fig. 2A–E).

3.3 Prognostic impact of ADRB1 in different cancers

To investigate the relationship between ADRB1 expression and prognosis, we analyzed its relationship with overall survival (OS), disease-free survival (DFS), disease-specific survival (DSS) and progression-free survival (PFS). For OS, Cox proportional hazard model analysis indicated that ADRB1 showed a significant different hazard ratio (HR) in cancers such as THYM ($P = 0.01$), SKCM ($P < 0.001$), SKCM-M ($P < 0.001$), BRCA ($P < 0.001$), pancreatic adenocarcinoma (PAAD) ($P = 0.01$) and LUAD ($P = 0.02$) (Fig. 3A). ADRB1 was identified as a protective factor in SKCM (HR = 0.91), SKCM-M (HR = 0.91), BRCA (HR = 0.89), PAAD (HR = 0.83) and LUAD (HR = 0.93). While it acted as a risk factor in THYM (HR = 1.71) (Fig. 3A, B). Kaplan–Meier survival analysis also indicated that patients with high ADRB1 expression in LUAD, SKCM, PAAD, BRCA and SKCM-M had significantly prolonged OS compared to those with low expression (Fig. 3C–G). External validation using the GEO database also confirmed that high ADRB1 expression was associated with prolonged OS in LUAD, PAAD, SKCM and BRCA (Fig. S1A–E).

We also analyzed the impact of ADRB1 on DFS. The HR of ADRB1 was found to be significant difference in cancers such as KIRP ($P = 0.0093$), PAAD ($P < 0.001$), THCA ($P < 0.001$), BRCA ($P < 0.001$), COAD ($P = 0.01$), colon adenocarcinoma/rectum adenocarcinoma esophageal carcinoma (COADREAD) ($P = 0.04$) and LUAD ($P = 0.05$). ADRB1 was found to be a protective factor in PAAD (HR = 0.60), THCA (HR = 0.59), BRCA (HR = 0.90), COAD (HR = 0.80), COADREAD (HR = 0.83) and LUAD (HR = 0.92), but a risk factor in KIRP (HR = 1.32) (Fig. S2A). Kaplan–Meier survival analysis showed that high ADRB1 expression was associated with better DFS in COAD, PAAD, COADREAD, LUAD and THCA, while low expression was linked to better prognosis in KIRP. In addition, expression of ADRB1 did not have significant effect on DFS in BRCA (Fig. S2B–H).

For DSS, ADRB1 exhibited significant different HR values in cancers such as SKCM ($P < 0.001$), SKCM-M ($P < 0.001$), BRCA ($P < 0.001$), PAAD ($P = 0.03$) and LUAD ($P = 0.04$) (Fig. S3A). Kaplan–Meier analysis confirmed that high ADRB1 expression correlated with favorable DSS in SKCM, BRCA, SKCM-M, PAAD and LUAD (Fig. S3B–F).

In analyzing PFS, ADRB1 showed significant HR in cancers such as PAAD ($P < 0.001$), BRCA ($P < 0.001$), THCA ($P < 0.001$), SKCM ($P = 0.01$), CHOL ($P = 0.01$), SKCM-M ($P = 0.02$) and LIHC ($P = 0.03$) (Fig. S4A). ADRB1 was identified as a protective factor in all of these except for LIHC, which no significant difference was observed (Fig. S4B–H).

Through prognostic analysis, we observed that ADRB1 showed a significant protective effect in cancers such as PAAD, LUAD, COAD, SKCM and SKCM-M. This suggests that ADRB1 may act as a tumor suppressor gene in these cancers. However, this hypothesis requires further experimental validation.

3.4 Relationship between ADRB1 and DNA methylation in cancers

DNA methylation is a common epigenetic modification that regulates gene expression. Abnormal DNA methylation patterns are considered an important factor in cancer development [19]. Studies have shown that the methylation was negatively correlated with gene expression [20]. We compared the methylation values of ADRB1 in normal and cancer tissues. The results showed that, in LUSC, SARC, PAAD, LIHC, KIRC, LUAD, HNSC, BRCA and KIRP, the promoter methylation level of ADRB1 was significantly increased. In metastatic SKCM tissues, the promoter methylation level of ADRB1 was significantly reduced compared to normal tissues (Fig. S5). Comparing these findings with the differential expression results in Fig. 1, the methylation levels in LUSC, SARC, PAAD, LIHC, KIRC, LUAD, HNSC, BRCA and KIRP were negatively correlated with ADRB1 expression. Above results align with the general relationship between promoter methylation and gene expression.

3.5 Mutation analysis of ADRB1 in cancers

Mutation in the genes can affect cancer prognosis and response to therapy [21]. Therefore, we analyzed the mutation status of ADRB1 using the cBioPortal website. The results showed that ADRB1 alterations were mainly in the form of mutations, amplifications and deep deletions across various cancers (Fig. 4A). Stomach adenocarcinoma (STAD) had the highest frequency of genetic alterations, followed by uterine corpus endometrial carcinoma (UCEC) and DLBC, primarily in the form of mutation and deep deletion. Missense were the main type of ADRB1 gene alterations. We also identified an E250K mutation in two cases of bladder urothelial carcinoma (BLCA) (Fig. 4B).

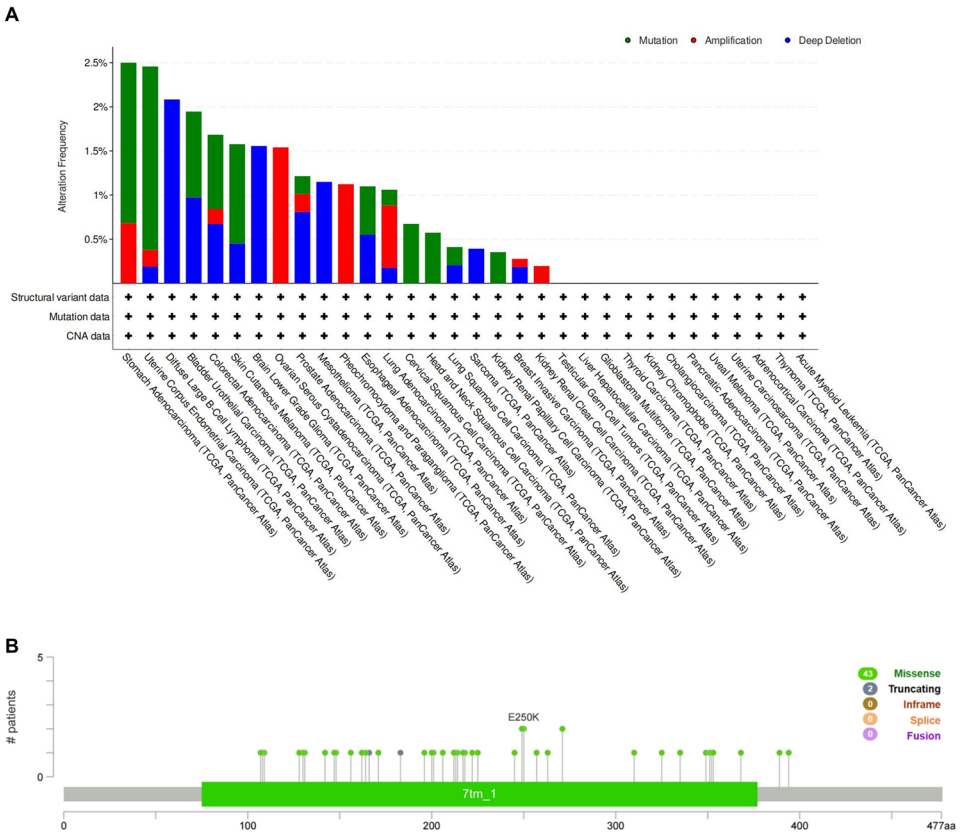

Fig. 4 Mutational characterization of the ADRB1 gene in various cancers. **A** Mutation frequency and type of mutation of the ADRB1 gene in various cancers. **B** Number of mutations in the ADRB1 gene in different cancer types from the TCGA database

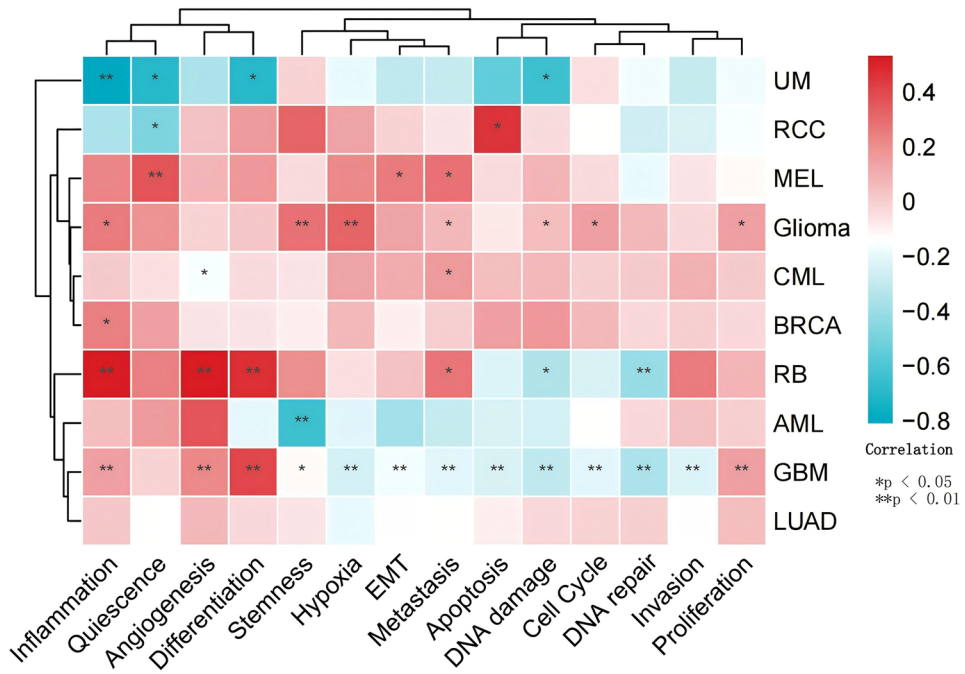

Fig. 5 The relationship between ADRB1 expression and different functional states in cancers was explored using the CancerSEA tool. *P < 0.05; **P < 0.01

We further analyzed the relationship between ADRB1 mutation and patient prognosis. The results showed no statistically significant difference in survival between patients with and without gene mutation (Fig. S6). This suggested that ADRB1 gene mutation was not the primary factor affecting patient prognosis.

3.6 Correlation between ADRB1 expression and various biological functions

Using the CancerSEA website, we obtained data on the correlation between ADRB1 and 14 functional states across 10 cancers, and visualized the results using the "pheatmap" package in R language. Figure 5 showed that expression of ADRB1 was negatively correlated with functions such as hypoxia, epithelial–mesenchymal transition (EMT), metastasis, apoptosis, DNA damage, cell cycle, DNA repair and invasion. But positively correlated with inflammation, angiogenesis, differentiation, stemness and proliferation in GBM. In uveal melanoma (UM), the expression of ADRB1 was negatively

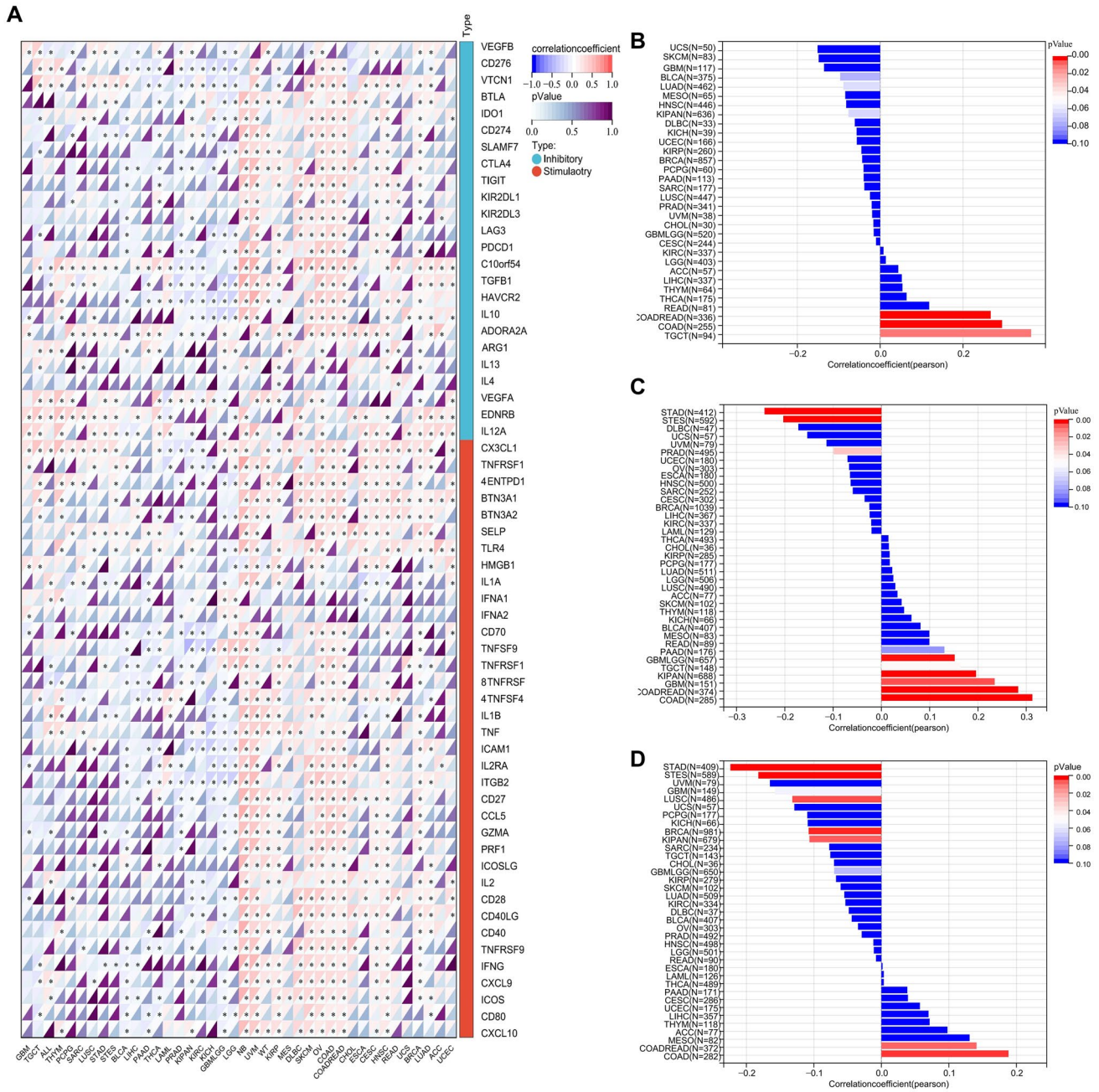

Fig. 6 The relationship between ADRB1 expression and tumor immune biomarkers. **A** The relationship between ADRB1 expression and ICP genes [inhibitory (24) and stimulatory (36)] in pan-cancer patients. Each small rectangular module represents the co-expression of immune-related genes and ADRB1 across various cancers. The color in the upper left corner represents the correlation coefficient (Cor). The star and color in the lower right corner represent the P-value. *P < 0.05. **B**–**D** The relationship between ADRB1 expression and **B** TMB, **C** MSI and **D** neoantigens

correlated with biological behaviors such as inflammation, quiescence, differentiation and DNA damage. Additionally, in retinoblastoma (RB), ADRB1 expression was negatively correlated with DNA damage and DNA repair, while positively correlated with biological processes such as inflammation, angiogenesis, differentiation and metastasis.

3.7 Correlation between ADRB1 expression and immune checkpoint genes, TMB, MSI and neoantigens

ICPs are composed of stimulatory and inhibitory molecules [22]. Their activation helps cancer cells evade immune surveillance [23]. We analyzed the correlation between ADRB1 expression and ICP genes. The results showed that the expression of ADRB1 was negatively correlated with the expression of most ICP genes in various cancers. However, in cancers such as high-risk Wilms tumor (WT), KIRP, COAD and COADREAD, the expression of ADRB1 was positively correlated with most immune checkpoint genes (Fig. 6A). This supports our hypothesis that high ADRB1 expression may have an anti-cancer effect in multiple cancer types.

TMB, MSI and neoantigens are promising tumor immune-related biomarkers for guiding immunotherapy [24–27]. Therefore, we analyzed the correlation between ADRB1 expression and TMB, MSI and neoantigens. The results showed that ADRB1 expression was positively correlated with TMB in COADREAD, COAD and TGCT, suggesting that low ADRB1 expression might inhibit cancer immune responses in these cancers (Fig. 6B). The expression of ADRB1 was also positively correlated with MSI in COAD, COADREAD, GBM, KIPAN, TGCT and GBMLGG, and negatively correlated in STAD, STES and PRAD (Fig. 6C). Additionally, ADRB1 expression was positively correlated with neoantigens in COAD and COADREAD, while showing a negative correlation in STAD, STES, GBM, BRCA and KIPAN (Fig. 6D). These findings further suggested that high ADRB1 expression might enhance the effectiveness of immune therapy in certain cancers, indicating its potential as a biomarker for immune therapy response.

3.8 Correlation analysis between ADRB1 expression and cancer immune infiltration

Immune infiltration is closely related to cancer occurrence, progression and metastasis [28, 29]. We used multiple algorithms, including TIMER, EPIC, MCPCOUNTER, CIBERSORT, QUANTISEQ, XCELL and TIDE to explore the correlation between ADRB1 expression and immune cell infiltration in various cancers. The results indicated that, in BRCA, CESC, COAD, KIRC and PAAD, $CD8^+$T cell infiltration was positively correlated with ADRB1 expression (Fig. 7A). In COAD, UVM, SKCM, READ and LUAD, macrophage infiltration was also significantly positively correlated with ADRB1 expression. Whereas in BLCA, LGG, KICH and PRAD, macrophage infiltration was significantly negatively correlated (Fig. 7B). In COAD, HNSC and KIRP, the expression of ADRB1 was positively correlated with myeloid dendritic cell infiltration. Whereas in LGG and THCA, a negative correlation was observed (Fig. 7C). In LIHC, TGCT, THYM and UVM, B cell infiltration was negatively correlated with ADRB1 expression. While in other cancers, a positive correlation was observed (Fig. 7D). In SARC and SKCM, $CD4^+$T cell infiltration was positively correlated with ADRB1 expression. Whereas in LGG, LUAD and LIHC, a negative correlation was observed (Fig. 7E). Neutrophils infiltration was positively correlated with ADRB1 expression in all cancers except LGG (Fig. 7F).

The ESTIMATE score is a method used to predict the stromal and immune cell content in malignant cancer tissues based on gene expression data. It is valuable for evaluating cancer diagnosis and prognosis [30, 31]. The ESTIMATE score includes three main scores: stromal score, immune score and estimate score. To further elucidate the relationship between ADRB1 expression and immune infiltration, we analyzed the ESTIMATE scores of ADRB1 across various cancers. The stromal score represents the presence of stroma in cancer tissues. In stromal score, we observed a significant correlation in 23 cancers. In LAML, BRCA, KIRP, COAD, COADREAD, THYM, WT, SKCM-P, SKCM, SKCM-M, NB, OV, UVM, TGCT and PCPG, the expression of ADRB1 was positively correlated with the stromal score. While in GBM, GBMLGG, LGG, PRAD, KIRC, BLCA, THCA and PAAD, it was negatively correlated (Fig. S7).

The immune score represents the degree of immune cell infiltration in cancer tissues, including various immune cells such as T cells, B cells and natural killer cells. In LAML, BRCA, COAD, COADREAD, HNSC, WT, SKCM, SKCM-M, NB, MESO, OV, UVM, ALL-R and DLBC, ADRB1 expression was positively correlated with the immune score. Whereas in GBM, GBMLGG, LGG, KIPAN, PRAD, BLCA, THCA and TGCT, it was negatively correlated (Fig. S8).

The estimate score is used to infer the cancer purity, namely the ratio of cancer cells to normal cells, which helps assess the malignancy and prognosis of the cancer. In LAML, BRCA, KIRP, COAD, COADREAD, HNSC, WT, SKCM-P, SKCM, SKCM-M, NB, OV, UVM, ALL-R and CHOL, ADRB1 was positively correlated with the estimate score. Whereas in GBM, GBMLGG, LGG, PRAD, KIRC, BLCA, THCA, PAAD, it was negatively correlated (Fig. S9).

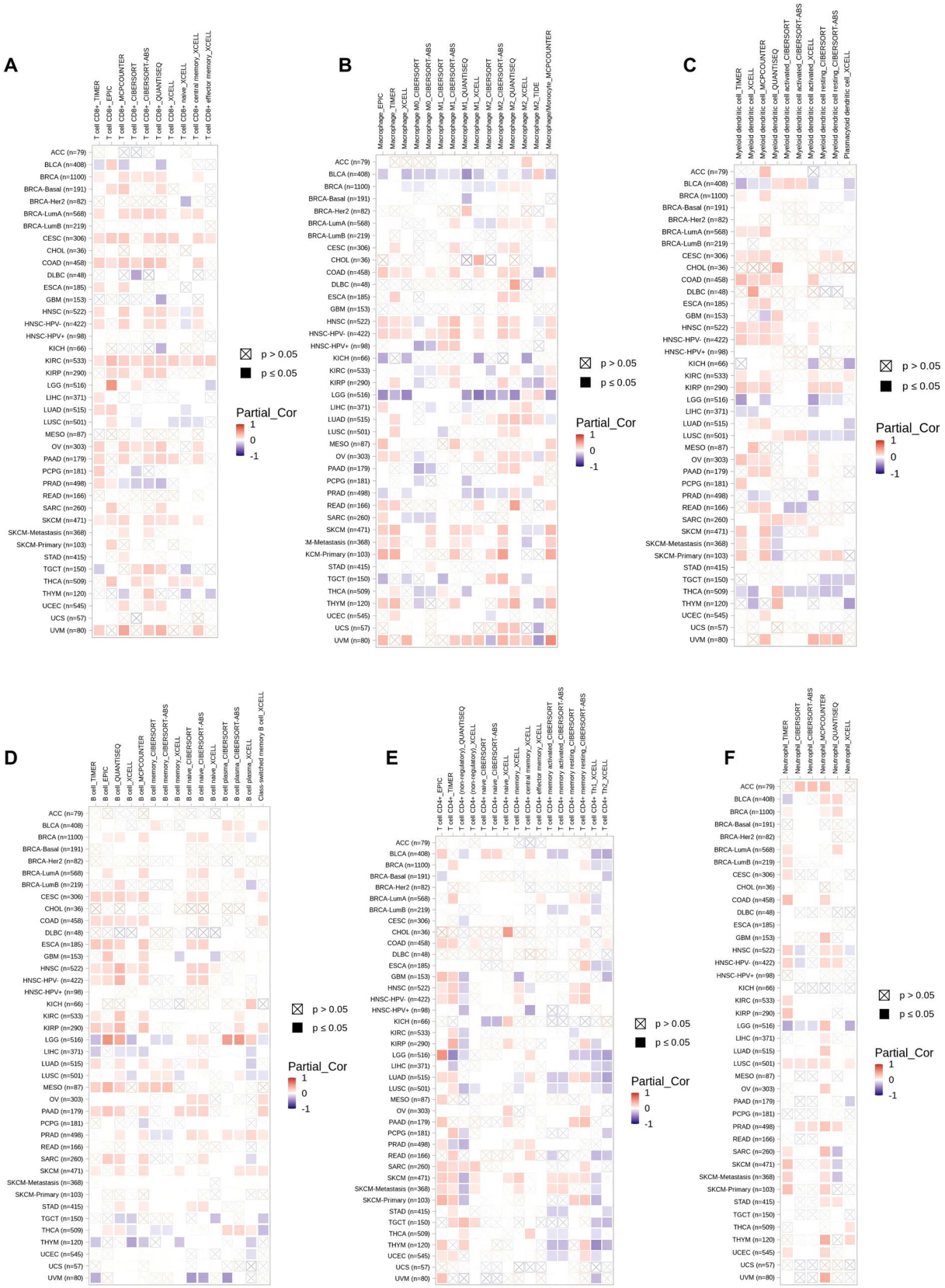

Fig. 7 Correlation between immune cells infiltration and ADRB1 expression. **A**–**F** The relationship of ADRB1 expression with $CD8^+$T cells (**A**), macrophages (**B**), myeloid dendritic cells (**C**), B cells (**D**), $CD4^+$T cells (**E**) and neutrophils (**F**) are described through the TIMER2.0 database

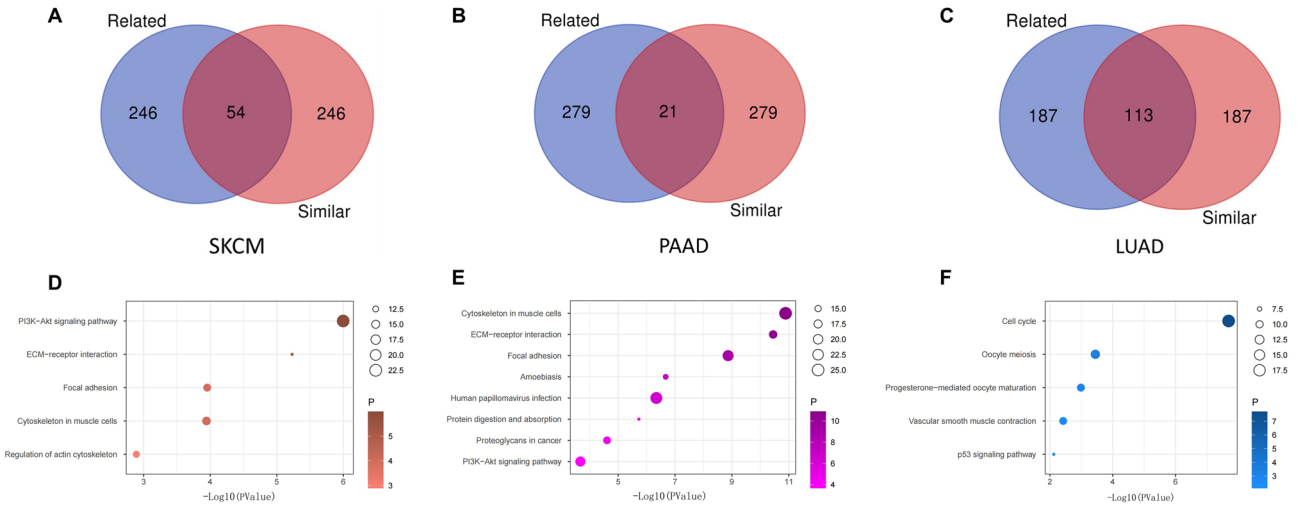

Fig. 8 Functional enrichment analysis of ADRB1 related genes. **A**–**C** Venn diagram shows the intersection between ADRB1 binding genes and ADRB1 related genes in SKCM (**A**), PAAD (**B**), LUAD (**C**) cancers, respectively. **D**–**F** KEGG pathway analysis results of ADRB1 binding genes and ADRB1 related genes in SKCM (**D**), PAAD (**E**), LUAD (**F**)

3.9 Functional enrichment analysis of ADRB1 related genes

Next, we utilized functional enrichment analysis to evaluate the potential molecular mechanism of ADRB1 in tumorigenesis and development. Venn diagrams were used to show the intersection of ADRB1 related genes and the similar genes of ADRB1 of three cancers, and the number of common genes of SKCM, PAAD and LUAD were 54, 21 and 113 respectively (Fig. 8A–C). After that, we combined the above two datasets and used DAVID to perform KEGG pathway function enrichment analysis of ADRB1 related genes and the similar genes of ADRB1. The results showed PI3K/Akt signaling pathway was enriched in SKCM and PAAD (Fig. 8D, E). ADRB1 was also involved in the occurrence of LUAD through cell cycle, oocyte meiosis and other signaling pathway (Fig. 8F).

4 Discussion

The sympathetic nervous system plays an important role in the progression of cancers [1]. Cancer tissues are innervated by nerves, and neurotransmitters may influence cancer development and metastasis [32, 33]. Clinical studies in malignant melanoma have shown that β-blockers can prolong patient survival [34], suggesting that β-adrenergic receptors may play a key role in cancer development. Both β_1-AR and β_2-AR are subtypes of β-ARs [6]. NE can activate both receptors [35]. Most research has focused on the role of β_2-AR in cancer development. In studies of breast cancer cell invasiveness, it was found that the incidence of stage T4 cancers did not significantly differ between users and non-users of the selective β_1-AR blocker atenolol, whereas the use of the non-selective β-ARs blocker propranolol significantly reduced cancer grade [36]. Zhang et al. demonstrated that blocking β_2-AR induces pancreatic cancer cells to arrest in the G1/S phase, leading to cancer cell death [37]. Zhang et al. showed that selective β_1-AR antagonists, such as atenolol, had little effect on cancer cell proliferation and invasion in vivo and in vitro, whereas β_2-AR antagonists could inhibit gastric cancer development, metastasis and angiogenesis [38]. These results suggest that NE released from sympathetic nerve endings promotes cancer development through β_2-AR. This may also explain why the role of β_1-AR in cancer progression has been largely neglected. Moreover, considering that β_1-AR has a higher affinity for NE than β_2-AR [39], understanding the role of β_1-AR in cancer progression and its impact on patient prognosis is crucial.

In this study, we investigated the differential expression of ADRB1 between cancer and normal tissues. Compared to normal tissues, ADRB1 expression was significantly increased in three cancers (CESC, KICH, PRAD). Whereas it was significantly reduced in many other cancers (BRCA, CHOL, COAD, GBM, HNSC, KIRC, KIRP, LIHC, LUAD, LUSC, READ, THCA, LGG). This suggested that ADRB1 might have cancer-suppressive effects in most cancers. Moreover, analysis

of ADRB1 in relation to clinical features indicated that ADRB1 expression generally decreased with the progression of cancer. Survival analysis showed that patients with low ADRB1 expression in LUAD, SKCM, PAAD and BRCA have a better prognosis. Therefore, we hypothesized that ADRB1 may be a protective factor.

We also focused that ADRB1 expression is upregulated in some cancers (CESC, KICH, PRAD) but downregulated in others. The dual effects of ADRB1 may arise from the different cellular mechanisms, microenvironment, and metabolic pathways of different types of cancers, so that the role of genes may exhibit very different effects in different cancers. For example, p53 may protect normal cells from carcinogenesis by promoting apoptosis [40, 41]. While in other cases, mutations in p53 may cause cells to lose their ability to apoptosis and thus promote tumor formation [42]. This difference in mechanisms allows us to understand the role of genes only in the context of specific cancers.

Subsequently, our methylation analysis indicated that ADRB1 promoter methylation levels were inversely correlated with ADRB1 expression in several cancers, such as LUSC, SARC, PAAD, LIHC, KIRC, LUAD, HNSC, BRCA and KIRP. This suggested that DNA methylation may contribute to the suppression of ADRB1 expression in above cancers. In cancers like LUAD and LIHC, the methylation results were consistent with differential expression analysis, clinical pathology and survival analysis results, further supporting the notion that ADRB1 has the potential to inhibit cancer progression. As for the clinical application of DNA methylation, in lung adenocarcinoma, DNA methylation profiling can identify different subgroups. These subgroups not only have significant differences in the composition of immune cells, but also have different DNA methylation ages and clinical outcomes. This finding provides a new idea for the individualized treatment of lung adenocarcinoma [43]. DNA methylation can also be used to accurately distinguish different histological stages of hepatocellular carcinoma; this differential ability is of great significance for the early diagnosis and treatment of liver cancer [44]. However, the clinical application of ADRB1 methylation in cancer therapy remains to be further studied.

Tumor immune microenvironment (TIME) is an important part of TME, which is closely related to tumor immunity in tumor progression [2, 45]. Globig et al. found that ADRB1 expression increased in exhausted $CD8^{+}$T cells and may serve as an immune checkpoint [46], suggesting that ADRB1 may play an important role in the immune system. In our results, ADRB1 is positively correlated with the immune score of some cancers, such as BRCA, COAD, CHOL, SKCM. These results suggest that ADRB1 may participate in tumor immune regulation by mediating immune infiltration. In addition, in the previous differential analysis, the expression of ADRB1 decreased in the above tumors, suggesting that ADRB1 expression in the above cancers may assist tumor cells in immune evasion. In addition, ADRB1 expression was positively correlated with most immune checkpoint genes in WT, KIRP, COAD, and COADREAD, suggesting that ADRB1 may be a tumor immunotherapy target.

Our study further elucidated the biological function of ADRB1 through gene function enrichment analysis. The results from the functional enrichment analysis of SKCM and PAAD indicated that ADRB1 potentially regulates tumor progression via the PI3K–Akt signaling pathway. The PI3K/AKT signaling pathway, a crucial pathway widely present in cells, regulates the invasion and metastasis of tumor cells [47]. In LUAD, the cell cycle pathway was enriched. In cells, cyclins and their regulatory proteins (CDKs) regulate the cell cycle, thereby influencing cell proliferation [48]. When β-ARs are activated, they modulate the expression of cyclins and their regulatory proteins by regulating downstream signaling pathways, subsequently regulating the progression of the cell cycle [7, 8]. This suggests that ADRB1 may influence the occurrence and progression of LUAD by regulating the cell cycle signaling pathway.

Our study demonstrated that ADRB1 acts as a protective factor in several cancers. The results suggested that ADRB1 may be a novel cancer suppressor gene capable of inhibiting cancer development in cancers such as LUAD and LIHC. These findings provided a new direction for future cancer treatment, but there are still many basic and clinical researches to carry out when it comes to clinical application.

Regarding the advantages, we screened the effects of ADRB1 on different cancer species based on public databases and data platforms. It provides a new idea for the follow-up tumor treatment research. We are also keenly aware of the limitations in the article. In this study, there is a lack of experimental verification of the role of ADRB1 in cancer, but the analysis of public data can help us quickly understand the cancer species in which ADRB1 has the potential to be a tumor suppressor gene and prognostic biomarker. Secondly, a variety of databases were jointly applied in this study. Differences in data sources may have skewed the results. However, when we specifically analyzed certain types of results, we used the same batch of data to ensure that there was no batch effect in the data. In addition, the results obtained by using different databases or platforms are consistent, which further confirms the reliability of our findings.

5 Conclusions

In conclusion, our study revealed the previously underestimated protective role of ADRB1 in multiple malignancies. ADRB1 expression is downregulated in most solid tumors and is significantly associated with the prognosis of tumor patients. In pancreatic cancer, lung adenocarcinoma and other cancers, high expression of ADRB1 can prolong the survival of patients and play a protective role. These findings suggest that ADRB1 has the potential to be a tumor suppressor gene and prognostic biomarker. However, further basic research needs to be carried out to verify its tumor suppressive potential.

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Data availability The datasets supporting the conclusions of this article are available in the website GEO (https://www.ncbi.nlm.nih.gov/geo/), TIMER2.0 (https://cistrome.shinyapps.io/timer/), GEPIA2 (http://gepia2.cancer-pku.cn/#analysis), Sangerbox (http://Sangerbox.com/Tool), UALCAN website (https://ualcan.path.uab.edu/analysis-prot.html), cBioPortal website (https://www.cbioportal.org/), and CancerSEA website (http://biocc.hrbmu.edu.cn/CancerSEA/home.jsp).

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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