ADRB2 Expression Predicts the Clinical Outcomes and is Associated with Immune Cells Infiltration in Lung Adenocarcinoma

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Research Article

Keywords: lung adenocarcinoma, ADRB2, immune cells infiltration, biomarker, prognosis

Posted Date: January 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1256564/v1

Abstract

The gene encoding beta2-adrenergic receptor (β2-AR), adrenoceptor beta 2 (*ADRB2*), has been reported to closely associated with various cancers. However, its role in lung adenocarcinoma (LUAD) remains controversial. This research shed light on the prognostic value of *ADRB2* in LUAD and further explored its association with immune cell infiltration. *ADRB2* was significantly decreased in LUAD. *ADRB2* expression in LUAD was significantly correlated with gender, smoking status, T classification, lymph node metastasis, and pathologic stage. Patients in the low *ADRB2* expression group presented with significantly poorer overall survival (OS) and disease-specific survival (DSS). Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Set Enrichment Analysis (GSEA) results showed that *ADRB2* participates in immune response. The expression of *ADRB2* was positively correlated with the infiltration level of most immune cells. Notably, *ADRB2* is involved in LUAD progression partly by regulating the immune microenvironment, which may potentially serve as a significant prognostic biomarker as well as a potential drug target.

Introduction

Lung cancer is the primary cause of malignant tumor mortality globally [1]. LUAD, one of the highest mortality rates and most aggressive forms of cancer, with a low 5-year survival rate <5% [2]. Late diagnosis may lead to difficulties in the treatment and prediction of prognosis. Thus, an in-depth study of the molecular mechanisms underlying LUAD progression is urgently needed. At present, there remains an unmet clinical need for tumor biomarkers, and the search for these could lead to more effective treatments and longer survival.

G-protein-coupled receptors (GPCRs) consist of a large family of integral membrane proteins with seven transmembrane helices. Adrenergic receptors (ARs), a member of GPCRs, are classically divided into two main groups: α-and β-adrenoceptors (β-AR, which is divided into β1, β2, and β3 subtypes) [3]. β-AR could facilitate cell proliferation, migration, invasion, inflammation, angiogenesis, apoptosis, cell immune response, and epithelial-mesenchymal transition by regulating multiple cancer-related cellular processes. Dysregulated expression of β2-AR was observed in various cancers, including breast cancer [4], hepatocellular carcinoma [5], prostate cancer [6], and ovarian carcinoma [7]. Moreover, abundant β2-AR expression was found to be closely linked with poor clinicopathological characteristics, tumor recurrence, metastasis, and poor prognosis. Although β2-AR is a carcinogenic biomarker; however, the clinical significance of its expression in patients with LUAD has not been thoroughly elucidated yet.

It is well known that the tumorigenesis, progression, OS, prognosis, and relapse of tumors are strongly linked to the expression of tumor genes. The gene encoding β2-AR, *ADRB2*, maps to human chromosome 5q31–q32 and is composed of a single exon of 2015 nucleotides [8]. The effect of *ADRB2* on lung cancer remains controversial. Mei et al. [9] identified *ADRB2* polymorphisms that were correlated with increased lung cancer risk. Nevertheless, Zheng et al.[1] found that *ADRB2* was underexpressed in LUAD tissues and low *ADRB2* expression is associated with poor clinical outcomes. The other research by Wang et al. reached the same conclusion [10].

Based on The Cancer Genome Atlas (TCGA) dataset, LUAD dataset was acquired for bioinformatics analysis to verify that *ADRB2* expression was significantly down-regulated in LUAD. Next, the relationship between *ADRB2* gene expression and clinical traits was further investigated. The expression of *ADRB2* was highly correlated with immune infiltration, which further confirmed that *ADRB2* could be used as a prognostic biomarker of LUAD.

Results

Patient characteristics

Patient characteristics are summarized in Table 1, including sex, age, smoking status, TNM stage, and pathologic stage.

Table 1
Demographic information of patients with lung LUAD

Characteristic		Number (%)
	535	
Gender	Female	286 (53.5%)
	Male	249 (46.5%)
Age	<=65	255 (49.4%)
	>65	261 (50.6%)
Smoker	No	75 (14.4%)
	Yes	446 (85.6%)
T stage	T1	175 (32.9%)
	T2	289 (54.3%)
	T3	49 (9.2%)
	T4	19 (3.6%)
N stage	N0	348 (67.1%)
	N1	95 (18.3%)
	N2	74 (14.3%)
	N3	2 (0.4%)
M stage	M0	361 (93.5%)
	M1	25 (6.5%)
Pathologic stage	Stage I	294 (55.8%)
	Stage II	123 (23.3%)
	Stage III	84 (15.9%)
	Stage IV	26 (4.9%)

ADRB2 Expression Level in LUAD

Based on the TCGA database, *ADRB2* mRNA expression level was analyzed in 594 tissues. Box plots showed *ADRB2* mRNA expression levels in 59 adjacent non-tumor tissues and 535 LUAD tissues. As shown in Figure 1A, *ADRB2* was down-expression in LUAD tissues compared with those in normal tissues (P<0.001, Figure 1A). Moreover, *ADRB2* was significantly lower in males (P<0.001, Figure 1C) and in patients with a smoking history (P<0.001, Figure 1D).

Association between ADRB2 and TNM Stages in LUAD Patients

To better understand the impact of *ADRB2* on LUAD patient prognosis, Kruskal–Wallis analysis was performed to determine the relationship between *ADRB2* expression and clinicopathological characteristics (pathologic and TNM stages). *ADRB2* expression was significantly decreased in LUAD patients (Figure 2A-D). It is noteworthy that *ADRB2* expression was inversely correlated with T stage (Figure 2B).

Relationship between ADRB2 and Clinical Characteristics

To further investigate the mechanism of *ADRB2* in LUAD, the associations between *ADRB2* expression and clinical characteristics were investigated. Based on the clinical data of 535 patients with LUAD, logistic regression analysis indicated that the expression level of *ADRB2* in LUAD was negatively correlated with gender (OR=0.502 for males vs. females, P<0.001); smoking status (OR=0.489 for yes vs. no, P=0.006); T classification (OR=0.603 for T2 vs. T1, P=0.009; OR=0.346 for T3 vs. T1, P=0.002; OR=0.232 for T4 vs. T1, P=0.007); lymph node metastasis (OR=0.648 for positive vs. negative, P=0.021); and pathologic stage (OR=0.513 for stage III vs. stage I, P=0.008, Figure 3).

Impact of ADRB2 on the Prognosis of LUAD

Survival curves were derived to assess the prognosis of high and low-*ADRB2* expression in LUAD patients. As displayed in Figure 3, patients in the low *ADRB2* expression group presented with significantly poorer OS (P=0.001, Figure 4A) and DSS (P=0.005, Figure 4B) than those in the high *ADRB2* expression group. However, PFI did not differ between the two groups (P=0.679, Figure 4C).

Effect of ADRB2 Expression on Survival Based on Univariate and Multivariate analyses

Univariate analysis revealed that pathological stage (HR, 2.664; 95% CI, 1.960-3.621; P<0.001); T stage (HR, 2.317; 95% CI, 1.591-3.375; P<0.001); N stage (HR, 2.601; 95% CI, 1.944-3.480; P<0.001); M stage (HR, 2.136; 95% CI, 1.248-3.653; P=0.006); and *ARDB2* expression (HR, 0.612; 95% CI, 0.456-0.821; P=0.095) were meaningful indicators of survival (Table 2). However, *ADRB2* expression was not an independent prognostic factor in patients with LUAD at multivariable analysis (HR, 0.703; 95% CI, 0.504-1.056; P=0.095, Table 2).

Table 2
Univariate and multivariate analyses of *ADRB2* and clinical pathological parameters associated with survival in patients with LUAD

Parameter	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*	HR	95% CI	*P*
Age	1.223	0.916-1.635	0.172	1.256	0.881-1.792	0.208
Smoker	0.894	0.592-1.348	0.591	0.896	0.536-1.498	0.676
Gender	1.070	0.803-1.426	0.642	0.969	0.681-1.379	0.861
Pathological stage	2.664	1.960-3.621	**<0.001**	1.341	0.805-2.233	0.260
T stage	2.317	1.591-3.375	**<0.001**	1.817	1.119-2.951	**0.016**
N stage	2.601	1.944-3.480	**<0.001**	2.086	1.387-3.139	**<0.001**
M stage	2.136	1.248-3.653	**0.006**	1.263	0.654-2.450	0.490
ADRB2	0.612	0.456-0.821	**0.001**	0.730	0.504-1.056	0.095

Evaluation of the Diagnostic Capacity of ADRB2 in LUAD

To explore the diagnostic value of *ADRB2* for LUAD, receiver operating characteristic (ROC) curve analysis was performed. The results of the ROC curves indicated that *ADRB2* was highly sensitive to the diagnosis of LUAD (AUC, 0.994; 95% CI: 0.989-0.999, Figure 5A). Additionally, the AUC was 0.598 for OS, which indicated that the prognostic model had good performance in predicting survival prognosis of patients with LUAD (95% CI: 0.548-0.648, Figure 5B).

Relationship of ADRB2 Expression Level with Immune Infiltration in LUAD

Pearson's analysis demonstrated that the infiltration of 19 types of immune cells was markedly related to *ADRB2* expression, which had a significantly positive relationship with activated DCs (aDCs) and a strongly-positive association with B cells, cytotoxic cells, dendritic cells (DCs), eosinophils, immature DCs (iDCs), macrophages, mast cells, neutrophils, natural killer (NK) cells, neutrophils, plasmacytoid DCs (pDCs), T cells, T helper cells, Tcm T central memory (Tcm), T effector memory (Tem), T follicular helper (TFH), type 1 Th cells (Th1), and type 17 Th cells (Th17) (P<0.001, Figure 6). However, T gamma delta (Tgd) and type 2 Th cells (Th2) (P<0.001, Figure 6) showed a negative association with *ADRB2*.

ADRB2 Associated Gene Set Enrichment in LUAD

To determine *ADRB2*-related signaling pathways, GSEA was performed between the high- and low-*ADRB2* groups. Significance was assessed using a normalized enrichment score (NES)≥1.5, P≤0.05, and false discovery rate (FDR)≤0.25. KEGG pathway enrichment analysis indicated that 13 important signaling pathways were significantly enriched in the highly expressed *ADRB2* phenotypes, including the JAK STAT signaling pathways, leukocyte trans-endothelial migration, chemokine signaling pathway, autoimmune, thyroid disease, Fc epsilon ri signaling pathway, intestinal immune network for iga production, cytokine receptor interaction, B cell receptor signaling pathway, NK cell-mediated cytotoxicity, allograft rejection, Mapk signaling pathway, T cell receptor signaling pathway, and NSCLC. Meanwhile, there were 13 eligible signaling pathways enriched in the low-*ADRB2* expression, including spliceosome, RNA polymerase, RNA degradation, citrate cycle (or TCA cycle), cell cycle, pentose phosphate pathway, basal transcription factors, oxidative phosphorylation, DNA replication, mismatch repair, cysteine and methionine metabolism, ubiquitin-mediated-proteolysis, and amino sugar and nucleotide sugar metabolism (Table 3, Figure 7). These results contribute to further exploration of *ADRB2* pathophysiological mechanisms.

Table 3
Gene sets enriched in the low and high *ADRB2* expression phenotypes.

Low expression				High expression			
Gene set name	NES	NOM p-value	FDR q-value	Gene set name	NES	NOM p-value	FDR q-value
KEGG_SPLICEOSOME	-2.229	0	0	KEGG_JAK_STAT_SIGNALING_PATHWAY	2.170	0	0.004
KEGG_RNA_POLYMERASE	-2.199	0	0	KEGG_LEUKOCYTE_ TRANSENDOTHELIAL_ MIGRATION	2.170	0	0.004
KEGG_RNA_DEGRADATION	-2.183	0	0.001	KEGG_CHEMOKINE_ SIGNALING_PATHWAY	2.037	0.003	0.010
KEGG_CITRATE_CYCLE_TCA_CYCLE	-2.126	0	0.002	KEGG_AUTOIMMUNE_ THYROID_DISEASE	2.036	0.002	0.008
KEGG_CELL_CYCLE	-2.103	0.001	0.002	KEGG_FC_EPSILON_RI _SIGNALING_PATHWAY	2.019	0.001	0.009
KEGG_PENTOSE_PHOSPHATE_PATHWAY	-2.075	0	0.003	KEGG_INTESTINAL_ IMMUNE_NETWORK_FOR_ IGA_PRODUCTION	2.009	0.004	0.009
KEGG_BASAL_ TRANSCRIPTION_FACTORS	-2.025	0	0.004	KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTER ACTION	1.996	0.007	0.010
KEGG_OXIDATIVE_PHOSPHORYLATION	-1.998	0.003	0.005	KEGG_B_CELL_RECEPT OR_SIGNALING_PATHWAY	1.969	0.006	0.010
KEGG_DNA_REPLICATION	-1.981	0	0.005	KEGG_NATURAL_KILLER_ CELL_MEDIATED_ CYTOTOXICITY	1.825	0.010	0.033
KEGG_MISMATCH_REPAIR	-1.896	0.012	0.031	KEGG_ALLOGRAFT_ REJECTION	1.825	0.012	0.031
KEGG_CYSTEINE_AND_ METHIONINE_METABOLISM	-1.798	0.001	0.025	KEGG_MAPK_SIGNALING_ PATHWAY	1.821	0.001	0.031
KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS	-1.743	0.014	0.037	KEGG_T_CELL_RECEPTOR_ SIGNALING_PATHWAY	1.805	0.022	0.031
KEGG_AMINO_SUGAR_AND_ NUCLEOTIDE_SUGAR_ METABOLISM	-1.654	0.025	0.056	KEGG_NON_SMALL_CELL_ LUNG_CANCER	1.708	0.014	0.049

Discussion

LUAD is a type of malignant lung tumor that originates from the bronchial mucosal glandular epithelium. Early diagnosis is difficult in most patients. LUAD is characterized by inconspicuous early symptoms, and LUAD is a lung tumor with a significant rate of malignant recurrence, metastasis, and unsatisfactory prognosis. Currently, targeted therapy, chemotherapy, immunotherapy, radiation therapy, and surgery remain the mainstays of therapy for LUAD, but the limitations of these therapies necessitate the development of effective approaches. Despite the remarkable importance of LUAD, a disease that affects millions of people worldwide, its exact pathogenesis is not fully characterized, and many aspects of the disease remain controversial.

ADRB2 is ubiquitously expressed in multiple tissues, including the smooth muscle of the human bronchi, cardiovascular system, central nervous system, and gastrointestinal tract. It has been proposed that gene expression, receptor function, and ligand response could be potentially affected by genetic polymorphisms. In recent years, polymorphisms in *ADRB2* gene at different loci have been studied in various diseases, and increasing evidence shows that *ADRB2* has a vital place in the occurrence and development of diverse range of cancers. Zhang et al. [11] found that the mRNA expressions of *ADRB2* were higher in gastric cancers compared with normal tissues. Moreover, patients with gastric cancer with positive *ADRB2* expression exhibited larger tumor size, late clinical stage, lower differentiation, and distant metastasis. In addition, high *ADRB2* expression can promote the angiogenic switch in prostate cancer

and prevent or delay the dominant role of pro-angiogenic factors, leading to tumor progression [12]. β2-AR is encoded by *ADRB2* and can bind specifically to endogenous catecholamines (such as adrenaline and noradrenaline), and promotes the production and release of cyclic adenosine phosphate (cAMP). cAMP can further activate and phosphorylate protein kinase A and C to activate downstream signal transduction pathways and promote the proliferation, migration, and metastasis of lung cancer cells [13]. The positive β2-AR expression can occur in several cancers, including hepatocellular carcinoma, colorectal cancer, melanoma, and gastric cancer, and is often indicative of poor prognosis [5,11,14–16]. Nevertheless, in oral squamous cell carcinoma, patients with higher β2-AR had a significant longer DSS and OS [17,18]. Yazawa et al.[19] retrospectively analyzed 328 surgically-resected patients with NSCLC and found that positive β2-AR expression was found in 29% of LUAD tissues, which markedly increased compared with in non-adenocarcinoma tissues. A high level of β2-AR expression was associated with vascular invasion, tumor cell proliferation, and poor prognosis in patients with LUAD. Nevertheless, Wang et al. [10] searched the gene expression synthesis (GEO) to obtain data showing that *ADRB2* is down-regulated in LUAD, and *ADRB2* mRNA levels declined with stage progression. *ADRB2* mRNA expression levels and its gene product, β2-AR, differ. Gene Polymorphism plays an indispensable function in tumorigenesis and prognosis of malignancies. Due to polymorphism, there is significant genetic variation in β2-AR structure in human populations. Dennis W. McGraw et al.[20] found that the C variant at rs1042711 is association with the down-regulation of β2-AR expression. Polymorphisms of *ADRB2* in the 5'UTR and promoter region would causes a down-modulation of *ADRB2* expression which in turn promotes onset of malignancies [21]. The *ADRB2* gene polymorphism affects gene expression, receptor function, and response to ligands[8]. Further studies are needed to analyze *ADRB2* mRNA and β2-AR expression in LUAD tissues and the biological mechanisms regulating the transcription and translation of the *ADRB2* gene in lung cancer cells.

The present study attempts to ascertain the prognostic significance of *ADRB2* expression in LUAD and further explore its correlation with immune cell infiltration. *ADRB2* was down-regulated in LUAD patients according to the TCGA database. Moreover, *ADRB2* levels were significantly lower in male patients and smokers. Premised on this, further studies revealed that *ADRB2* in LUAD was strikingly associated with sex, smoking status, T classification, lymph node metastasis, and pathologic stage.

The survival analysis revealed a favorable survival in high *ADRB2* expression group compared to those with low expression. Univariate analysis revealed that pathological stage, T stage, N stage, M stage, and *ADRB2* expression influenced OS. To further determine the diagnostic capacity of *ADRB2* in LUAD, ROC curves were used to confirm that *ADRB2* is sensitive to the diagnosis and prognosis of LUAD. Altogether, these findings illustrate that *ADRB2* is a potential prognostic marker for LUAD.

The tumor immune microenvironment (TME) is very important in cancer pathogenesis [22]. Immune cells are vital elements of the TME [23]. The correlation between *ADRB2* expression and the infiltration of 24 immunocytes was further explored to elucidate the mechanisms responsible for *ADRB2* to predict clinical prognosis. Further correlation analysis indicated that the infiltration of 19 immune cells was significantly associated with *ADRB2* expression. *ADRB2* expression was positively correlated with aDCs, B cells, cytotoxic cells, DCs, eosinophils, iDCs, macrophages, mast cells, neutrophils, NK cells, pDCs, T cells, T helper cells, Tcm, Tem, TFH, Th1 cells, and Th17 cells. B cells are dominant in the progression of lung cancers [24,25] and can be observed at the individual stages of carcinogenesis [26]. B cells can prolong the survival of cancer patients by inhibiting tumor progression and preventing metastasis. In addition, antibodies produced by B cells are essential mediators of tumor cell death [27]. Cytotoxic T lymphocytes (TILs) are major players in antitumor immunity and can lead to apoptosis of cancer cells through a series of steps; therefore, high infiltration of TILs is a favorable prognostic marker for many cancers. DCs are the most effective antigen-presenting cells to induce primary tumor immune response (20), and in NSCLC patients, an increased DC count was significantly associated with an increase in DSS (21). The role of macrophages in cancer progression is still controversial. Tumor-associated macrophages promote tumor progression by facilitating tumor stroma formation and angiogenesis [28]. In patients with NSCLC with prolonged survival, macrophage-infiltrating tumors are mainly of the M1 type [29]. Mast cells, which have cytotoxic effects on cancer cells, can enhance the immunity of patients with LUAD against cancer cells and improve their postoperative prognosis [30]. NK cells are cytotoxic and it is essential in the immune monitoring of cancers [31]. Carrega et al.[32] found that in resected LUAD tissues, increasing numbers of infiltrating NK cells were associated with favorable patient survival outcomes. T cells are the most abundant monocytes infiltrating the NSCLCs [33]. T cells can secrete cytokines to inhibit tumor stroma formation and use cytotoxic molecules to kill epithelial nuclear stromal cells. Al-Shibli et al.[34] reported that T cell infiltration is associated with better DSS, and T cells are an independent indicator of survival. T helper cells play an important role in cancer immunity by secreting cytokines [35]. Both Th1 and Th17 cells produce proinflammatory factors, and their extensive infiltration can significantly improve clinical outcomes in a variety of cancers [36–38]. Large infiltration of cytotoxic T cells in tumor tissues is associated with longer survival [36]. Studies have shown that TFH has an antitumor response, and IL-21 secreted by TFH induces the activation, proliferation, and differentiation of B cells [39,40]. *ADRB2* expression may up-regulate the levels of infiltrating immune cells to limit the development of LUAD. In contrast, *ADRB2* expression was negatively correlated with Th2 cells and Tgd. Th2 cells have many pro-neoplastic activities and take part in cancer progression by cytokine release. Th2 cells are dominant in lymphocytes from malignant pleural effusion in patients with lung cancer [41,42]. Current studies have revealed that Tgd has a pro-tumor effect, which can inhibit innate and adaptive immunity by inducing immunosenescence [43–45].

To summarize, low *ADRB2* expression is associated with poor prognosis of LUAD. These results show that *ADRB2* expression level affects the immunity activity in the TME, and *ADRB2* might be a valuable biomarker for the immune status in LUAD patients.

To further explore the mechanism of *ADRB2* in LUAD, the signaling pathways involved in *ADRB2* was screened. In the *ADRB2* high-expression group, *ADRB2* associated genes were significantly enriched in immune signaling pathways (such as B cell receptor signaling pathway, T cell receptor signaling pathway, and NSCLC, NK-cell-mediated cytotoxicity, chemokine signaling pathway, and Jak STAT signaling pathway), in KEGG analysis. Those are significant in the tumorigenesis, development, and invasion of malignancies [46]. On the other hand, in the *ADRB2* low-expression group, *ADRB2* correlated genes were enriched in metabolism-related pathways, including RNA polymerase, citrate cycle, pentose phosphate pathway, oxidative phosphorylation, cysteine and methionine metabolism, and amino sugar and nucleotide sugar metabolism, implying that *ADRB2* up-regulated the signaling pathways associated with

immune response and induced antitumor efficiency. Therefore, *ADRB2* expression was down-regulated as LUAD progressed, and the TME switched from an immune-active state to a metabolic state. The *ADRB2* expression can be considered a biomarker to predict immune response.

At present, most studies on the relationship between *ADRB2* and the occurrence and progression of LUAD are based on its gene expression product, β2-AR, and its signaling pathway. This study revealed *ADRB2* as a key gene in the immune microenvironment of LUAD by performing a bioinformatics analysis, to provide evidence for *ADRB2* as a potential prognostic marker for LUAD. However, some limitations arise in the research. Firstly, the present research was limited by the small number of cases, and a large cohort is needed to validate the results of this research. Secondly, this research primarily focused on the expression of *ADRB2* mRNA from TCGA, and without involving β2-AR levels in LUAD tissues. Thus, this study still needs large-sample, multi-center, multi-ethnic clinical trials and basic experimental studies to prove the prognostic value of *ADRB2* in LUAD.

Conclusion

In summary, *ADRB2* expression was significantly down-regulated in patients with LUAD. *ADRB2* is involved in LUAD progression partly by regulating the immune microenvironment, which may potentially serve as a significant prognostic biomarker as well as a potential drug target.

Materials And Methods

TCGA Data

On or before November 13, 2021, the mRNA profile was extracted from TCGA (https://cancergenome.nih.gov/), including 535 LUAD samples and 59 normal samples. Relevant clinical information was derived from TCGA. The relevant data TCGA provided is open-access, no additional approval from the Ethics Committee were required. All methods were performed in accordance with the relevant guidelines and regulations.

ADRB2 Expression and Survival Analyses

The original expression data downloaded from TCGA were processed using the Perl programming language. The differential *ADRB2* expression were analyzed by Mann-Whitney U test or Kruskal-Wallis test when appropriate, and the results were visualized using the “limma” and “beeswarm” package of R software. Survival data were extracted and analyzed using the Perl programming language, and patients without complete survival state and time were removed. Subsequently, we matched the complete survival data with *ADRB2* expression data and obtained 499 patients’ data. In survival analysis, the *ADRB2* mRNA expression level was split into two groups by the median expression value, and OS, DSS, and progression-free interval (PFI) were evaluated with Kaplan-Meier analysis and log- rank test. A Kaplan-Meier survival curve was constructed by the survival package of R software. The diagnostic capacity of *ADRB2* was evaluated using ROC curve.

Univariate and Multivariate Cox Regression Analyses

Both univariate and multivariate analyses of clinical pathological parameters were performed adopting Cox proportional hazards analysis. In addition, we quantitatively evaluated the independent predictive value of clinicopathological parameters and *ADRB2* expression for survival and explored the prognostic effect of *ADRB2* on survival after adjusting for other confounding factors. Meanwhile, when matching it with *ADRB2* expression data, incomplete clinical information was excluded.

Evaluation of Immune Infiltration

First, GSEA method from the R package “GSVA” was used to present infiltration enrichment of 24 common immune cells in each sample, including mast cells, DCs, iDCs, macrophages, eosinophils, TFH, Th1, neutrophils, pDCs, T cells, NK cells, B cells, aDCs, Tem, T helper cells, cytotoxic cells, Tcm, $CD8^+$ T cells, regulatory T cells (Treg), NK CD56 bright cells, Th17, NK CD56dim cells, Tgd, and Th2. After that, Pearson’s analysis was used to investigate the relationship between *ADRB2* expression level and 24 immune cell infiltration in LUAD. Wilcoxon rank test was used to compare the levels of immune cell infiltration between different *ADRB2* expression groups.

Gene Set Enrichment Analysis (GSEA)

All LUAD patients in TCGA dataset were allocated into high and low group based on the expression of *ADRB2*. GSEA was used as a signaling pathway analysis tool to explore the signaling pathways related to *ADRB2* in LUAD. GSEA between high and low *ADRB2* expression was performed using GSEA 3.0. Phenotypes were determined based on *ADRB2* expression levels. The gene set “c2 all.v6.0 symbols.gmt” was used for the enrichment analysis. KEGG analysis was performed to explore the significant pathways associated with *ADRB2* expression.

Statistical Analysis

The differential *ADRB2* expression was analyzed by Mann-Whitney U test or Kruskal-Wallis test. The correlation between *ADRB2* expression and clinicopathological parameters was analyzed using the Chi-square test and logistic regression. Survival curves were analyzed with Kaplan–Meier analysis. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA) and R version 4.0.4, and the level of statistical significance was defined as a $P<0.05$.

Declarations

Data availability

The datasets generated and/or analysed during the current study are available in The Cancer Genome Atlas (TCGA) dataset (https://cancergenome.nih.gov/).

Author contributions

L.Y. J. wrote the original draft, F. X. prepared the figures and tables, J. T. Z. and W. D. Ch. analyzed the raw data, X. Y. and X. B. C. downloaded the raw data from TCGA database, X. L. and M. H. G. reviewed the relevant literature, Q. Q. W. made contribution to the language, Z. T. C. edited the manuscript and made revisions. All authors reviewed the manuscript.

Funding

The present study was supported by the National Natural Science Foundation of China (grant No. 82004281), the China Postdoctoral Science Foundation (grant No. 2021T140427 and 2021M691986), and the Development Plan of Shandong Medical and Health Technology (grant No. 2019WS581).

Competing interests

The authors declare no competing interests.

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Figures

Figure 1

The *ADRB2* expression and its relationship with clinical characteristics based on TCGA data. (A) Boxplot of *ADRB2* expression between the LUAD and normal tissues. The expression of *ADRB2* is grouped by age (B), gender (C), and smoking status (D). ***P<0.001, ns: not significance

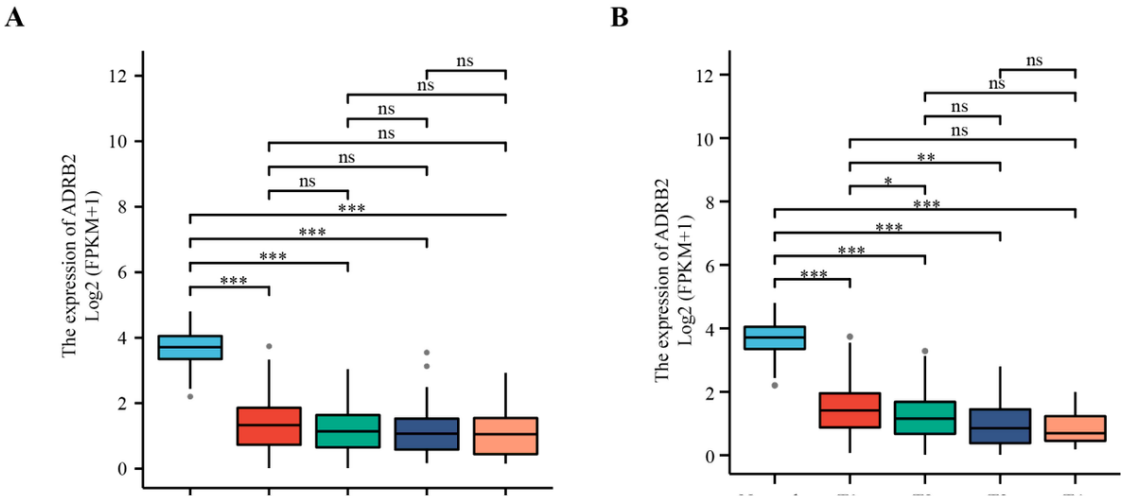

Figure 2

The expression of *ADRB2* is grouped by pathological stage (A), T stage (B), T stage (C), and M stage (D). *P<0.05, **P<0.01, ***P<0.001, ns: not significance

Characteristics	Total (N)	OR (95% CI)	P value
Gender			
Male vs. Female	535	0.502 (0.355−0.707)	**<0.001**
Age			
>65 vs. <=65	516	1.187 (0.840−1.677)	0.332
Smoker			
Yes vs. No	521	0.489 (0.290−0.809)	**0.006**
T stage			

Figure 3

Relationship between *ADRB2* and clinical characteristics

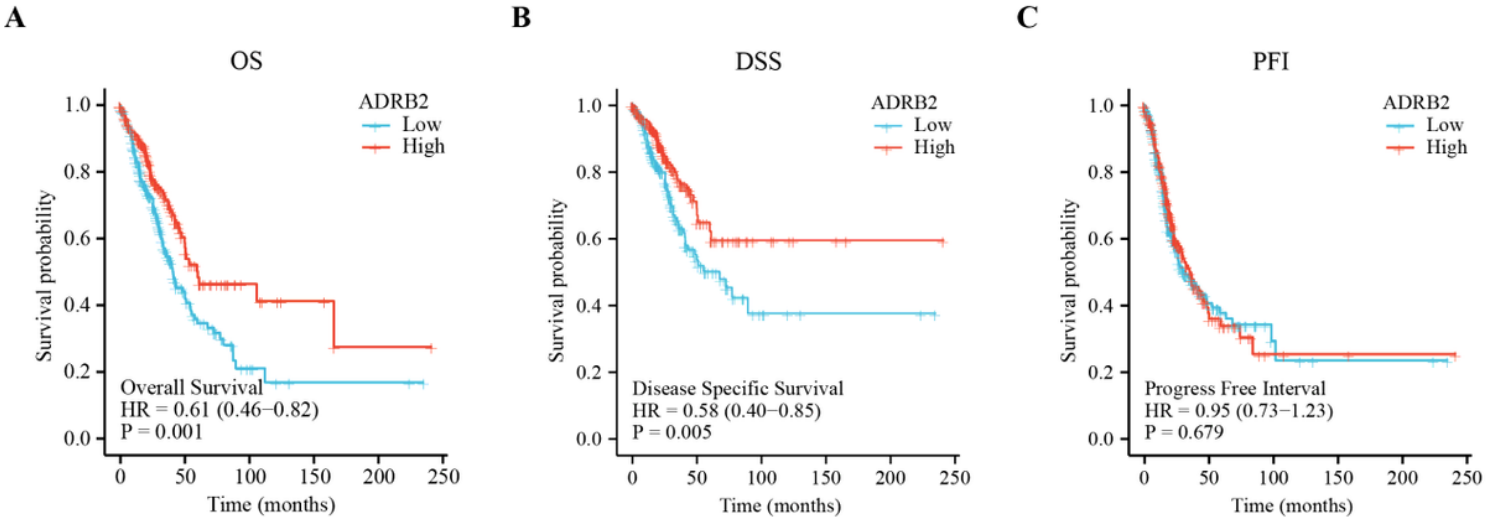

Figure 4

Survival analysis of *ADRB2* expression in LUAD patients: OS (A), DSS (B), and PFI (C)

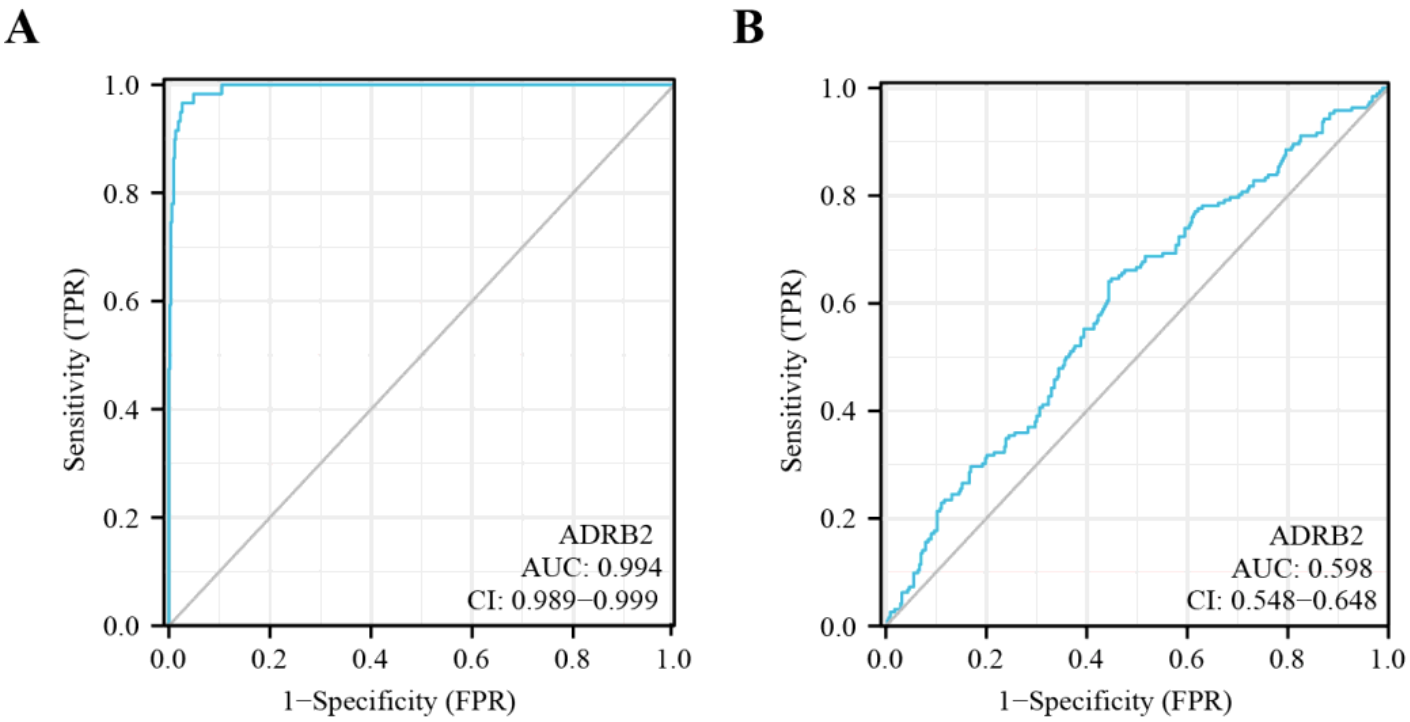

Figure 5

The ROC curve of *ADRB2*-associated diagnostic model (A). The ROC curve for predicting OS (B) in LUAD patients

Figure 6

The relationship between immune cell infiltration and *ADRB2* expression (A). The infiltration levels of immune cell populations in lung adenocarcinoma (LUAD) patients with different *ADRB2* expression (B). *P<0.05, **P<0.01, ***P<0.001

Figure 7

Enrichment plots from gene set enrichment analysis (GSEA)