ADRB2 expression predicts the clinical outcomes and is associated with immune cells infiltration in lung adenocarcinoma

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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, 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.

Lung cancer is the primary cause of malignant tumor mortality globally1. LUAD, one of the highest mortality rates and most aggressive forms of cancer, with a low 5-year survival rate < 5%. 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.

ADRB2 encodes beta-2-adrenergic receptor (β2-AR) which is a member of the G protein-coupled receptor superfamily (GPCRs). 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 ADRB2 was observed in various cancers, including breast cancer4, hepatocellular carcinoma5, prostate cancer6, and ovarian carcinoma7. Moreover, abundant ADRB2 expression was found to be closely linked with poor clinicopathological characteristics, tumor recurrence, metastasis, and poor prognosis. Although ADRB2 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 nucleotides8. 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,

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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\(^9\). 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.** The clinical data of 535 LUAD patients included gender, age, smoking status, T stage, N stage, M stage, pathological stage, OS event, and DSS event (Table 1). Chi-square test revealed that ADRB2 was significantly down-regulated with T stage \( (P < 0.001) \), gender \( (P = 0.005) \), smoking \( (P = 0.004) \), OS events \( (P < 0.001) \) and DSS events \( (P = 0.005) \). There was no significant correlation between ADRB2 expression and other clinicopathological features.

**ADRB2 expression level in LUAD.** Based on the TCGA database, ADRB2 mRNA expression level was analyzed in 594 tissues. As showcased in Fig. 1a, it evaluated the ADRB2 mRNA expression levels in TCGA pan-cancer. The results indicated that the expression level of ADRB2 in the LUAD samples was much higher than that in the normal tissue samples \( (P < 0.001) \). Box plots showed ADRB2 mRNA expression levels in 59 adjacent non-tumor tissues and 535 LUAD tissues. As shown in Fig. 1b, ADRB2 was down-expression in LUAD tissues compared with those in normal tissues \( (P < 0.001, \) Fig. 1b). Figure 1c shows a pairwise boxplot of the same out-

### Table 1. Demographic and clinicopathological parameters of high and low ADRB2 expression group patients with lung adenocarcinoma in TCGA-LUAD.

| Characteristic               | Low expression of ADRB2 | High expression of ADRB2 | P      |
|------------------------------|-------------------------|--------------------------|--------|
| n                            | 267                     | 268                      |        |
| Gender, n (%)                |                         |                          | 0.005  |
| Female                       | 126 (23.6%)             | 160 (29.9%)              |        |
| Male                         | 141 (26.4%)             | 108 (20.2%)              |        |
| Age, n (%)                   |                         |                          | 0.723  |
| < = 65                       | 131 (25.4%)             | 124 (24%)                |        |
| > 65                         | 129 (25%)               | 132 (25.6%)              |        |
| Smoker, n (%)                |                         |                          | 0.004  |
| No                           | 25 (4.8%)               | 50 (9.6%)                |        |
| Yes                          | 233 (44.7%)             | 213 (40.9%)              |        |
| T stage, n (%)               |                         |                          | <0.001 |
| T1                           | 68 (12.8%)              | 107 (20.1%)              |        |
| T2                           | 151 (28.4%)             | 138 (25.9%)              |        |
| T3                           | 33 (6.2%)               | 16 (3%)                  |        |
| T4                           | 13 (2.4%)               | 6 (1.1%)                 |        |
| N stage, n (%)               |                         |                          | 0.299  |
| N0                           | 167 (32.2%)             | 181 (34.9%)              |        |
| N1                           | 52 (10%)                | 43 (8.3%)                |        |
| N2                           | 43 (8.3%)               | 31 (6%)                  |        |
| N3                           | 1 (0.2%)                | 1 (0.2%)                 |        |
| M stage, n (%)               |                         |                          | 1.000  |
| M0                           | 187 (48.4%)             | 174 (45.1%)              |        |
| M1                           | 13 (3.4%)               | 12 (3.1%)                |        |
| Pathologic stage, n (%)      |                         |                          | 0.052  |
| Stage I                      | 132 (25%)               | 162 (30.7%)              |        |
| Stage II                     | 66 (12.5%)              | 57 (10.8%)               |        |
| Stage III                    | 51 (9.7%)               | 33 (6.3%)                |        |
| Stage IV                     | 14 (2.7%)               | 12 (2.3%)                |        |
| OS event, n (%)              |                         |                          | <0.001 |
| Alive                        | 152 (28.4%)             | 191 (35.7%)              |        |
| Dead                         | 115 (21.5%)             | 77 (14.4%)               |        |
| DSS event, n (%)             |                         |                          | 0.005  |
| Alive                        | 173 (34.7%)             | 206 (41.3%)              |        |
| Dead                         | 73 (14.6%)              | 47 (9.4%)                |        |

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comes (P < 0.001). Figure 1d shows 100 normal samples and 94 LUAD samples from the GEO database, which shows that ADRB2 is lowly expressed in LUAD patients (P < 0.001).

**Association between ADRB2 and TNM stages in LUAD patients.** To better understand the impact of ADRB2 on LUAD patient prognosis, Kruskal–Wallis analysis and Spearman correlation analysis were performed to determine the relationship between ADRB2 expression and clinicopathological characteristics (pathologic and TNM stages). The ADRB2 expression was significantly decreased in LUAD patients, and was significantly correlated with pathological stage, T stage and N stage. (Fig. 2a–d, Supplementary Table S1).

**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 0.603 for males vs. females, P = 0.004); smoking status (OR 0.457 for yes vs. no, P = 0.003); T classification (OR 0.581 for T2 vs. T1, P = 0.005; OR 0.308 for T3 vs. T1, P < 0.001; OR 0.293 for T4 vs. T1, P = 0.018); and pathologic stage (OR 0.527 for stage III vs. stage I, P = 0.011, Fig. 3).
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.

Figure 3. Relationship between ADRB2 and clinical characteristics.
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 Fig. 4, patients in the low ADRB2 expression group presented with significantly poorer OS (HR 0.65(0.48–0.87), P = 0.004, Fig. 4a) and DSS (HR 0.61(0.42–0.88), P = 0.008, Fig. 4b) than those in the high ADRB2 expression group. However, PFI did not differ between the two groups (HR 0.90(0.69–1.17), P = 0.412, Fig. 4c). Subgroup analysis suggested that the link between lower ADRB2 expression and worse OS was statistically significant in most subgroups, especially in the stage I subgroup of pathologic stage (HR 0.61(0.38–1), P = 0.048), N0 subgroup of N stage (HR 0.62(0.40–0.94), P = 0.025), and M0 subgroup of M stage (HR 0.62(0.44–0.88), P = 0.007).

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 1.387–3.139; P < 0.001); M stage (HR 2.136; 95% CI 1.248–3.653; P = 0.006); and ADRB2 (HR 0.612; 95% CI 0.456–0.821; P = 0.001) were significantly associated with worse OS. Multivariate analysis further demonstrated that pathological stage (HR 1.341; 95% CI 0.805–2.233; P = 0.260), T stage (HR 1.817; 95% CI 1.119–2.951; P = 0.016), N stage (HR 2.086; 95% CI 1.387–3.139; P < 0.001), and ADRB2 (HR 0.730; 95% CI 0.504–1.056; P = 0.095) were independently associated with survival in the multivariate model.
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, and multivariate Cox risk regression analysis showed that T stage (HR 1.817; 95% CI 1.119–2.951; P = 0.016) and N stages (HR 2.086; 95% CI 1.387–3.139; P < 0.001) were independent prognostic factors for OS (Table 2).

Evaluation of the diagnostic capacity of ARDB2 in LUAD. To explore the diagnostic value of ARDB2 for LUAD, receiver operating characteristic (ROC) curve analysis was performed. The results of the ROC curves indicated that ARDB2 was highly sensitive to the diagnosis of LUAD (AUC, 0.994; 95% CI 0.989–0.999, Fig. 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 (AUC, 0.590; 95% CI 0.548–0.648, Fig. 5b). The time-dependent accuracy of ARDB2 in predicting OS in 1, 3, and 5 years was also assessed through a time-dependent ROC analysis, AUC < 0.5 indicates that the expression of ARDB2 is opposite to the occurrence trend of OS events in LUAD patients (Fig. 5c). Figure 5d shows that the survival rate of patients in the high-risk group is poor, and the risk of death is high. With the decrease of ARDB2 expression, the risk score tends to increase gradually.

Relationship of ARDB2 expression level with immune infiltration in LUAD. Figure 6a shown the relationships between expression of ARDB2 and 28 types of tumor-infiltrating lymphocytes (TILs) across human cancers in the TISIDB database. In TCGA database, pearson’s analysis demonstrated that the infiltration of 24 types of immune cells was markedly related to ARDB2 expression, which had a significantly positive relationship with CD8 T cells (P = 0.049), type 17 Th cells (Th17) (P = 0.021), and regulatory T cells (TReg) (P = 0.025), and a strongly-positive association with activated DCs (aDCs), B cells, cytotoxic cells, dendritic cells (DCs), eosinophils, immature DCs (iDCs), macrophages, mast cells, neutrophils, natural killer (NK) cells,
Figure 6. Relations between the expression of ADRB2 and 28 types of TILs across human cancers (a). The relationship between immune cell infiltration and ADRB2 expression (b). The infiltration levels of immune cell populations in lung adenocarcinoma (LUAD) patients with different ADRB2 expression (c). ADRB2 was correlated with abundance of B cells, CD4 T cells, CD8 T cells, macrophage, NK cells, and mast cells (d–i). *P < 0.05, **P < 0.01, ***P < 0.001.
neutrophils, plasmacytoid DCs (pDCs), T cells, T helper cells, Tcm T central memory (Tcm), T effector memory (Tem), T follicular helper (TFH), and type 1 Th cells (Th1) (P < 0.001, Fig. 6b,c). However, T gamma delta (Tgd) and type 2 Th cells (Th2) (P < 0.001, Fig. 6b,c) showed a negative association with ADRB2. As can be seen in Fig. 6d–i, the expression of ADRB2 was correlated with adundance of B cells (r = 0.334, P = 7.59e−15), CD4 T cells (r = 0.125, P = 0.00448), CD8 T cells (r = 0.27, P = 5.52e−10), macrophage (r = 0.403, P < 2.2e−16), NK cells (r = 0.424, P < 2.2e−16), mast cells (r = 0.527, P < 2.2e−16) in the TISIDB database.

### 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, allotment 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, Fig. 7). These results contribute to further exploration of ADRB2 pathophysiological mechanisms.

### Discussion
LUAD is a type of malignant lung tumor that originates from the bronchial mucosal glandular epithelium. LUAD is characterized by inconspicuous early symptoms, and LUAD is a lung tumor with a significant rate of malignant recurrence, metastasis, and unsatisfactory prognosis. Early diagnosis is difficult in most patients.

ADRB2 is ubiquitously expressed in multiple tissues, including the smooth muscle of the human bronchi, cardiovascular system, central nervous system, and gastrointestinal tract. In recent years, increasing evidence shows that ADRB2 has a vital place in the occurrence and development of diverse range of cancers. Zhang et al. 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.

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

| Gene set name                          | Low expression | High expression |
|----------------------------------------|----------------|-----------------|
|                                       | Gene set name  | NES  | NOM p-value | FDR q-value | NES  | NOM p-value | FDR q-value |
| KEGG_SPLICEOSOME                      | −2.229         | 0    | 0           |             | 2.170 | 0            | 0.004       |
| KEGG_RNA_POLYMERASE                   | −2.199         | 0    | 0           |             | 2.170 | 0            | 0.004       |
| KEGG_RNA_DEGRADATION                  | −2.183         | 0    | 0.001       |             | 2.037 | 0.003        | 0.010       |
| KEGG_CITRATE_CYCLE_TCA_CYCLE          | −2.126         | 0    | 0.002       |             | 2.036 | 0.002        | 0.008       |
| KEGG_CELL_CYCLE                       | −2.103         | 0.001| 0.002       |             | 2.019 | 0.001        | 0.009       |
| KEGG_PENTOSE_PHOSPHATE_PATHWAY        | −2.075         | 0    | 0.003       |             | 2.009 | 0.004        | 0.009       |
| KEGG_BASEAL_TRANSCRIPTION_FACTORS     | −2.025         | 0    | 0.004       |             | 1.996 | 0.007        | 0.010       |
| KEGG_OXIDATIVE_PHOSPHORYLATION        | −1.998         | 0.003| 0.005       |             | 1.969 | 0.006        | 0.010       |
| KEGG_DNA_REPLICATION                  | −1.981         | 0    | 0.005       |             | 1.825 | 0.010        | 0.033       |
| KEGG_MISMATCH_REPAIR                  | −1.896         | 0.012| 0.031       |             | 1.825 | 0.012        | 0.031       |
| KEGG_CITRINE_AND_METHIONINE_METABOLISM| −1.798         | 0.001| 0.025       |             | 1.821 | 0.001        | 0.031       |
| KEGG_OXIDATIVE_PHOSPHORYLATION        | −1.743         | 0.014| 0.037       |             | 1.805 | 0.022        | 0.031       |
| KEGG_NON_SMALL_CELL_LUNG_CANCER      | −1.708         | 0.014| 0.049       |             | 1.708 | 0.014        | 0.049       |
and is often indicative of poor prognosis\textsuperscript{5,11,14-16}. The regulation of β2-AR on tumorigenesis may be twofold, depending on the type of tumor and the stage of cancer progression. A study revealed that activation of ADRB2 results in inhibition tumor cell growth, as well as induction of apoptosis and tumor regression, where this activation results in the inactivation of the Raf-1/Mek-1/Erk1/2 pathway by a cAMP-dependent activation of protein kinase A\textsuperscript{17}. In oral squamous cell carcinoma, patients with higher ADRB2 had a significant longer DSS and OS\textsuperscript{18}. Caparica et al.\textsuperscript{19} found that a high ADRB2 expression may be a favorable prognostic factor in patients with HER2 + early breast cancer and evidence that a possible association with antiproliferative, antiangiogenic, and immunogenic effects of ADRB2. Yazawa et al.\textsuperscript{20} retrospectively analyzed 328 surgically-resected patients with NSCLC and found that positive ADRB2 expression was found in 29% of LUAD tissues, which markedly increased compared with non-adenocarcinoma tissues. A high level of ADRB2 expression was associated with vascular invasion, tumor cell proliferation, and poor prognosis in patients with LUAD. Nevertheless, Wang et al.\textsuperscript{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. The exact mechanism is still controversial. This study used high-throughput RNA sequencing data obtained from TCGA database to further ascertain the expression and the prognostic significance of ADRB2 in LUAD and explore its correlation with immune cell infiltration.

The low expression of ADRB2 in tumor tissues of LUAD patients is correlated with gender, smoking, OS events and DSS events, indicating that ADRB2 is a potential biomarker for prognosis and diagnosis. Data mining from TCGA database showed that the expression of ADRB2 was correlated with pathological stage, T stage and N stage, and the disease tended to progress with the decrease of ADRB2 expression. Premised on this, logistic regression analysis showed that ADRB2 is significantly correlated with gender, smoking status, T stage and pathological 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 biomarker for LUAD.

The tumor immune microenvironment (TME) is very important in cancer pathogenesis\textsuperscript{21}. Immune cells are vital elements of the TME\textsuperscript{22}. 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, CD8+ T cells, cytotoxic cells, DCs, eosinophils, ILCs, macrophages, mast cells, neutrophils, NK cells, pDCs, T cells, T helper cells, Tcm, Tem, TFH, Th1 cells, Th17 cells, and TReg. B cells are dominant in the progression of lung cancers\textsuperscript{13,24} and can be observed at the individual stages of carcinogenesis\textsuperscript{22}. 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\textsuperscript{26}. Cytotoxic T lymphocytes (CTLs) are major players in antitumor immunity and can lead to

![Figure 7. Enrichment plots from gene set enrichment analysis (GSEA).](image-url)
apoptosis of cancer cells through a series of steps; therefore, high infiltration of CTLs is a favorable prognostic marker for many cancers. DCs are the most effective antigen-presenting cells to induce primary tumor immune response, and in NSCLC patients, an increased DC count was significantly associated with an increase in DSS. The role of macrophages in cancer progression is still controversial. Tumor-associated macrophages promote tumor progression by facilitating tumor stroma formation and angiogenesis. In patients with NSCLC with prolonged survival, macrophage-infiltrating tumors are mainly of the M1 type. 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. NK cells are cytotoxic and it is essential in the immune monitoring of cancers. NK cells can secrete cytokines to inhibit tumor stroma formation and use cytokotoxic molecules to kill epithelial nuclear stromal cells. Al-Shibli et al. 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. Both Th1 and Th17 cells produce proinflammatory factors, and their extensive infiltration can significantly improve clinical outcomes in a variety of cancers. Large infiltration of cytotoxic T cells in tumor tissues is associated with longer survival. Studies have shown that TGF has an antitumor response, and IL-21 secreted by Th1 induces the activation, proliferation, and differentiation of B cells. 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. Current studies have revealed that Tgd has a pro-tumor effect, which can inhibit innate and adaptive immunity by inducing immunosuppression.

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. 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, 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, multicenter, multi-ethnic clinical trials and basic experimental studies to prove the prognostic value of ADRB2 in LUAD. Low expression of ADRB2 is associated with poor prognosis in LUAD patients, which may be related to immunocompromised.

**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**

**Data acquisition.** On or before November 13, 2021, the mRNA profile (HTSeq-Counts and HTSeq-FPKM) was extracted from TCGA (https://cancergenome.nih.gov/), including 535 LUAD samples and 59 normal samples. Relevant clinical information was derived from TCGA. RNAseq data in FPKM (fragments Per Kilobase Per Million) format were converted to TPM (transcripts Per Million reads) format and log2 transformed. ADRB2 expression data from datasets GSE40791 was downloaded from the GEO database (https://www.ncbi.nlm.nih.gov/gds/). The relevant data TCGA and GEO 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 (version: strawberry-perl-5.32.1.1-64bit.mis, https://strawberryperl.com/). GEOquery package (version: 2.54.1, https://cran.r-project.org/web/packages/GEOquery/index.html) is used to download GEO database expression data. The differential ADRB2 expression were analyzed by Mann–Whitney U test, Dunn’s test, Kruskal–Wallis test or Spearman correlation analysis when appropriate, and the results were visualized using the ggplot2 R package (version: 3.3.2, https://cran.r-project.org/web/packages/
Analysis was performed to explore the significant pathways associated with ADRB2 expression. Expression levels. The gene set “c2 all.v6.0 symbols.gmt” was used for the enrichment analysis. KEGG pathway analysis 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. Figure 6a is drawn online in TISIDB (http://cis.hku.hk/TISIDB/index.php). GSEA method from the R package “GSVA (version:1.34.0, http://bioconductor.org/packages/release/bioc/html/GSVA.html), clusterProfiler (version:3.18.0, http://bioconductor.org/packages/release/bioc/html/clusterProfiler.html), and rtracklayer (http://bioconductor.org/packages/release/bioc/html/rtracklayer.html)” was used to present enrichment 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, TRex, 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. Pearson correlation test and the independent-samples T test were used to compares the levels of immune cell infiltration between different ADRB2 expression groups. Spearman’s test was used to evaluate the relationship between ADRB2 expression and the abundance of TILs.

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 (http://www.gsea-msigdb.org/gsea/index.jsp). 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. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp, Armonk, NY, USA) and R (version 4.0.2, https://www.r-project.org/) and the level of statistical significance was defined as a P < 0.05.

Data availability
The datasets generated and/or analysed during the current study are available in TCGA dataset (https://cancergenome.nih.gov/) and GEO database (https://www.ncbi.nlm.nih.gov/geo/).

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References
1. Zheng, Q., Min, S. & Zhou, Q. Identification of potential diagnostic and prognostic biomarkers for LUAD based on TCGA and GEO databases. Biosci. Rep. https://doi.org/10.1042/bsr202004370 (2021).
2. Lu, X. et al. Phosphorylation-specific phospholipase Cγ2 activation-driven autophagy caused cell death in human lung adenocarcinoma A549 cells in vivo and in vitro. Int. J. Biol. Sci. 16, 1427–1440. https://doi.org/10.7150/ijbs.42962 (2020).
3. Ruffolo, R. R. Jr. & Hieble, J. P. Alpha-adrenoceptors. Pharmacol. Ther. 61, 1–64. https://doi.org/10.1016/0163-7258(94)90058-2 (1994).
4. Qin, J. et al. Adrenergic receptor beta2-activation by stress promotes breast cancer progression through macrophages M2 polarization in tumor microenvironment. BMC Rep. 48, 295–300. https://doi.org/10.5483/bmbrep.2015.48.5.008 (2015).
5. Chen, D. D. et al. The beta2-adrenergic receptor is a potential prognostic biomarker for human hepatocellular carcinoma after curative resection. Ann. Surg. Oncol. 19, 3556–3565. https://doi.org/10.1245/s10434-012-2396-1 (2012).
6. Ramberg, H. et al. Hormonal regulation of beta2-adrenergic receptor level in prostate cancer. Prostate 68, 1133–1142. https://doi.org/10.1002/pros.20778 (2008).
7. Thaker, P. H. et al. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. Nat. Med. 12, 939–944. https://doi.org/10.1038/nm1447 (2006).
8. Litonjua, A. A. et al. Very important pharmacogene summary ADRB2. Pharmacogenet. Genom. 20, 64–69. https://doi.org/10.1097/ FPC.0b013e328333dace6 (2010).
9. Mei, L., Huang, C., Wang, A. & Zhang, X. Association between ADRB2, IL33, and IL2RB gene polymorphisms and lung cancer risk in a Chinese Han population. Int. Immunopharmacol. 77, 105930. https://doi.org/10.1016/j.intimp.2019.105930 (2019).
Author contributions
L.Y.J. wrote the original draft, F.X. and S.T. prepared the figures and tables, J.T.Z. and W.D.C. 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 references.
literature, Q.Q.W. made contribution to the language, Z.T.C. edited the manuscript and made revisions. All authors reviewed the manuscript.

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**Competing interests**
The authors declare no competing interests.

**Additional information**
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