

IL-11RA is a Prognostic Biomarker and Correlated with Immune Infiltration in Lung Adenocarcinoma

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Research

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Abstract

Background: Interleukin-11 receptor subunit alpha (IL-11RA) contributes to multiple biological processes in various tumors. However, the role of IL-11RA in Lung adenocarcinoma (LUAD) is still undetermined. The study aims to explore the role of IL-11RA in LUAD via an integrated bioinformatics analysis.

Methods: TIMER, GEPIA, TCGA and HPA databases analysis were used to detect IL-11RA expression. UALCAN database was used to analysis the correlation between IL-11RA expression and clinicopathological parameters of LUAD. Kaplan-Meier Plotter, TCGA and GEO databases were used to analysis overall survival (OS) and progression-free survival (PFS) of the LUAD patients. Univariate Cox regression analysis was used to assess the prognostic value of IL-11RA in different clinical characteristics. GSEA, and TIMER were used to investigate the relationship between IL-11RA and immune infiltration.

Results: The expression of IL-11RA was down-regulated in LUAD tissues. Furthermore, IL-11RA expression was closely associated with clinical stage, lymph node stage and smoking habits. The patients with lower IL-11RA expression had poorer overall survival (OS) and progression-free survival (PFS). Lower IL-11RA expression was significantly associated with its hypermethylation, and the hypermethylation of CpG site at cg14609668 and cg21504624 was obviously correlated with poorer OS. Then, we found that IL-11RA may play an important role in LUAD progression and immune regulations. Notably, High expression of IL-11RA may suppress the progression of LUAD through inhibiting cell proliferation and immune cell infiltration, especially in B cells, CD4⁺ T cells, and Dendritic Cell.

Conclusions: Decreased IL-11RA expression correlates with poor prognosis and immune infiltration in LUAD. Our work highlights IL-11RA might be a potential biomarker for prognosis and provide a new therapeutic target for LUAD patients.

Introduction

Lung cancer is one of the most prevalent types of malignancies worldwide, with a high morbidity and mortality [1]. Histologically, lung cancer is divided into several different types. Lung adenocarcinoma (LUAD) is the most frequent type, accounting for more than 50% of all lung cancers [2]. Currently, surgical resection combined with chemotherapy and radiotherapy is a primary method for treating LUAD [3]. In recent years, lung cancer immunotherapy has become a new research hot spot, which can activate the patients' immune system, exert an antitumor effect [4], and improve the clinical symptoms and overall survival [5]. Despite the advance of immunotherapy, the prognosis of patients with LUAD remains poor [6]. Therefore, in the era of precision medicine, it is necessary to explore a reliable immune-related biomarker that can predict the prognosis of LUAD and become a novel target for LUAD immunotherapy.

IL-11 is a hematopoietic cytokine engaged in numerous biological processes and validated as a target for the treatment of various cancers. Permyakov and his colleagues found that aside from interleukin-11 receptor subunit alpha (IL-11RA) and gp130 receptors, IL-11 interacts with calcium sensor protein S100P

[7]. IL-11RA is a member of the cytokine receptors family, which can be found in cytoplasm and membrane, indicating that IL-11RA not only has functions that are ligand-dependent but also ligand-independent [8–9]. Currently, IL-11RA is known to be associated with tumor progressivity, cell growth, and differentiation from several malignant tumors [10–11], suggesting its potential involvement in human tumorigenesis. However, the relationship between IL-11RA and LUAD is still poorly understood.

Here, we used publicly available databases, such as the TCGA, GEO, GEPIA, TIMER, Kaplan-Meier plotter, HPA and UALCAN, to evaluate the expression of IL-11RA in LUAD and its prognostic role as well as its possible molecular mechanism. IL-11RA expression in LUAD was validated by TIMER, GEPIA, TCGA and HPA databases. Prognosis and clinical characteristics were analyzed using Kaplan-Meier plotter, TCGA and GEO databases. Subsequently, TCGA databases were used to analyze the relationship between methylation and IL-11RA expression in LUAD. Moreover, we also investigated the association of IL-11RA expression with tumor-infiltrating immune cells in LUAD. The present study revealed the important function of IL-11RA in LUAD and explain the potential relationship and mechanism of the interaction between IL-11RA and tumor progressivity.

Materials And Methods

Data collection

The TCGA database containing transcriptomic data and clinical data of 59 normal and 526 lung adenocarcinoma tissues were downloaded from the UCSC Xena website (<http://xena.ucsc.edu/>). After excluding duplicate patients, 59 normal and 513 lung adenocarcinoma tissues and 57 pairs of lung adenocarcinoma and normal lung tissues were included for IL-11RA expression level analysis, and 504 LUAD samples had complete OS survival information. Moreover, the DNA methylation data of 32 normal and 471 lung adenocarcinoma tissues was also downloaded from the UCSC Xena website, and 460 LUAD patients were included for OS survival analysis. The GSE72094 data set containing the expression and survival data of 398 LUAD patients was downloaded from The Gene Expression Omnibus dataset (GEO, <https://www.ncbi.nlm.nih.gov/geo/>).

GEPIA database analysis

Gene Expression Profiling Interactive Analysis (GEPIA, <http://gepia.cancer-pku.cn/detail.php>) is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects, using a standard processing pipeline [12]. This study performed a differential IL-11RA mRNA expression analysis in 483 LUAD and 347 normal tissues.

HPA database analysis

The Human Protein Atlas (HPA, <http://www.proteinatlas.org>) database provides abundant transcriptome and proteome data via immunohistochemistry and RNA-sequencing analyses. In this study, the protein

expression levels of IL-11RA were determined by immunohistochemistry.

UALCAN database analysis

the UALCAN database (<http://ualcan.path.uab.edu>) uses TCGA level 3 RNA-seq and clinical data from 31 cancer types [13]. In this study, we used the UALCAN database to study the correlation between IL-11RA mRNA expression level and clinicopathological parameters of LUAD.

Kaplan–Meier plotter database analysis

The correlation between IL-11RA expression and survival in LUAD was analyzed by Kaplan-Meier plotter (<http://kmplot.com/analysis/>). 719 LUAD patients were divided into high expression groups and low expression groups based on the median expression levels of the IL-11RA. The prognostic value of IL-11RA was assessed by overall survival (OS) and progression-free survival (PFS) using the hazard ratio (HR), 95% confidence intervals (CI), and log-rank p-value. Meanwhile, univariate cox regression analysis was used to assess the prognostic value of IL-11RA in different clinical characteristics. Besides, the prognostic significance of IL-11RA in LUAD was also analyzed in TCGA-LUAD and GSE72094 data set through “survminer” and “survival” packages in R.

Analysis of IL-11RA methylation

The methylation level of IL-11RA between 32 normal and 471 LUAD patients and the prognostic value of IL-11RA DNA CpG sites in 460 LUAD patients were analyzed by R software. The correlation between IL-11RA gene expression and its methylation levels were analyzed by the Pearson method.

Gene set enrichment analysis

In order to clarify the possible mechanism of IL-11RA in lung adenocarcinoma, TCGA datasets of LUAD with a functional gene set file (c5.all.v7.2) gene set were analyzed by GSEA. The 504 LUAD patients were divided into a high IL-11RA expression group and a low IL-11RA expression group based on the median expression levels. Gene sets with adjust pvalue < 0.05 were considered statistically significant. The normalized enrichment score (NES) was acquired by analyzing with 1000 times permutations [14].

Calculation of Immune Scores

Immune scores were calculated by the ESTIMATE algorithm of the downloaded data for each LUAD sample [15]. Subsequently, comparison of Immune scores between the high and low IL-11RA expression groups was analyzed.

TIMER database analysis

Tumor Immune Estimation Resource (TIMER, <https://cistrome.shinyapps.io/timer/>) is a comprehensive resource for systematical analysis of immune infiltrates across diverse cancer types. We used TIMER to analyze the expression of IL-11RA in multiple tumors and its association with several immune infiltrated

cells (B cells, CD4⁺ T cells, CD8⁺ T cells, neutrophils, Myeloid Dendritic cell and macrophages) in LUAD. In addition, the correlation analysis between IL-11RA and related genes and markers of immune cells were analyzed in TIMER.

Statistical analysis

R version 4.0.3 software were used for statistical analyses. Paired t-test and unpaired t-test were used to compare the expression of IL-11RA between normal and tumor groups. Survival analyses in the TCGA-LUAD and GSE72094 cohort were conducted between high and low IL-11RA expression groups through Kaplan-Meier analysis with the log-rank test. Univariate Cox analysis was used to evaluate the influence of IL-11RA expression and other clinicopathological factors (gender, stage, smoke) on survival. The correlation between IL-11RA expression and methylation was assessed by Pearson's correlation. The correlation between IL-11RA expression and immune cells and its markers was assessed by Spearman's correlation. P-value < 0.05 was set up as the cut-off criterion.

Results

The expression of IL-11RA is down-regulated in lung adenocarcinoma

To evaluate the IL-11RA expression in various tumours, we searched Transcriptome-seq data in the TIMER database (Fig. 1A). The result revealed that the IL-11RA mRNA expression in liver hepatocellular carcinoma (LIHC), pheochromocytoma, and paraganglioma (PCPG) were higher compared to normal tissues, while it was considerably lower in bladder urothelial carcinoma (BLCA), invasive breast carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), esophageal carcinoma (ESCA), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), kidney renal papillary cell carcinoma (KIRP), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), prostate adenocarcinoma (PRAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA) and uterine corpus endometrial carcinoma (UCEC) than in the normal tissue. These data show that IL-11RA has a significant differential expression between tumour tissues and normal tissues, and it may be regarded as oncogenic gene, particularly in LUAD. To further investigate the relevance of IL-11RA expression level with human LUAD, we performed a detailed analysis of RNA-Seq data from the GEPIA and TCGA databases. The quantitative evaluation of the expression level of IL-11RA mRNA in LUAD tissues was significantly lower than that in normal tissues (Fig. 1B and Fig. 1C). Data from tumor and matched normal tissues of LUAD patients in the TCGA further verified these results (Fig. 1D). Besides, we further analyzed the protein level of IL-11RA in LUAD tissues from the Human Protein Atlas (HPA) database. Immunohistochemical results indicated that the protein expression level of IL-11RA exhibited significant upregulation in LUAD tissues. Overall, these results

suggest that the expression of IL-11RA is decreased in various types of cancer, particularly in LUAD, demonstrating that IL-11RA may suppress LUAD tumorigenesis.

The correlation between IL-11RA mRNA expression level and clinicopathological parameters of LUAD

In order to clarify the correlation between IL-11RA mRNA expression level and clinicopathological parameters of LUAD, we investigated IL-11RA expression on the basis of patients' different clinical pathological parameters, such as age, gender, stage, lymph -node status, smoking habits using UALCAN database. Results showed that IL-11RA expression was much lower in LUAD patients than normal tissues in subgroup analysis based on age and gender (Fig. 2A and Fig. 2B). For cancer stages and lymph node status, IL-11RA expression was lower expressed in different subclasses than that in normal lung tissues (Fig. 2C and Fig. 2D). It is highly intriguing that the expression of IL-11RA decreases as the stage increases between stage 1 and stage 3 (Fig. 2C), and IL11RA also decreases with the increase of the lymph node stage between N0 and N2 (Fig. 2D). Besides, IL-11RA expression was closely associated with smoking habits (Fig. 2E). An analysis of subclass showed that IL-11RA expression was much lower in lung adenocarcinoma patients who smoke or those who have smoked for more than 15 years than lung adenocarcinoma patients who do not smoke or those who have smoked for less than 15 years. All these data together indicated that low IL-11RA expression was widely correlated with lung adenocarcinoma.

Low expression of IL-11RA is associated with poor prognosis of LUAD

The Kaplan-Meier plotter database was utilized to explore the correlation between IL-11RA expression and prognosis in LUAD. Firstly, according to the IL-11RA expression median value, LUAD samples were divided into IL-11RA high (top 50%) expression group and IL-11RA low (bottom 50%) expression group. Low expression of IL-11RA was associated with poor overall survival (OS) in patients with LUAD (hazard ratio [HR] = 0.54, 95% confidence interval [CI] = 0.42–0.69, log-rank P = 4.7e-07) (Fig. 3A). Similarly, low IL-11RA expression was significantly associated with reduced progression-free survival (PFS) (Fig. 3B, HR = 0.37, 95% CI = 0.26–0.57, log-rank P = 8.8e-10). Furthermore, low expression of IL-11RA, which was capable of predicting poor OS, was also validated in TCGA database (Fig. 3C, HR = 0.64, 95% CI = 0.48–0.85, P = 0.001) and GSE72094 database (Fig. 3D, HR = 0.37, 95% CI = 0.25–0.54, P < 0.001). Based on this large-sample validation analysis, these results suggest that low IL-11RA expression implies reduced survival in LUAD.

To better understand the effect of decreased expression of IL-11RA on survival, the Kaplan-Meier plotter database was accessed to analyze the correlation between IL-11RA expression and clinical characteristics of patients by univariate cox regression analysis (Table 1). The low expression of IL-11RA corresponded with worse OS and PFS in women, male, smoking or nonsmoking patients (P < 0.05). For

clinical staging, downregulation of IL-11RA was linked to worse OS in stage 1, stage N0, and stage M0 in LUAD ($P < 0.05$) and worse PFS in stage 1 ($P < 0.05$).

Table 1. Correlation of IL-11RA mRNA expression and clinical prognosis in LUAD by Kaplan-Meier plotter

	Overall survival (n = 719)			Progression-free survival (n = 641)		
	N	HR (95% CI)	p-value	N	HR (95% CI)	p-value
Gender						
Female	317	0.45 (0.3-0.68)	8.4e-05	235	0.51 (0.32-0.82)	0.0043
Male	344	0.51 (0.36-0.71)	7.1e-05	226	0.3 (0.19-0.49)	1.5e-07
Stage						
1	370	0.21 (0.13-0.35)	8.7e-12	283	0.38 (0.23-0.63)	0.00011
2	136	0.76 (0.47-1.22)	0.25	103	0.9 (0.52-1.55)	0.69
3	24	0.7 (0.26-1.87)	0.47	10	-	-
4	4	-	-	0	-	-
T stage						
1	123	0.55 (0.29-1.03)	0.057	47	0.73 (0.16-3.25)	0.67
2	105	0.72 (0.41-1.26)	0.25	93	1.39 (0.74-2.64)	0.31
3	4	-	-	2	-	-
4	0	-	-	0	-	-
N stage						
0	184	0.54 (0.33-0.89)	0.013	102	0.87 (0.4-1.87)	0.72
1	44	0.83 (0.38-1.81)	0.63	38	1.1 (0.44-2.76)	0.83
2	3	-	-	2	-	-
M stage						
0	231	0.51 (0.34-0.77)	0.0011	142	0.77 (0.44-1.37)	0.38
1	1	-	-	0	-	-
Smoke						
Ever	246	0.37 (0.22-0.61)	5.8e-05	243	0.43 (0.27-0.68)	0.00021
Never	143	0.19 (0.07-0.56)	0.00079	143	0.4 (0.21-0.77)	0.0047

Table 2
Correlation analysis between IL-11RA and relate genes and markers of immune cells in
TIMER

Description	Gene Markers	None adjusted		Tumor purity adjusted	
		Cor	P-value	Cor	P-value
Activated B cell	CD19	0.21	1.52e-06	0.226	4.11e-07
	CD79A	0.105	1.74e-02	0.101	2.52e-02
	GNG7	0.456	8.78e-28	0.456	1.02e-26
	BLK	0.301	3.09e-12	0.323	1.88e-13
	CLEC9A	0.242	2.73e-08	0.232	1.96e-07
Immature B cell	CD22	0.388	5.62e-20	0.425	4.57e-23
	FCRL1	0.329	1.9e-14	0.349	1.52e-15
	FAM129C	0.345	7.45e-16	0.374	9.05e-18
	TXNIP	0.284	5.19e-11	0.281	2.01e-10
	STAP1	0.276	1.98e-10	0.277	4.02e-10
Memory B cell	FCER1A	0.309	6.95e-13	0.305	4.39e-12
	SOX5	0.228	1.67e-07	0.232	1.99e-07
	TLR9	0.137	1.77e-03	0.134	2.82e-03
	CCNA2	-0.408	4.95e-22	-0.42	1.53e-22
	CDKN3	-0.389	4.39e-20	-0.407	4.03e-21
Th1	T-bet (TBX21)	0.199	5.07e-06	0.19	2.15e-05
	STAT4	0.231	1.19e-07	0.221	6.95e-07
	TNF-a (TNF)	0.17	1.01e-04	0.148	9.77e-04
	STAT1	-0.082	6.17e-02	-0.125	5.39e-03
Th2	STAT6	0.257	3.15e-09	0.269	1.22e-09
	STAT5A	0.278	1.3e-10	0.27	1.11e-09
	IL13	0.165	1.67e-04	0.144	1.39e-03
	GATA3	0.104	1.82e-02	0.072	1.12e-01
Tfh	BCL6	0.236	6.11e-08	0.232	1.89e-07
	IL21	-0.042	3.39e-01	-0.069	1.26e-01

Description	Gene Markers	None adjusted		Tumor purity adjusted	
		Cor	P-value	Cor	P-value
Th17	STAT3	0.129	3.28e-03	0.126	4.92e-03
	IL17A	-0.063	1.51e-01	-0.07	1.23e-01
Treg	STAT5B	0.329	1.89e-14	0.333	3.26e-14
	FOXP3	0.135	2.16e-03	0.12	7.81e-03
	CCR8	0.084	5.65e-02	0.063	1.64e-01
T cell exhaustion	CTLA4	0.147	7.97e-04	0.12	7.55e-03
	TIM-3 (HAVCR2)	0.12	6.61e-03	0.082	7.03e-02
	GZMB	-0.186	2.25e-05	-0.243	4.76e-08
	PD-1 (PDCD1)	0.073	9.91e-02	0.045	3.24e-01
CD8⁺ T cell	CD8A	0.043	3.35e-01	0.013	7.74e-01
	CD8B	0.055	2.17e-01	0.031	4.92e-01
M1 Macrophage	IRF5	0.213	1.02e-06	0.195	1.27e-05
	INOS (NOS2)	0.058	1.9e-01	0.044	3.27e-01
	COX2 (PTGS2)	-0.094	3.27e-02	-0.115	1.06e-02
M2 Macrophage	CD163	0.103	1.89e-02	0.063	1.6e-01
	VSIG4	0.143	1.18e-03	0.112	1.24e-02
	MS4A4A	0.156	3.72e-04	0.123	6.32e-03
Neutrophil	CD66b (CEACAM8)	0.278	1.33e-10	0.279	2.7e-10
	CD11b (ITGAM)	0.256	3.89e-09	0.234	1.54e-07
	CCR7	0.271	3.9e-10	0.289	6.16e-11
Dendritic cell	HLA-DPB1	0.382	2.36e-19	0.39	2.35e-19
	HLA-DPA1	0.326	3.13e-14	0.326	1.15e-13
	BDCA-1 (CD1C)	0.329	1.95e-14	0.323	2.07e-13
	CD11C (ITGAX)	0.315	2.45e-13	0.308	2.84e-12
TAM	IL10	0.17	1.04e-04	0.144	1.37e-03
	CCL2	0.075	8.93e-02	0.045	3.16e-01
	CD68	0.098	2.64e-02	0.071	1.13e-01

Analysis of IL-11RA methylation profile in LUAD patients

To further identify the potential mechanism leading to the downregulation of IL-11RA in LUAD, we firstly analyzed the DNA methylation levels of IL-11RA in the 471 LUAD samples and 32 normal samples from TCGA database. We found that the DNA methylation levels of IL-11RA were significantly higher in LUAD tissues than in normal lung tissues (Fig. 4A, $P = 2.079e-12$). Consecutively, the correlation between DNA methylation level and expression of IL-11RA in LUAD was assessed. The result indicates that the mRNA expression level of IL-11RA was negatively correlated with its DNA methylation level (Fig. 4B, $Cor = -0.22$, $P = 2.2e-06$). These results showed that the hypermethylation of IL-11RA might contribute to the downregulation of IL-11RA in LUAD. Furthermore, we discovered that LUAD patients with the higher methylation level of CpG sites cg14609668 (Fig. 4C) and cg21504624 (Fig. 4D) had a worse OS. These results suggest that the methylation and expression level of IL-11RA could influence the prognosis of LUAD patients.

IL-11RA is involved in immune infiltration and proliferation in LUAD

To further explore the biological function of IL-11RA in LUAD, we performed Gene Set Enrichment Analysis (GSEA) analysis. Previously, research has shown that the occurrence and development of tumor are closely related to immune infiltration levels [16]. Therefore, we utilize GSEA to explore the correlation of IL-11RA and immune infiltration. Enrichment results showed that many gene sets related to immune activation, such as activation of immune response (Fig. 5A), immune receptor activity (Fig. 5B), leukocyte mediated cytotoxicity (Fig. 5C), T cell activation (Fig. 5D), B cell activation (Fig. 5E), Macrophage activation (Fig. 5F), were enriched in the IL-11RA high expression group, which suggests that IL-11RA low expression may promote the progression of LUAD through immune inhibition. Furthermore, we found that several cell cycle-related gene sets were enriched in the IL-11RA low expression group (Fig. 5G-5I), which indicates that IL-11RA low expression may also be involved in facilitating cell proliferation in LUAD.

Correlation analysis between IL-11RA expression and six main infiltrating immune cells and its immune marker genes

To further clarify the correlation between IL-11RA expression and immune infiltration of LUAD, we firstly calculated the immune scores of the LUAD samples by the ESTIMATE algorithm using the data from TCGA database to predict the presence of infiltrating immune cells. The results found that the immune scores were lower in the IL-11RA low group (Fig. 6A), which indicated that IL-11RA low expression might be involved in the immune inhibition of LUAD.

Then, considering that IL-11RA is a crucial mediator of immune cell activation, a correlation analysis between IL-11RA expression and six types of immune-infiltrating cells, including B cells, $CD4^+$ T cells,

macrophages, CD8⁺ T cells, neutrophil and myeloid dendritic cell was performed via the TIMER database. The results showed that IL-11RA expression levels were significantly positively correlated with levels of infiltrating B cells, CD4⁺ T cells, and myeloid dendritic cell in LUAD (Fig. 6B-6H). Taken all together, the above evidence indicates that IL-11RA may be involved in the immune response of patients with LUAD by affecting the immune cells.

Lastly, to expand the understanding of the crosstalk between IL-11RA and multiple marker genes of immune cells, we did correlation analysis between them via the TIMER database. The results found that 44/52 (84.62%) immune cell markers were significantly associated with IL-11RA expression ($P < 0.05$), of which the number of positive correlations was 40/52 (76.92%), and the negative was 4/52 (7.69%). The results showed that the expression level of IL-11RA was significantly correlated with most immune markers of immune cells in LUAD.

Discussion

With the aggravation of environmental pollution and abuse of tobacco products, the incidence of LUAD has increased year by year, which is a subject of concern [17]. Although remarkable advances were achieved in the diagnosis and therapeutic strategies over the past decades, the overall prognosis of LUAD patients is still poor [18]. Multiple studies have reported that abnormal gene expression is involved in cancer-related processes and correlated with these patients' prognosis [19–20]. However, the potential role of IL-11RA in the development and prognosis of LUAD remains ambiguous. In the present study, we demonstrated that IL-11RA is significantly downregulated in patients with LUAD via bioinformatics analysis based on multiple databases and significantly predicts a poor prognosis; also, the higher tumor stage got a lower expression. Univariate Cox analyses indicated that the lower expression of IL-11RA might be defined as a risk factor that affects the OS and PFS of LUAD patients. Moreover, the hypermethylation of IL-11RA might contribute to the downregulation of IL-11RA and poorer OS in LUAD. Lastly, we analyzed a correlation analysis between IL-11RA and immune infiltration or immune markers, finding that IL-11RA was related to most of the immune marker genes, which may be the mechanism by which IL-11RA affects prognosis. These results suggest that IL-11RA may play a role in the development of LUAD and serve as a possible prognostic biomarker and a novel immune-related therapeutic target in LUAD.

IL-11RA was previously reported as a molecule that is highly associated with tumorigenesis [9]. The abnormal expression of IL-11RA was observed in multiple tumor tissues, such as gastric carcinoma, colon cancer, breast cancer, prostate cancer, osteosarcoma, and melanoma [21–22]. Our results found that the mRNA and protein expression level of IL-11RA is significantly downregulated in patients with LUAD. The expression of IL-11RA decreases with the increase of stages between stage 1 and stage 3 or between stage N0 and N2, but no further decreases in the stage 4 or N3, which may be due to the extremely limited advanced cases, so expanding the advanced sample size for further study is needed. Furthermore, the relationships between lung cancer and duration of smoking and type of smoking have quantified by epidemiologist [23]. We found that compared with non-smokers, the expression of IL-11RA

in smokers was significantly lower, and there was a consistent trend in the comparison between patients who had smoked for less than 15 years and those who had smoked for more than 15 years. We guess that the down-regulation of IL-11RA may be one of the potential mechanisms of lung cancer caused by smoking.

Regarding prognosis, in the Kaplan-Meier plotter, TCGA and GEO databases, the survival analysis found matching prognostic value that low IL-11RA expression was associated with poor outcomes in LUAD. Moreover, downregulation of IL-11RA was linked to worse OS of LUAD in stage 1, stage N0, and stage M0. These results robustly reflected that IL-11RA serve as a possible prognostic biomarker in lung adenocarcinoma.

Abnormal DNA methylation occupies an essential role in the induction and progression of LUAD [24]. DNA methylation is correlated with carcinogenesis by repressing the tumor suppressor gene's expression and promoting oncogenes' expression [25–26]. Therefore, we guess that hypermethylation of anti-oncogenes such as IL-11RA led to their low expression, and may be an important cause of tumorigenesis. In our analysis, a negative correlation between IL-11RA methylation and IL-11RA mRNA expression existed in LUAD tissues. In addition, we found that hypermethylation of CpG sites cg14609668 and cg21504624 had a worse OS. These results well demonstrated our guess.

Another significant finding from this study is that IL-11RA expression correlates with immune infiltration levels in LUAD. In the tumor microenvironment of lung cancer, the landscape of immune cell types is T cells, followed by B cells, macrophages, dendritic cells and natural killer cells [27]. A great variety of immune cells provide an anti-tumor effect by migrating close to tumors when tumour arise [28]. Our results demonstrate that the immune infiltrating was lower in the IL-11RA low group and IL-11RA expression has significantly positive relationships with the infiltration level of CD4⁺ T cells, B cell and myeloid dendritic cell. IL-11RA expression also strongly correlated with majority of the molecular markers of immune infiltrating cells in LUAD. In addition, the results that IL-11RA may be involved in immune response and cell proliferation was validated by GSEA. Most studies indicate that dendritic cells contribute to the activation of antitumor T lymphocytes [29–30], and higher levels of T cells and B cells were closely related with better prognosis in cancer patients [31–33]. Therefore, these findings indicate that IL-11RA expression is closely related with recruitment and regulation of immune infiltrating cells and may be involved in the immune response to LUAD development, resulting in a worse prognosis in LUAD patients.

It is worth noting that some inevitable limitations exist in our analysis. First, although we preliminarily investigated the biological process of IL-11RA in LUAD through GSEA enrichment analysis, the detailed mechanism that correlation between IL-11RA low expression and LUAD progression requires further biomedical experiments. Then, more prospective data were needed for proving the clinical utility of it.

Conclusions

In conclusion, we suggest that decreased IL-11RA expression correlates with poor prognosis and immune infiltration in LUAD by applying integrated bioinformatics approaches. Our work highlights IL-11RA might be a potential biomarker for prognosis and provide a new immunotherapeutic target for LUAD patients.

Abbreviations

CI: Confidence intervals; **DEGs:** Differentially expressed genes; **ECM:** Extracellular matrix; **FC:** Fold change; **FDR:** False Discovery Rates; **GEO:** Gene Expression Omnibus; **GEPIA:** Gene Expression Profiling Interactive Analysis; **GSEA:** Gene set enrichment analysis; **GTEX:** Genotype-Tissue Expression; **HPA:** The Human Protein Atlas; **HR:** Hazard ratio; **IL-11RA:** Interleukin-11 receptor subunit alpha; **KM plotter:** Kaplan-Meier plotter; **LUAD:** Lung adenocarcinoma; **NES:** Normalized enrichment score; **OS:** Overall survival; **PFS:** Progression-free survival; **PPI:** Protein–protein interaction; **TCGA:** The Cancer Genome Atlas; **TIMER:** Tumor Immune Estimation Resource; **UCSC:** University of California santa cruz.

Declarations

Ethical Approval and Consent to participate

Not applicable

Consent for publication

All authors agree to publish this article in Hereditas

Availability of supporting data

Transcriptomic data, clinical and DNA methylation data of LUAD patients were downloaded from the UCSC Xena website (<https://xenabrowser.net/datapages>), GSE72094 data were downloaded from GEO (<https://www.ncbi.nlm.nih.gov/geo/>) database.

Competing interests

The authors confirm that there are no conflicts of interest.

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Authors' contributions

Meng Xu conceived this study, Ai tao Nai and SHOAI BASHIR were responsible for the acquisition and analysis of data. Ling Jin contributed to the literature search for the manuscript. Zirui He and Shuwen

Zeng wrote and revised the manuscript. All authors reviewed the manuscript and approved the manuscript for publication.

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Figures

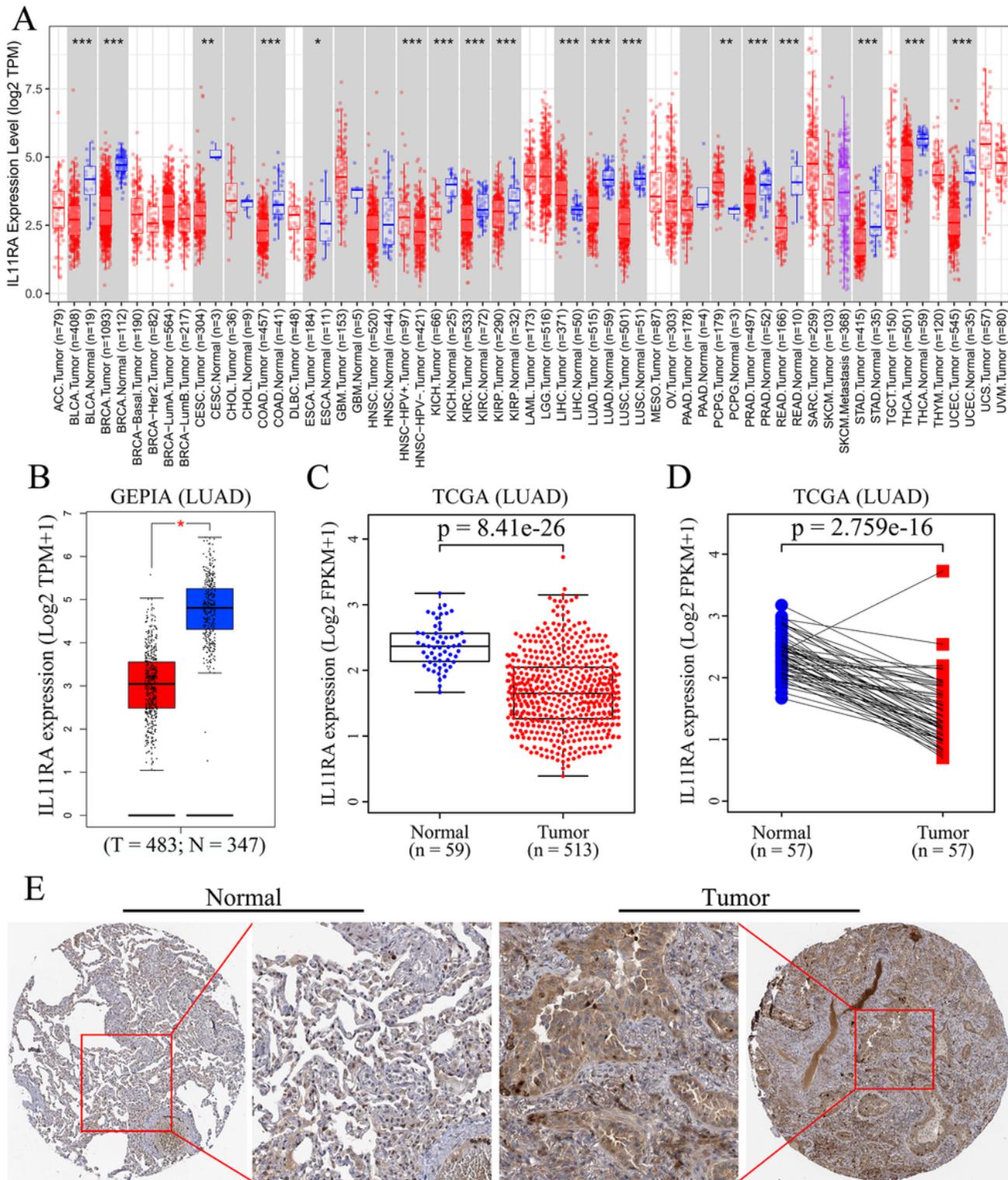


Figure 1

The expression of IL-11RA is down-regulated in lung adenocarcinoma (LUAD). (A) Human IL-11RA expression levels in different tumor types from TCGA database were determined by TIMER; (B) IL-11RA expression in LUAD and normal tissues based on the TCGA and GTex data analysed by GEPIA, * $P < 0.05$; (C) IL-11RA expression in LUAD and normal tissues in the TCGA dataset; (D) Expression levels of IL-11RA

in paired LUAD samples in the TCGA dataset; (E). Representative immunohistochemistry staining of IL-11RA expression in normal lung tissues and LUAD tissues. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

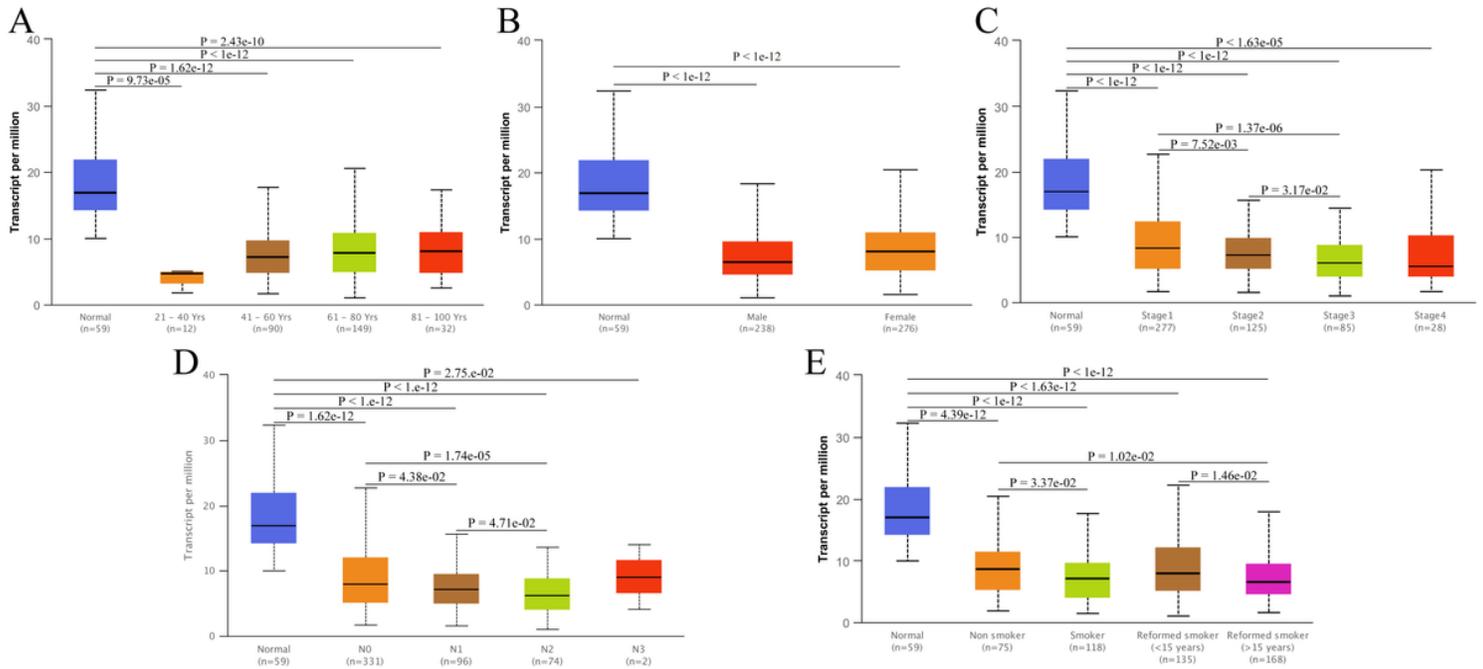


Figure 2

UALCAN analysis for the correlation between IL-11RA mRNA expression level and clinicopathological parameters of LUAD. (A) Age. (B) Gender. (C) Stage. (D) Lymph -node status. (E) Smoking habits

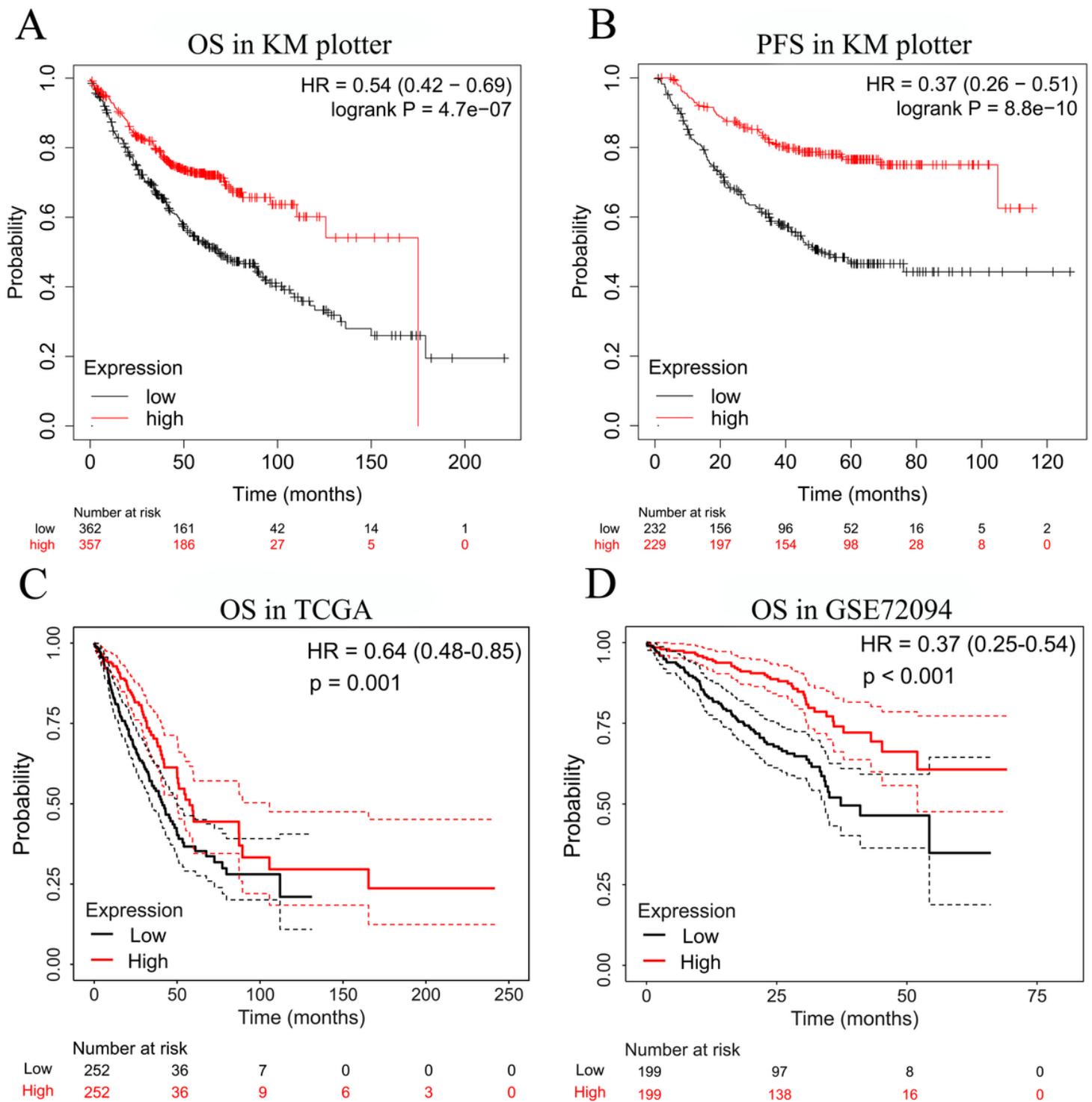


Figure 3

Low expression of IL-11RA is associated with a poor prognosis of LUAD. (A-B) Low IL-11RA expression was correlated with worse OS and PFS in Kaplan Meier plotter database; (C) Low IL-11RA expression was correlated with worse OS in the LUAD from TCGA datasets; (D) Low IL-11RA expression was correlated with worse OS in the LUAD from GSE72094 datasets. P<0.05 was considered significant.

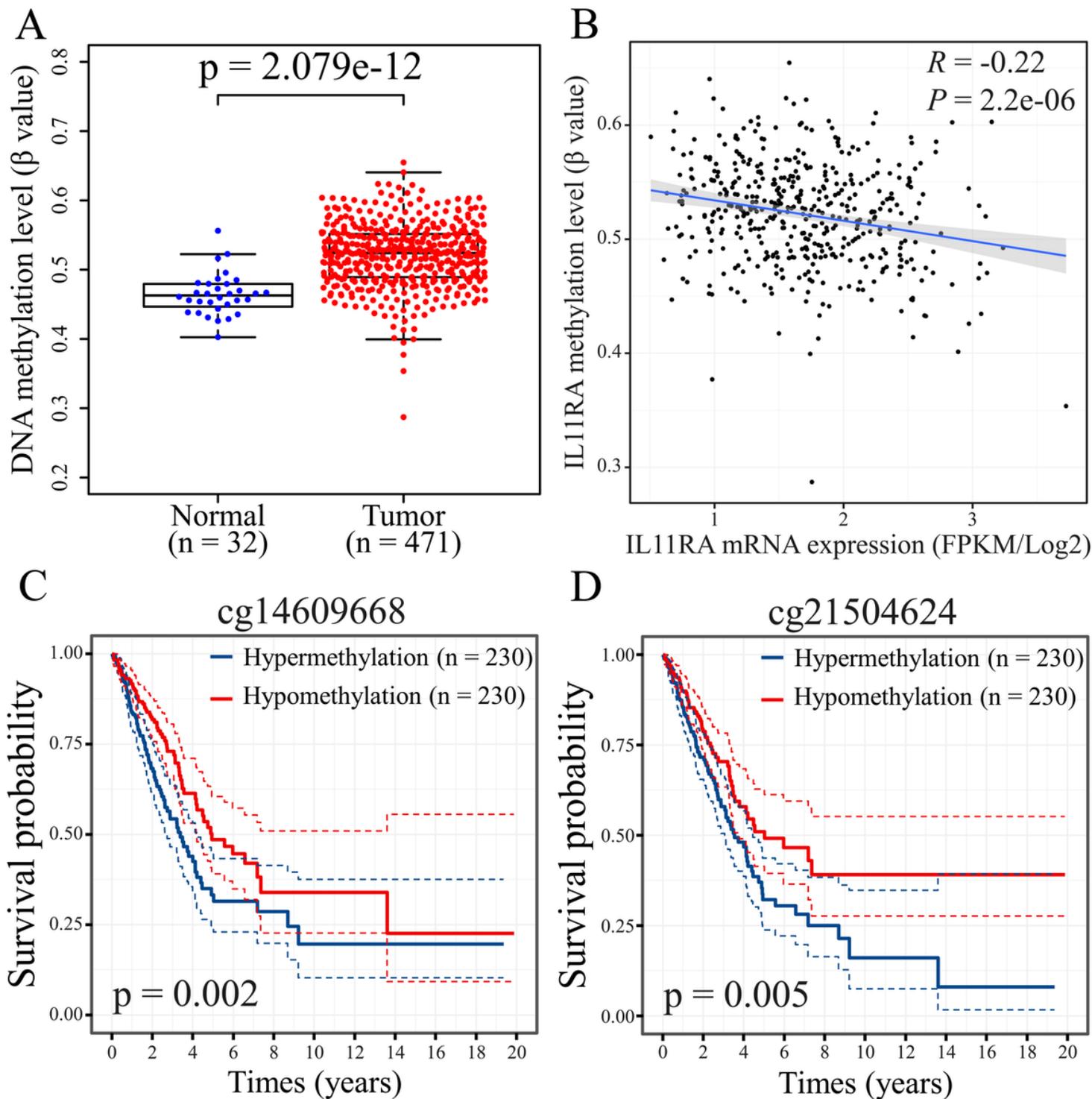


Figure 4

Analysis of IL-11RA methylation profile in LUAD patients. (A) The methylation level of IL-11RA in the normal and LUAD tissues. (B) The correlation between gene expression and methylation of IL-11RA. (C) Survival curve of the LUAD patients with differently methylated cg14609668. (D) Survival curve of the LUAD patients with differently methylated cg21504624.

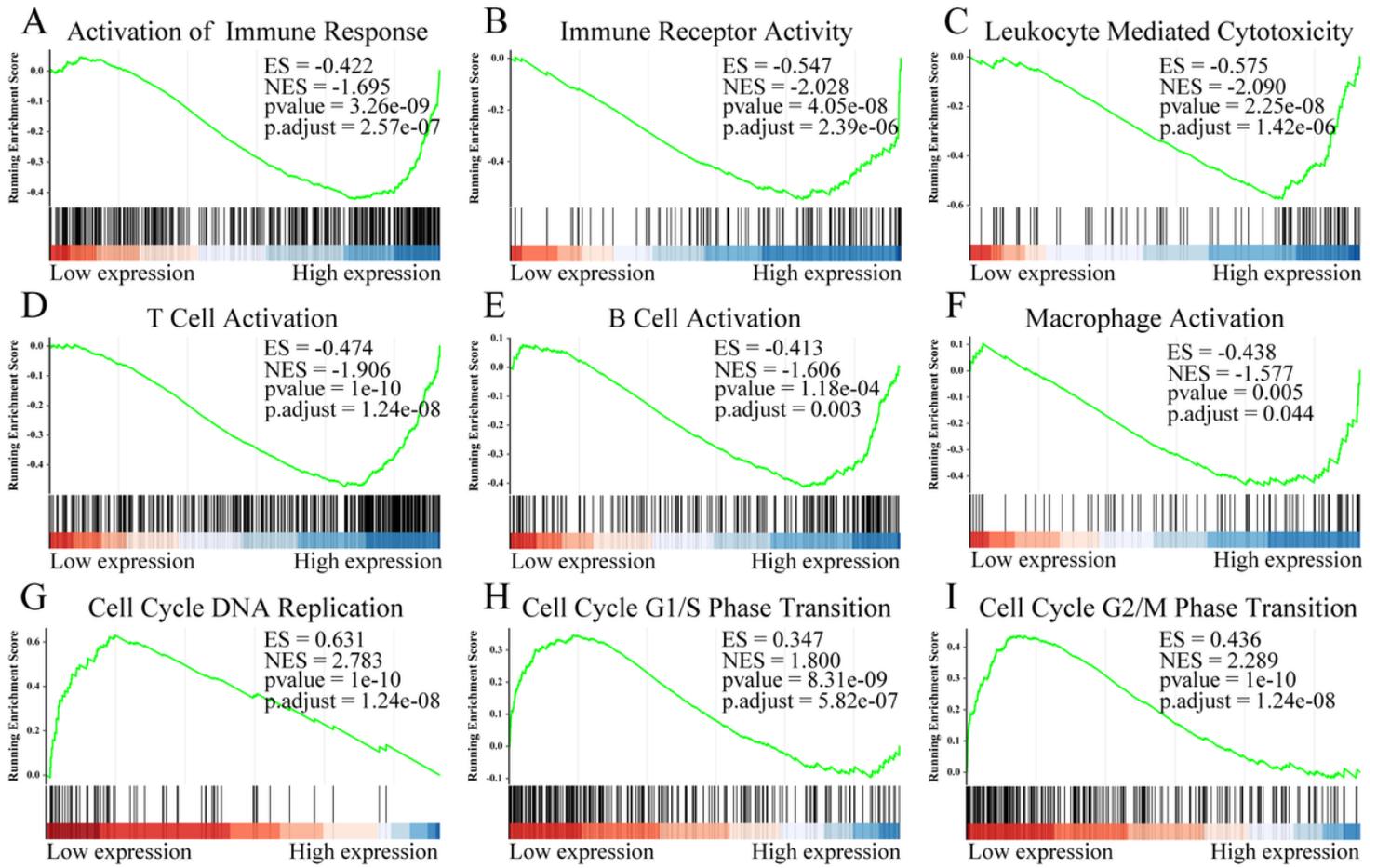


Figure 5

Immune activation and cell cycle processes were enriched according to IL-11RA expression in LUAD. (A-F) Immune activation processes were enriched in the IL-11RA high expression group of LUAD. (G-I) Cell cycle processes were enriched in the IL-11RA low expression group of LUAD.

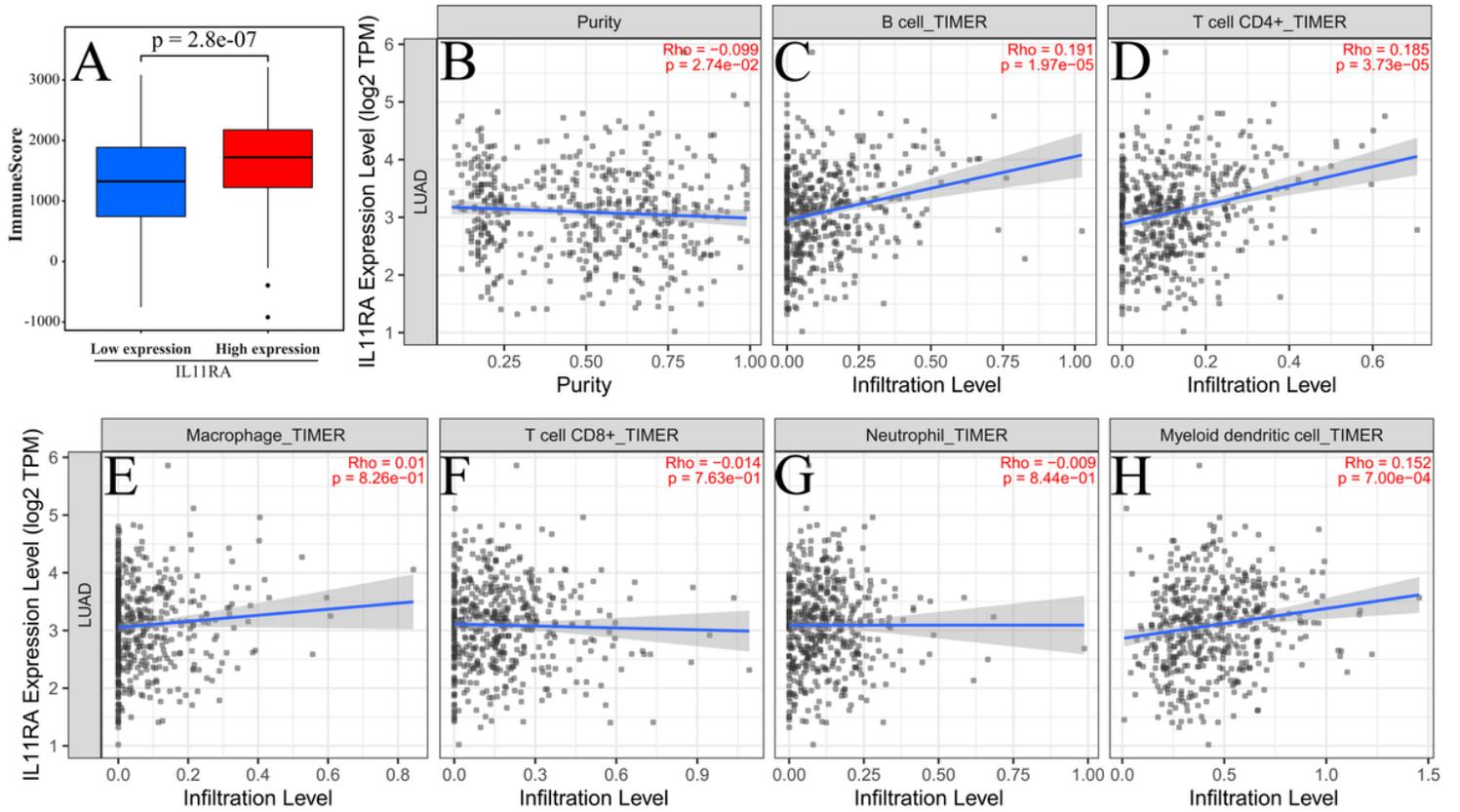


Figure 6

Correlation of IL-11RA expression with immune infiltration level in LUAD. (A) Comparison of immune score between the high and low IL-11RA expression groups of LUAD. (B-H) IL-11RA is associated with tumor purity and immune infiltration levels of LUAD by TIMER.