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Hsa-miR-21 promoted the progression of lung adenocarcinoma by regulating *LRIG1* expression

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Abstract

Lung cancer is the foremost cause of cancer-related fatalities globally, and lung adenocarcinoma (LUAD) is one of the common types of lung cancer with significant molecular heterogeneity. Leucine rich repeats and immunoglobulin like domains 1 (*LRIG1*) has been demonstrated to be down-regulated in lung cancer and related to prognosis of patients. The purpose of this work is to explore the targeting miRNAs of *LRIG1*, and the related regulatory mechanisms in LUAD. The data of LUAD patients were collected from The Cancer Genome Atlas and Gene Expression Omnibus databases. The differential expression analysis and gene set enrichment analysis (GSEA) were performed using "limma" and "clusterProfiler" function package, respectively. The levels of hsa-miR-21 mRNA and *LRIG1* mRNA and LRIG1 protein expressions were analyzed using RT-qPCR and western blot analysis. The infiltration of immune cells was determined using CIBERSORT software. In LUAD patients, hsa-miR-21 expression was observably related to *LRIG1* expression. Hsa-miR-21 might negatively modulate the *LRIG1* expression in LUAD. LUAD patients with hsa-miR-21 up-regulation exhibited inferior prognosis. In addition, those with LUAD who had high hsa-miR-21 expression but low *LRIG1* expression had a worse prognosis, whereas those with low hsa-miR-21 expression but high *LRIG1* expression had a better prognosis. Functional enrichment analysis indicated that metabolic related signaling pathways (EGFR tyrosine kinase inhibitor resistance) were significantly activated in LUAD patients with *LRIG1* up-regulation. Finally, we found that relative content of naive B cells, plasma cells and resting CD4+T cells were significantly increased and regulatory T cells and Macrophages M0 were decreased in *LRIG1* high expression group and hsa-miR-21 low expression group. We firstly reported that hsa-miR-21 might regulate the *LRIG1* expression in LUAD, thereby effecting the onset and progression of LUAD.

Clinical trial number: Not applicable.

Keywords Lung adenocarcinoma, *LRIG1*, hsa-miR-21, Prognosis, Immune cell infiltration

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Introduction

In recent years, the incidence of lung cancer has increased, with the second incidence and the first mortality rate, seriously threatening human life and health [1]. Smoking, age, genetic factors, ethnic factors are all linked to the occurrence of lung cancer [2–4]. As the histological type of lung cancer corresponds with tumor behavior and prognosis, lung cancers (Small-cell lung cancer (SCLC) and non-small lung cancer (NSCLC)) are frequently categorized according to histological types [3]. NSCLC is responsible for 85% of all lung cancer diagnosis [5], with the predominant subtypes being lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) [3]. LUAD, a severe subtype of lung cancer, affects approximately 40% of all patients diagnosed with this disease [6]. One of the primary molecular distinctions between LUAD and LUSC lies in their genetic alterations. LUAD is often associated with mutations in genes such as EGFR, KRAS, and BRAF, which are critical for targeted therapies [7]. For instance, EGFR mutations are detected in approximately 10%~30% of LUAD patients, with higher prevalence rates in specific populations, such as Chinese patients, where rates can reach up to 50% [8]. In contrast, LUSC typically shows a higher frequency of TP53 mutations and fewer actionable mutations, making targeted therapies less effective for this subtype [9]. The presence of these mutations in LUAD allows for the use of targeted therapies, such as EGFR tyrosine kinase inhibitors (TKIs), which have significantly improved outcomes for patients with these specific genetic alterations [10, 11]. Moreover, LUAD faces issues related to tumor heterogeneity and resistance mechanisms. The heterogeneity within LUAD tumors can lead to varied responses to treatment, complicating the management of the disease. For example, LUAD can exhibit different histological patterns, such as lepidic, acinar, and micropapillary, each associated with distinct prognoses and treatment responses [12]. This intratumoral heterogeneity can result in the presence of subclonal mutations that may not be effectively targeted by standard therapies, leading to treatment resistance [13].

The leucine rich repeats and immunoglobulin like domains (LRIG) protein family consists of three members (LRIG1, LRIG2, LRIG3) that are type I single-transmembrane proteins [14]. *LRIG1* is abundantly expressed in numerous human tissues, exhibiting notably elevated levels in the colon and brain [15]. It has been demonstrated that *LRIG1* negatively regulates the ERBB receptor tyrosine kinases (RTKs), such as EGFR/ERBB1/HER1 and ERBB3/HER3 [16, 17]. RTKs are critical regulators of differentiation, metabolism, proliferation and motility of cells. During the process of tumorigenesis, the expression and function of RTK can become dysregulated, ultimately leading to the acquisition of oncogenic functions [18–20]. In addition, considerable evidence supports that the expression of *LRIG1* is down-regulated in most cancers, for instance colorectal tumor [21], breast cancer [22], lung cancer [23], thyroid cancer [24], hepatocellular carcinoma [25]. In NSCLC, the high *LRIG1* expression is correlated with better prognosis of patients [26, 27]. In addition, The *LRIG1* overexpression could inhibit clonal capacity and tumor growth in human lung cancer cells [15]. Additionally, LRIG1 is a tumor suppressor in lung cancer.

MicroRNAs (miRNAs), non-coding RNA molecules mediated by approximately 22 nucleotides long, are the basis of epigenetic regulation of gene expression [28]. miRNAs modulate the expression and function of their linked target genes, influencing the biological behaviors of cancer cells and stromal cells, which could potentially contribute to tumor progression [29]. In glioblastoma, the high expression of miR-183 could down-regulate the *LRIG1* and increase the EFGR/Akt activity, resulting in the promoted radioresistance of tumor [30], and miRNA-19a down-regulation could restrain the growth of glioma cells via modulating *LRIG1* expression [31]. In oral verrucous carcinoma, *LRIG1* is the key target of miR-181b [32]. MiR-20a directly targeted the 3′-UTR of *LRIG1* to inhibit *LRIG1* expression, and miR-20a was inversely expressed with *LRIG*1 in specimens of gastric cancer [33]. However, in LUAD, whether *LRIG1* expression is regulated by miRNAs to impact the progression of tumors is still undocumented. Accordingly, in this study, we explored the targeting miRNAs of *LRIG1*, and analyzed the regulatory mechanism of *LRIG1* by miRNAs in LUAD.

Methods

Data

In The Cancer Genome Atlas (TCGA, https://tcga-data.nci.nih.gov/tcga/) database, we downloaded mRNA expression profiling data of 585 LUAD patients, including 525 LUAD samples and 60 para-cancerous samples. Among which, totally 501 samples included complete survival information (Table 1). In TCGA database, we also collected miRNA expression profiling data from 564 LUAD patients, including 518 LUAD samples and 46 para-cancerous samples and collected the mutation annotation format (MAF) files of LUAD for subsequent analysis.

After downloading the dataset, the probe information was converted into GeneSymbol or miRNA numbers according to the corresponding annotation file. This allows for direct used in analysis without the need for further standardization or additional analyses.

Moreover, in Gene Expression Omnibus (GEO, https://www.ncbi.nlm.nih.gov/geo/) database, we extracted GSE29248 dataset, which included 6 LUAD samples and

Table 1 Clinicopathological characteristics of patients with LUAD from TCGA-LUAD database

Characteristics		Patients(*N* = 501)	
		NO.	%
Gender	Female	271	54.09%
	Male	230	45.91%
Age	≤ 66(Median)	259	51.70%
	> 66(Median)	242	48.30%
Grade	I	269	53.69%
	II	119	23.75%
	III	80	15.97%
	IV	25	4.99%
	Unknown	8	1.60%
Survival Time	Long(> 5 years)	251	50.10%
	Short(< 5 years)	52	10.38%
OS status	Dead	182	36.33%
	Alive	319	63.67%
Radiation	Yes	416	83.03%
	No	71	14.17%
	Unknown	14	2.79%
Tobacco	Yes	58	11.58%
	No	361	72.06%
	Unknown	82	16.37%

6 para-cancerous samples. The miRNA expression profiling data in GSE29248 dataset were downloaded through Illumina Human v2 MicroRNA expression beadchip.

Cell line source and culture

Human bronchial epithelial cell line (BEAS-2B) and two human LUAD cell lines (NCI-H1975, NCI-H441) were purchased from Bena Culture Collection (Beijing, China) and Procell (Wuhan, China), respectively. BEAS-2B was cultured in DEMO medium (PM150210, Procell, Wuhan, China), NCI-H1975 and NCI-H441 were seeded in RPMI-1640 medium (PM150110, Procell, Wuhan, China). All medium were supplemented with 10% fetal bovine serum (PBS, 164210, Procell, Wuhan, China) and 1% penicillin/streptomycin (P/S) under a humidified environment of 5% CO2 at 37 °C.

The hsa-miR-21 inhibitor and inhibitor NC were purchased from RiboBio Co., Ltd. (Guangzhou, China). The NCI-H1975 cells were inoculated in six-well plates and cultured for 24 h. Next, the cells were transfected with hsa-miR-21 inhibitor and inhibitor NC using Lipofectamine 3000 (L3000075, Invitrogen) at 37 °C for 48 h following the manufacturer's protocol. Transfection efficiency was determined by RT-qPCR.

Differential expression analysis and gene set enrichment analysis (GSEA)

The R language (version 4.1.0, the same below) "limma" [34] function package was applied to identify differentially expressed genes (DEGs) or differentially expressed miRNAs (DEmiRNAs) between two groups according to the $p < 0.05$ and $|Log2FC| > 1$. These DEGs were then subjected to GSEA employing "clusterProfiler" package [35] in R language. The significantly enriched pathways were identified via $p < 0.05$.

Survival analysis

Based on the Kaplan Meier (KM) method, R language "survival" and "survminer" package (https://CRAN.R-project.org/package=survival) was applied to calculate the overall survival of patients. The log-rank test was used to compare survival between groups and to calculate the *p*-value. The "ggsurvplot" function was used to visualize the KM curve with confidence interval and to label the *p*-value. Multivariate Cox regression model was employed to explore whether the gene could predict the survival of LUAD patients independently of other factors.

Immune cell infiltration analysis

The infiltration of 22 immune cells were determined via CIBERSORT software [36]. These immune cell types include naive B cells, memory B cells, plasma cells, CD8 + T cells, naive CD4 + T cells, activated CD4 + T cells, memory T cells, resting CD4 + T cells, regulatory T cells (Tregs), follicular helper T cells, γδ T cells, resting natural killer (NK) cells, activated NK cells, resting mast cells, activated mast cells, M0 macrophages, M1 macrophages, M2 macrophages resting dendritic cells (DCs), activated DCs, neutrophils, eosinophils, and monocytes. By utilizing the 547 predefined barcode genes within the deconvolution algorithm, CIBERSORT can accurately characterize the composition of immune infiltrating cells based on gene expression matrices. It is important to note that the sum of all estimated proportions in each sample equals 1.

RT-qPCR analysis

The total RNA was extracted from cells using TRNzol Universal (DP424, TIANGEN, Beijing, China) and detected using an ultraviolet spectrophotometer (SMA2000, ThermoFish, USA). The RNA was converted to cDNA using HiFiScript cDNA Synthesis Kit (CW2569M, Cowin Biotech. CO.,Ltd, Beijing, China) following the prescribed protocol. The quantitative PCR was performed using All-in-One qPCR (Qp001, GeneCopoeia, USA) on a qPCR fluorescence quantification system (FQD-96 A, Hangzhou bogi, Hangzhou, China). GAPDH was considered as internal control, and the primers were shown in Table 2. The $2^{-\Delta\Delta Ct}$ was used to calculate the fold change of gene mRNA [37].

Western blot

The proteins were extracted using RIPA lysate and PMSF. Western blot assay was performed as described previously [38]. The primary antibody used in this study was

Table 2 Primer sequences for qPCR

Genes	Forward Primer (5′-3′)	Reverse Primer (5′-3′)
LRIG1	CTGCATGAGTTGGTCCTGTCC	TGTGGCTGATGGAATTGTGG
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
hsa-miR-21	ACAGCCCATCGACTGGTGTTG	
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT

Anti- LRIG1 (PA5-52860, 04 ug/mL, Thermo, USA), the secondary antibodies were HRP-labeled Coat anti-rabbit IgG (H + L) (ZB-2301, 1: 10000, Beijing Zhong Shan -Golden Bridge Biological Technology CO.,Ltd, Beijing, China) and HRP-labeled Goat anti-mouse IgG (H + L) (ZB-2301, 1: 10000, Beijing Zhong Shan -Golden Bridge Biological Technology CO.,Ltd, Beijing, China). The internal reference was GAPDH (60004-1-Ig, 1:50000, Proteintech, Beijing, China). Image J was employed to quantify the grayscale value of each band. The primary images of the blots were shown in the Supplementary Fig. 1.

Results

Hsa-miR-21 expression exhibited negative correlation with LRIG1 expression in LUAD

Firstly, we analyzed the DEmiRNAs between LUAD vs. para-cancerous samples in the TCGA-LUAD cohort. We found that 92 miRNAs were significantly up-regulated and 30 miRNAs were significantly down-regulated in LUAD samples in the TCGA-LUAD cohort (Fig. 1A, LUAD vs. para-cancerous). In the miRNA database mirwalk (http://mirwalk.umm.uni-heidelberg.de/), we predicted the target miRNAs for the gene *LRIG1* and obtained 1462 target miRNAs of *LRIG1* (Table S1). The cross-over analysis showed that among these 1462 miRNAs, 72 miRNAs were up-regulated in LUAD samples (Fig. 1B). These results indicated that there were 72 target miRNAs of *LRIG1* in LUAD.

Among these 72 miRNAs, hsa-miR-21 is correlated with metastasis of NSCLC [39]. NSCLC subtypes can be classified based on the expression of hsa-miR-21 [40]. Therefore, we analyzed the expression of hsa-miR-21 in LUAD samples. The results showed that hsa-miR-21 was significantly up-regulated in LUAD samples (Fig. 1C, D, LUAD vs. para-cancerous) in the GSE29248 dataset and the TCGA-LUAD cohort. We also found that hsa-miR-21 expression exhibited a significant negative association with *LRIG1* expression (Fig. 1E). Therefore, we selected hsa-miR-21 for subsequent analyses.

In the TCGA-LUAD cohort, the *LRIG1* expression was decreased in LUAD samples (Fig. 1F, LUAD vs. para-cancerous). Furthermore, hsa-miR-21 expression was up-regulated in NCI-H1975 and NCI-H441 cells compared to BEAS-2B cell by RT-qPCR analysis (Fig. 2A). Meanwhile, the levels of LRIG1 mRNA and protein expression were observably reduced in NCI-H1975 and NCI-H441 cells than that in BEAS-2B cell (Fig. 2B and C). These results indicated that hsa-miR-21 expression showed a prominent negative correlation with *LRIG1* expression in LUAD.

To investigate whether hsa-miR-21 regulates the expression of *LRIG1*, we constructed the hsa-miR-21 knockdown NCI-H1975 cells (hsa-miR-21 inhibitor group). Compared to the inhibitor NC group, the expression of hsa-miR-21 was significantly reduced (Fig. 2D). In addition, both the mRNA and protein levels of *LRIG1* were significantly increased in the hsa-miR-21 inhibitor group (Fig. 2E and F). These results suggest that hsa-miR-21 may negatively regulate the expression of *LRIG1* in LUAD.

Hsa-miR-21 could effectively predict the prognosis of LUAD patients through modulating LRIG1 expression

We then divided the LUAD patients into two groups according to the median expression level of hsa-miR-21 (18.45299679): hsa-miR-21 high expression group and hsa-miR-21 low expression group. In the TCGA cohort, the hsa-miR-21 high expression group was correlated with worse prognosis (Fig. 3A, high vs. low). In the TCGA-LUAD cohort, the area under curves (AUCs) for 1-, 3-, and 5-year overall survival in were 0.55, 0.57 and 0.64, respectively (Fig. 3B). These results suggest that hsa-miR-21 may effectively predict the prognosis of LUAD patients.

Moreover, the LUAD patients were classified into *LRIG1* high expression group and *LRIG1* low expression group, based on the median value of *LRIG1* expression (11.84392105) in the TCGA-LUAD cohort. Then, we classified LUAD patients into hsa-miR-21 high expression and *LRIG1* high expression group, hsa-miR-21 high expression and *LRIG1* low expression group, hsa-miR-21 low expression and *LRIG1* high expression group, and hsa-miR-21 low expression and *LRIG1* low expression group. In the TCGA-LUAD cohort, we found that the hsa-miR-21 high expression and *LRIG1* low expression group was correlated with a poorer prognosis of LUAD patients (Fig. 3C). Conversely, hsa-miR-21 low expression and *LRIG1* high expression group was correlated with a better prognosis of LUAD patients (Fig. 3C). The tumor somatic mutation (TMB) analysis showed that in both hsa-mir-21 high expression and hsa-miR-21 low expression groups, gene *TP53* and *TTN* had the highest mutation rates (Fig. 3D and E). The mutation rate was significantly increased in the hsa-miR-21 high expression group compared to the hsa-miR-21 low expression group. The above results suggest that hsa-miR-21 may effectively predict the prognosis of LUAD patients by regulating *LRIG1* expression.

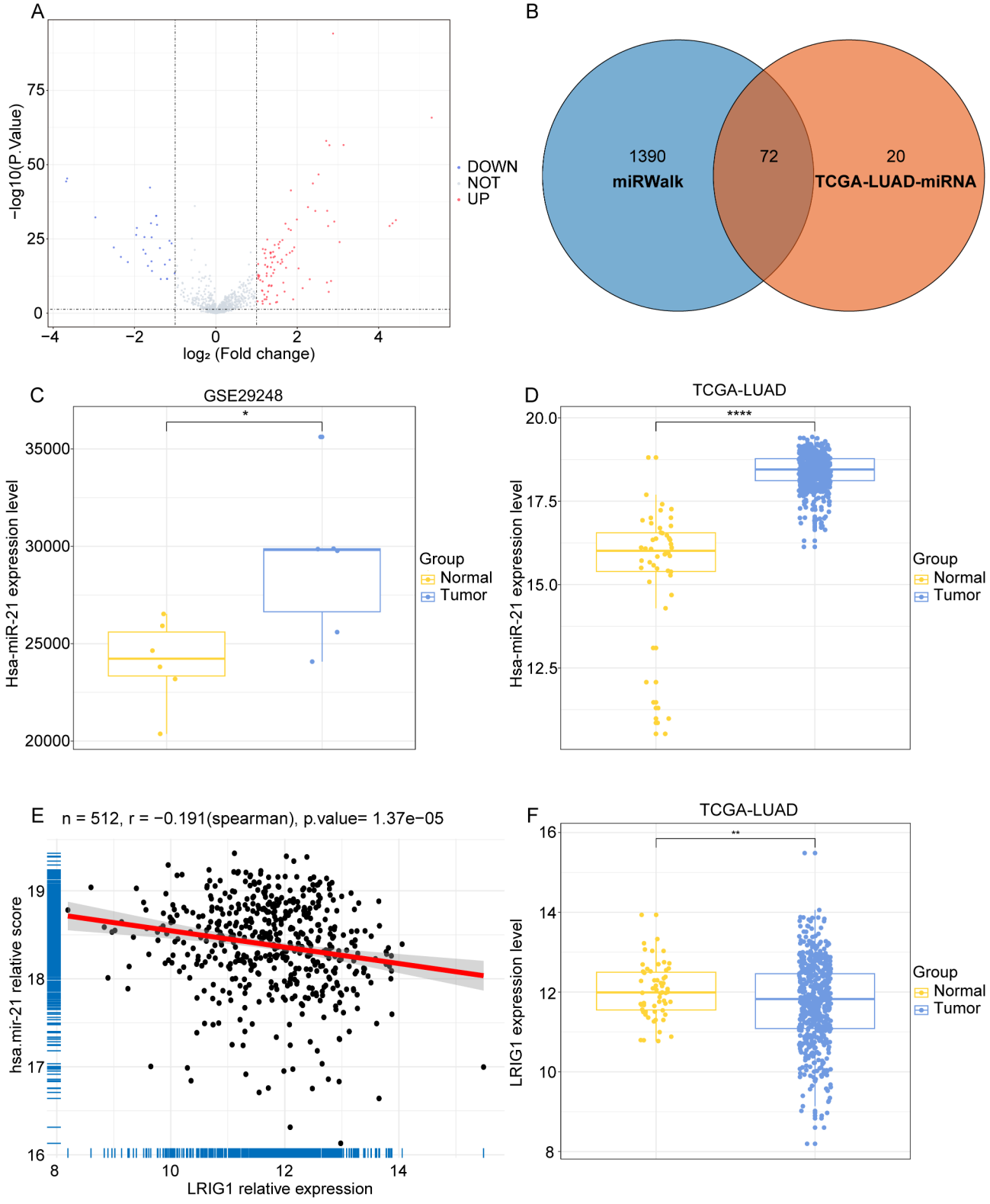

Fig. 1 Hsa-miR-21 expression exhibited negative correlation with *LRIG1* expression in LUAD. **(A)** The differentially expressed miRNAs (DEmiRNAs) between LUAD and para-cancerous samples in TCGA cohort. **(B)** The overlapping miRNAs between TCGA-LUAD-miRNA (up-regulated) and miRWalk groups. **(C-D)** The expression of hsa-miR-21 in LUAD samples in GSE29248 and TCGA-LUAD. **(E)** The correlation of hsa-miR-21 with *LRIG1*. **(F)** The *LRIG1* expression in LUAD samples. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$

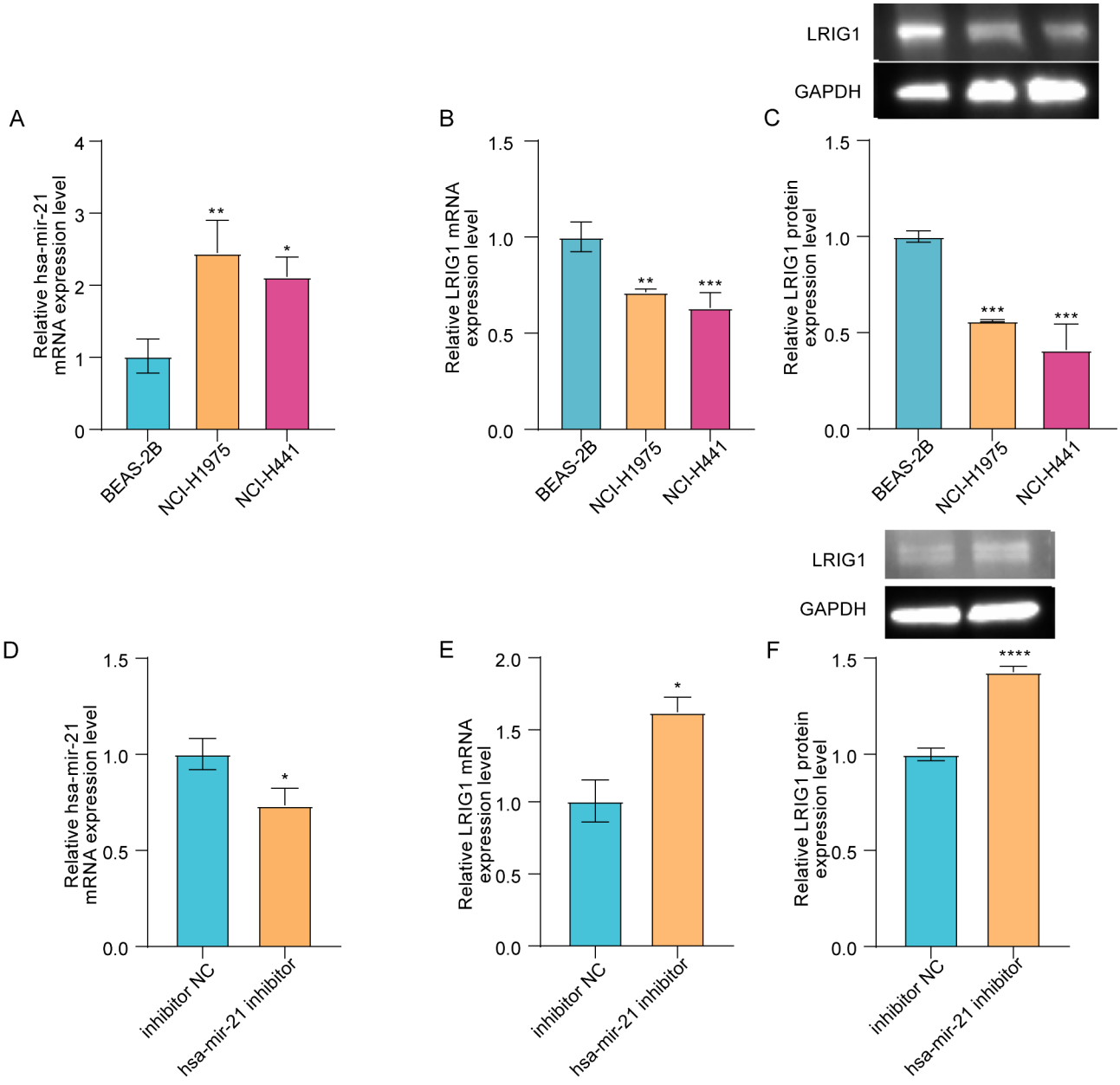

Fig. 2 Hsa-miR-21 might negatively regulate the expression of LRIG1 in LUAD. The expression of hsa-miR-21 **(A)**, LRIG1 mRNA **(B)** and protein **(C)** in BEAS-2B, NCI-H1975 and NCI-H441 cells. The expression of hsa-miR-21 **(D)**, LRIG1 mRNA **(E)** and protein **(F)** in hsa-miR-21 inhibitor group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

The signaling pathways correlated with hsa-miR-21 and *LRIG1* in LUAD

GSEA revealed that in the TCGA-LUAD cohort, 80 and 65 signaling pathways were significantly activated and suppressed, respectively, in the hsa-miR-21 high expression group (high vs. low, Table S2, Fig. 4A). Meanwhile, we found that in the *LRIG1* high expression group, a total of 143 signaling pathways were observably activated in the KEGG pathway (Table S3). The top 20 enriched KEGG pathways are shown in Fig. 4B. Notably, the metabolic-related pathway, EGFR tyrosine kinase inhibitor resistance was significantly activated in *LRIG1* high expression group (Fig. 4C), nevertheless this signaling pathway was not significantly enriched in the hsa-miR-21 high expression group (Fig. 4D).

The correlation of immune cell infiltration with hsa-miR-21 and *LRIG1*

Finally, we calculated the relative content of 22 immune cells' infiltration in 496 LUAD patients expressing hsa-miR-21 and *LRIG1* (Fig. 5A). As shown in Fig. 5B and C, the relative content of naive B cells, plasma cells and

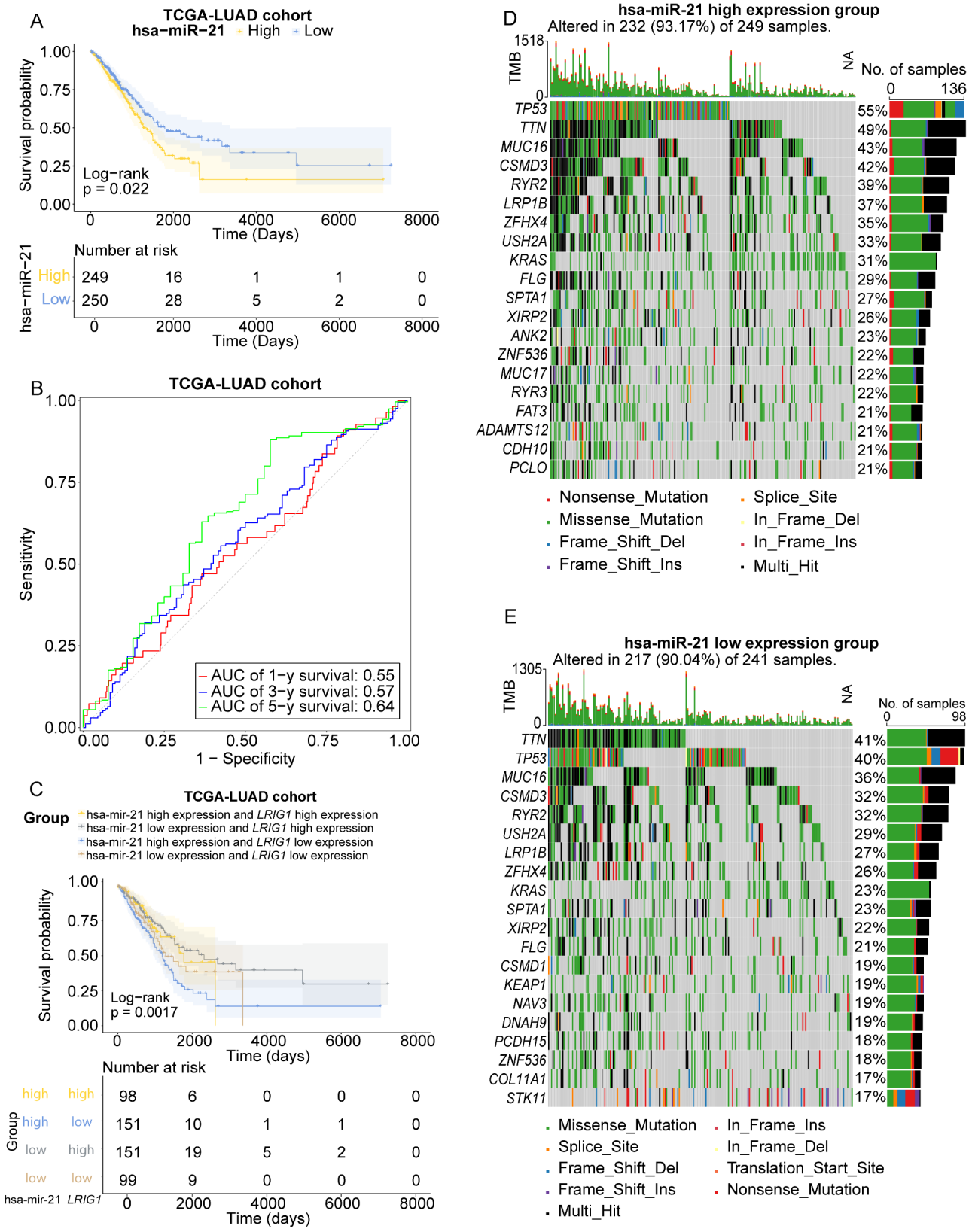

Fig. 3 Hsa-miR-21 could effectively predict the prognosis of LUAD patients through modulating *LRIG1* expression. **(A)** The survival rate of patients with hsa-mir-21 high and low expression groups in TCGA-LUAD cohort. **(B)** The area under curves (AUCs) of 1-, 3-, and 5-year overall survival in TCGA-LUAD cohort. **(C)** The survival rate of patients in the different groups in TCGA-LUAD cohort. **(D-E)** The tumor somatic mutation (TMB) in hsa-mir-21 high and low expression groups

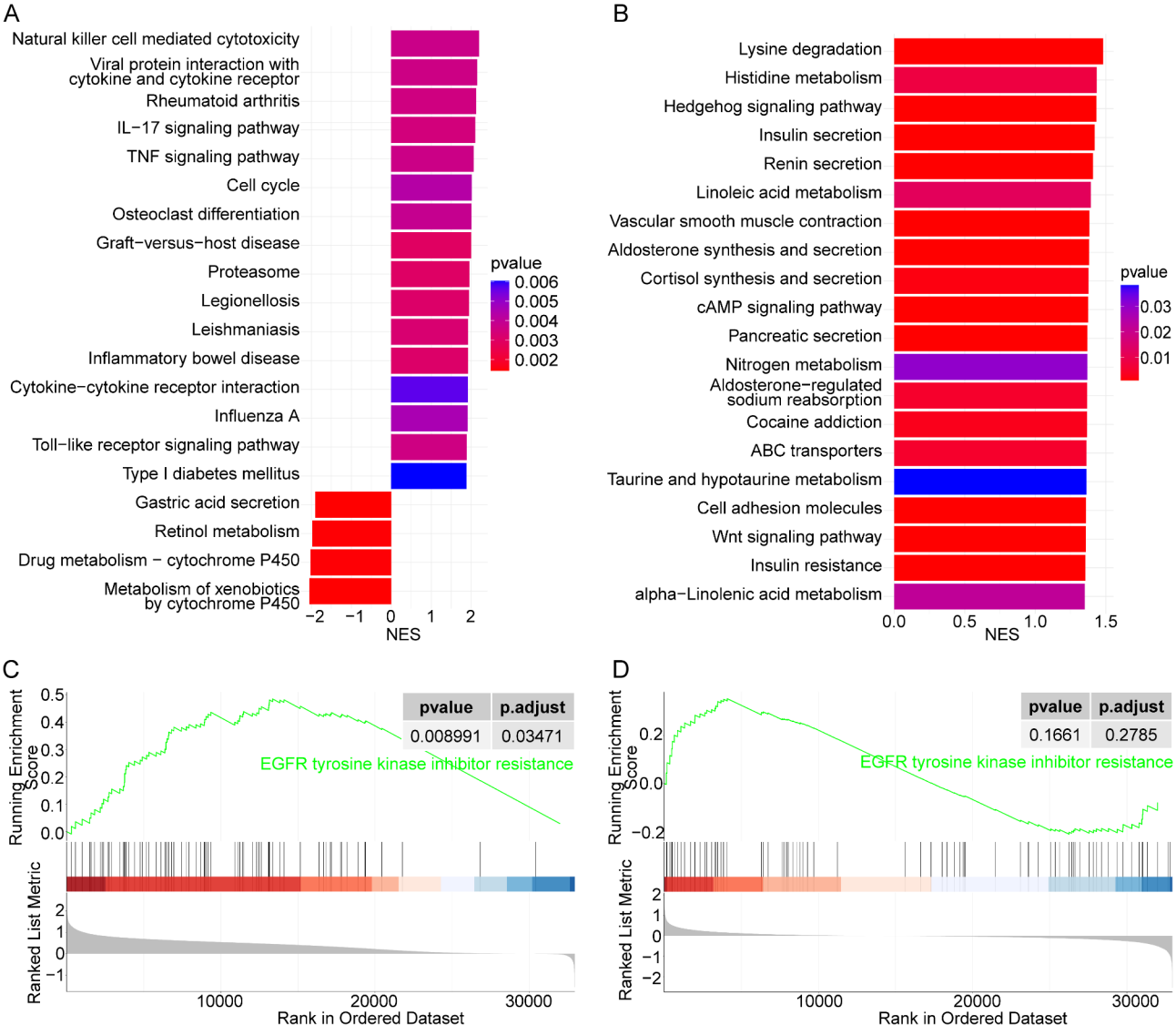

Fig. 4 The signaling pathways correlated with hsa-miR-21 and *LRIG1* in LUAD. The top 20 significantly enriched signaling pathways in hsa-mir-21 high expression group **(A)** and *LRIG1* high expression group **(B)**. Activation of EGFR tyrosine kinase inhibitor resistance pathway in *LRIG1* high expression group **(C)** and hsa-mir-21 high expression group **(D)**

resting CD4 + T cells was significantly increased in the *LRIG1* high expression group and the hsa-miR-21 low expression group. The relative content of Tregs and M0 macrophages were significantly decreased in the *LRIG1* high expression group and the hsa-miR-21 low expression group (Fig. 5B and C).

Discussion

According to reports, *LRIG1* could suppress the growth of different types of malignant tumor [41], and the expression and function of *LRIG1* were modulated by miRNAs in some cancers [30, 42]. Research indicates that hsa-miR-21 is significantly upregulated in LUAD and facilitates tumor progression through various mechanisms [43]. For instance, hsa-miR-21 inhibits the Hippo signaling pathway by targeting *KIBRA*, thus promoting the progression of LUAD [44]. Additionally, hsa-miR-21 enhances the proliferation, migration, and invasion of LUAD cells by targeting genes such as *WWC2* and *SET* [45, 46]. Although hsa-miR-21 has been extensively studied in LUAD, the specific interaction between miR-21 and *LRIG1* in the context of tumorigenesis has yet to be elucidated. Herein, we firstly reported that hsa-miR-21 could regulate the *LRIG1* expression in LUAD, thereby effecting the onset and progression of tumor. This regulatory axis may offer valuable insights for the treatment and diagnosis of LUAD patients.

MiRNAs are small, non-coding RNA molecules that play crucial roles in the regulation of gene expression, particularly in the context of cancer, including LUAD [47,

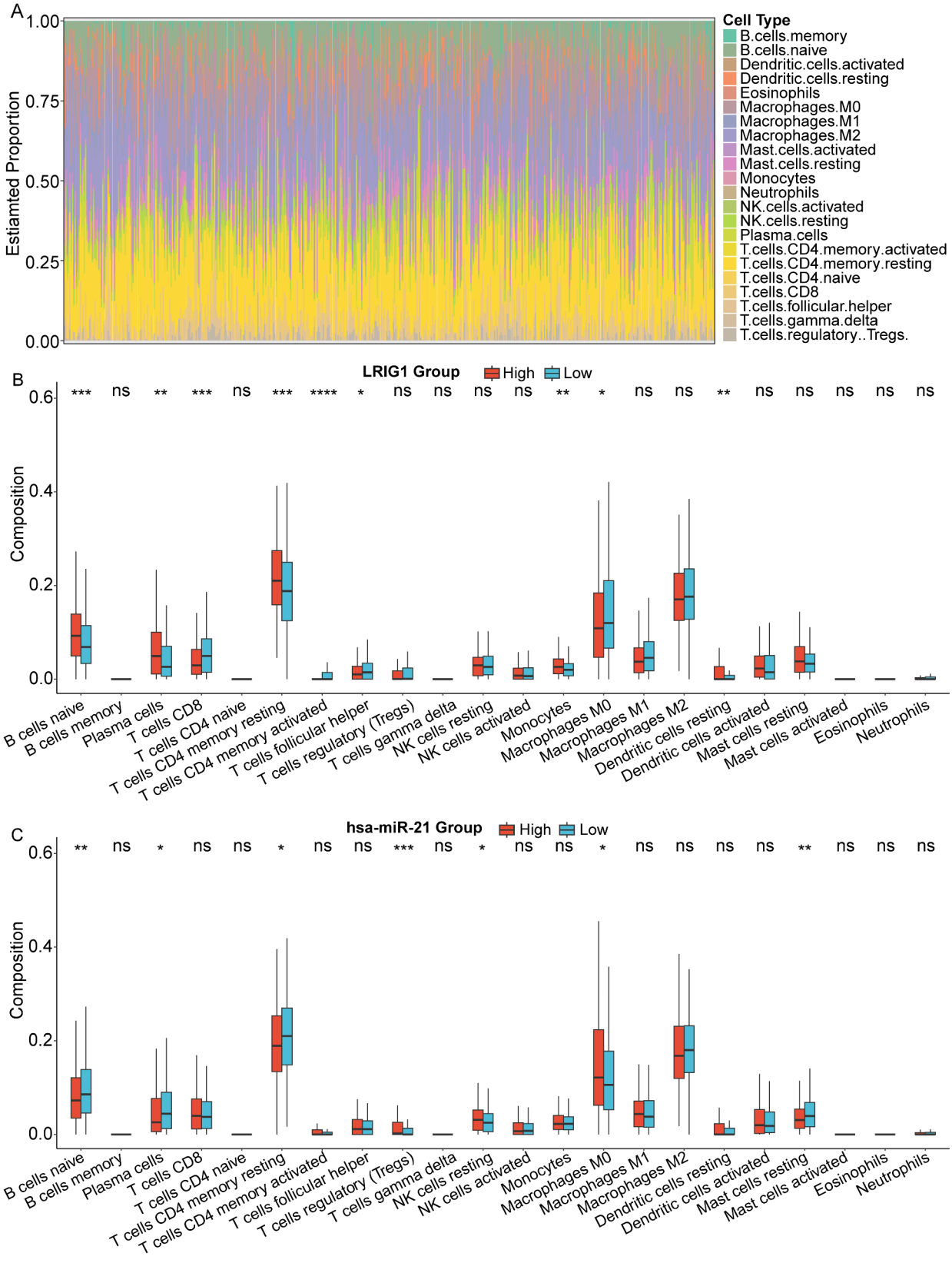

Fig. 5 The correlation of immune cell infiltration with hsa-miR-21 and *LRIG1*. **(A)** The relative content of 22 immune cell infiltration in LUAD samples. **(B)** The relative content of 22 immune cell infiltration in *LRIG1* high and *LRIG1* low expression groups. **(C)** The relative content of 22 immune cell infiltration in hsa-miR-21 high and low expression groups. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

48]. In LUAD, several specific miRNAs have been identified that either promote or suppress tumorigenesis. For instance, hsa-miR-33b has been shown to be downregulated in LUAD tissues and cell lines, and its restoration can inhibit cell proliferation, migration, and invasion, as well as epithelial-mesenchymal transition (EMT) in vitro [49]. Hsa-miR-294 is upregulated in tumor-initiating Sca-1(+) CD34(+) cells during LUAD development. It has been shown to bind to the 3' UTR of matrix metalloproteinase 3 (MMP3), downregulating its expression and thereby inhibiting the migration and invasion of cancer cells [50]. Additionally, hsa-miR-206 has been shown to inhibit cell viability and migration in LUAD by directly targeting the MET gene, further illustrating the diverse roles of miRNAs in regulating cancer-related pathways [51]. This evidence suggests that miRNAs function in LUAD through multiple mechanisms, including direct targeting of mRNAs involved in key pathways of tumor growth and metastasis. In this study, we first identified 122 DEmiRNAs between LUAD and para-cancerous samples. Among which, 72 miRNAs were targeting miRNAs of LRIG1. Of these, hsa-miR-21 was negatively correlated with *LRIG1* in LUAD. Hsa-miR-21 is known to play a crucial role in driving tumorigenesis by blocking negative regulators of the RAS/MEK/ERK pathway and inhibiting apoptosis in NSCLC [52]. The elevated expression of hsa-miR-21 was remarkably related to worse survival and recurrence of NSCLC patients [53]. The human primary squamous cell lung carcinoma patients with high hsa-miR-21 expression were related to short survival time [54], which was consistent with the results of this work that the LUAD patients with high hsa-miR-21 expression were correlated with inferior prognosis. Furthermore, the elevated expression of hsa-miR-21 was correlated with distal metastasis and advanced TNM stage of NSCLC [55]. Dong and colleagues have suggested that hsa-miR-21 expression was elevated in NSCLC patients with brain metastases, and the hsa-miR-21 inhibitors could decrease the proliferation of NSCLC cells in vitro [56]. These evidences suggested that miRNA-21 might be a diagnostic and prognostic marker for lung cancer. Wei et al. have indicated that the elevated levels of plasma hsa-miR-21 may correlate with the susceptibility of NSCLC to platinum drugs [57]. By suppressing hsa-miR-21, the resistance to multiple drugs demonstrated by lung cancer cells was abated, as the repression influenced the gene expression of multidrug resistance-associated genes and impeded the Akt signaling pathway [58]. Consequently, hsa-miR-21 can serve as an effective marker for anticipating the sensitivity of lung cancer to drug-based chemotherapy. In addition, increasing numbers of researches reported that hsa-miR-21 could regulate the target genes to involve in the prognosis of lung cancer. Collectively, targeting hsa-miR-21 in LUAD presents significant potential for enhancing diagnostic accuracy, improving therapeutic efficacy, overcoming drug resistance, and facilitating personalized treatment strategies. Continued investigation into the mechanisms of miR-21 and its interactions with various signaling pathways will be essential for translating these potential applications into clinical practice. Moreover, hsa-miR-21 was capable of suppressing the expression of *PTEN*, a tumor suppressor gene, and thereby stimulating the growth and invasion of NSCLC [55]. We found that hsa-miR-21 expression exhibited negative correlation with *LRIG1* expression in LUAD. LUAD patients showing elevated hsa-miR-21 levels alongside diminished *LRIG1* expression demonstrated a poorer prognosis. Conversely, patients displaying reduced hsa-miR-21 expression and elevated *LRIG1* expression exhibited a better prognosis. It has been demonstrated that high *LRIG1* expression was related to better prognosis of patients [26, 27]. However, to the best of our knowledge, there have been no reports of hsa-miR-21 regulation of *LRIG1* expression in lung cancer. Therefore, compared with the results of previous studies, this study is the first to report that hsa-miR-21 may regulate the progression of LUAD by regulating the expression of *LRIG1*.

In NSCLC, studies have shown that *LRIG1* expression is often decreased compared to non-malignant lung tissue [59], which is consistent with our findings. This reduction in *LRIG1* correlates with advanced disease stages and poorer patient outcomes. For instance, one study found that *LRIG1* protein expression was an independent prognostic factor in NSCLC, with higher levels associated with improved patient survival rates [26]. Furthermore, the expression of *LRIG1* mRNA was also linked to patient survival, reinforcing its role as a potential biomarker for prognosis in lung cancer [26]. Moreover, the expression of *LRIG1* has been linked to the clinical characteristics of lung cancer patients. Studies have shown that *LRIG1* expression correlates with tumor type, clinical stage, and lymph node metastasis, suggesting its potential utility as a biomarker for assessing disease progression and treatment response [60]. Thus, the ability of *LRIG1* to serve as a prognostic marker is particularly valuable in the context of personalized medicine, where understanding the molecular underpinnings of a patient's cancer may guide treatment decisions. EGFR signaling activity was linked to the advancement of renal cell carcinoma malignancy [61]. In NSCLC, the mutation of EGFR was the second frequently oncogenic driver event [62]. In NSCLC cells harboring mutant EGFR, the *LRIG1* could inhibit the proliferative, migratory and invasive ability of cancer cells [59]. *LRIG1* is a negative regulator of EGFR signaling and has been shown to modulate this pathway in LUAD [16]. *LRIG1* functions by promoting the degradation of EGFR, thereby reducing

its availability on the cell surface and limiting its signaling capacity [16, 17]. This regulatory mechanism is crucial, as excessive EGFR signaling can lead to increased tumor aggressiveness and resistance to therapies targeting the EGFR pathway [63–65]. Studies have indicated that high levels of *LRIG1* expression are associated with better clinical outcomes in LUAD [26], suggesting that *LRIG1* may serve as a tumor suppressor by inhibiting the oncogenic effects of EGFR signaling. It has been reported that hsa-miR-21 overexpression is associated with acquired resistance of EGFR-TKI in NSCLC [65]. We discovered that the metabolic related pathway, EGFR tyrosine kinase inhibitor resistance was significantly activated in $LRIG1^{high}$ group, nevertheless this signaling pathway was not enriched in hsa-miR-21^{high} group. Collectively, it appears that hsa-miR-21 was not involved in the negative regulation of EGFR by *LRIG1* in LUAD.

Furthermore, the relative content of naive B cells, plasma cells and resting CD4 + T cells were significantly increased in *LRIG1* high expression group and hsa-miR-21 low expression group. The relative content of Tregs and Macrophages M0 were remarkably decreased in *LRIG1* high expression group and hsa-miR-21 low expression group. B cells respond to infected cells or tumor cells by differentiating into memory B cells or plasma cells. Plasma cells can secrete immunoglobulins (also known as antibodies) which bind to and neutralize target antigens [66]. In regards to the enhancement of antitumor immunity, various T cell types, contribute to T cell-mediated immune responses in the tumor microenvironment (TME) [67]. The infiltration level of Treg cells in LUAD is relatively high, exhibiting complex regulatory effects [68]. Treg cells suppress the activity of other immune cells through the secretion of inhibitory cytokines, such as IL-10 and TGF-β, thereby potentially contributing to an immunosuppressive role within the TME [68]. Hao et al. have indicated that the proportion of naive B cells decreases in LUAD, suggesting a potential relationship between naive B cells and the immunosuppressive state of the TME [69]. Additionally, there is a significant increase in plasma cells in LUAD, which exhibit high differentiation and enhanced antibody-producing capabilities. Notably, plasma cells coexist with Tregs and exhausted T cells (Tex), while showing a negative correlation with cytotoxic T cells (CTLs) [69]. This observation implies that plasma cells may influence the TME of LUAD through their interactions with these immune cell populations. Macrophages are a diverse group of phagocytic cells that possess complex phenotypic and functional properties within the TME. They possess the ability to eliminate malignant cells via phagocytosis or by producing soluble factors that induce apoptosis in tumor cells [70]. In LUAD, the infiltration of M2 macrophages within the tumor parenchyma is correlated with a poor prognosis, whereas a high infiltration of M1 macrophages is linked to a better prognosis [71, 72]. Accordingly, the expression patterns of *LRIG1* and hsa-miR-21 in LUAD may influence the immune landscape and treatment responses of tumors by modulating the infiltration of immune cells within the TME. Future research should further investigate their specific mechanisms in LUAD and evaluate their potential as targets for immunotherapy.

This study is the first to report the regulation of LRIG1 by hsa-miR-21 in LUAD, and providing insights into its impact on prognosis and immune cell infiltration. However, it is important to acknowledge certain limitations of this study that should be considered. The primary limitation of this study is the sample size. In comparison to TCGA-LUAD, the sample size of GSE29248 is relatively small, which limits the granularity of our validation analysis and may introduce variability in the results. Additionally, further in vivo experiments and clinical studies are necessary to confirm whether hsa-miR-21 influences the occurrence, progression, and prognosis of LUAD through the regulation of *LRIG1*. Lastly, while this study established a correlation between hsa-miR-21 and *LRIG1*, as well as with certain immune cells, it did not comprehensively elucidate their complex interactions within the broader immune microenvironment. Future research should aim to further investigate the role of hsa-miR-21 and *LRIG1* in mediating the interactions between immune cells and tumor cells.

Conclusions

We have firstly reported that hsa-miR-21 is responsible for regulating the *LRIG1* expression, thereby contributing to the progression of LUAD. Hsa-miR-21 was highly expressed in LUAD while *LRIG1* was lowly expressed in LUAD cells. Meanwhile, high hsa-miR-21 expression indicates worse prognosis of LUAD patients. Moreover, Hsa-miR-21 and *LRIG1* expression were correlated with infiltration of immune cell infiltration in LUAD. These findings provide more information for researches to understand the regulatory mechanism of *LRIG1* by miRNAs in the onset of LUAD. Moreover, targeting the hsa-miR-21/*LRIG1* axis in LUAD therapy holds promise as a novel therapeutic strategy. By inhibiting hsa-miR-21, it may be possible to restore the expression and function of *LRIG1*, thereby promoting tumor suppression. This approach could lead to improved therapeutic outcomes for LUAD patients, particularly those with high hsa-miR-21 expression. However, further research is needed to explore the efficacy and safety of such targeted therapies in clinical settings.

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Conceptualization: Li Liu, Xinhua Liu; Methodology: Li Liu, Chengpeng Gao, Meijuan Liu; Software: Xinhua Liu, Meijuan Liu, Leqiang Wang; Validation: Li Liu, Chengpeng Gao, Mengmeng Peng; Formal analysis: Xinhua Liu, Meijuan Liu, Leqiang Wang; Investigation: Li Liu, Chengpeng Gao, Mengmeng Peng; Resources: Li Liu, Meijuan Liu, Leqiang Wang; Data Curation: Xinhua Liu, Chengpeng Gao, Mengmeng Peng; Writing - Original Draft: Li Liu, Xinhua Liu, Meijuan Liu; Writing - Review & Editing: Chengpeng Gao, Mengmeng Peng, Leqiang Wang; Visualization: Xinhua Liu, Meijuan Liu; All authors read and approved the final manuscript.

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Data availability
Publicly available datasets were analyzed in this study. The data can be found in the following databases: The Cancer Genome Atlas (TCGA, https://tcga-data.nci.nih.gov/tcga/) and the Gene Expression Omnibus (GEO, ID: GSE29248).

Declarations

Ethics approval and consent to participate
This manuscript is not a clinical trial, hence the ethics approval and consent to participation are not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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