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A prognostic model based on ferroptosis-related long non-coding RNA signatures and immunotherapy responses for non-small cell lung cancer

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Abstract. – OBJECTIVE: Non-small cell lung cancer (NSCLC) ranks high in the incidence of malignant tumors, with limited treatment options and poor prognosis. Ferroptosis is a newly discovered cell death mechanism based on iron and reactive oxygen species (ROS). The role of ferroptosis-related long non-coding RNAs (lncRNAs) and associated prognostic mechanisms in NSCLC require investigation.

MATERIALS AND METHODS: We constructed a prognostic multi-lncRNA signature based on ferroptosis-related differentially expressed lncRNAs in NSCLC. The levels of ferroptosis-related lncRNA in normal lung cells and lung adenocarcinoma cells were verified by RT-PCR.

RESULTS: We identified eight differentially expressed lncRNAs associated with NSCLC prognosis. The expression of *AC125807.2*, *AL365181.3*, *AL606489.1*, *LINC02320*, and *AC099850.3* was upregulated, while *SALRNA1*, *AC026355.1*, and *AP002360.1* were downregulated in NSCLC cell lines. Kaplan-Meier analysis showed that a high-risk patient group was associated with poor NSCLC prognosis. A risk assessment model based on ferroptosis-related lncRNAs was superior to NSCLC prognosis based on traditional clinicopathological features. Gene Set Enrichment Analysis (GSEA) identified immune- and tumor-related pathways in low-risk group patients. In addition, The Cancer Genome Atlas (TCGA) showed that T cell function during APC co-inhibition, APC co-stimulation, chemokine receptor (CCR), MHC class I, parainflammation, T cell co-inhibition, and check-point expression differed significantly between low- and high-risk groups. M6A-related mRNA comparisons between these groups also revealed significant differences in *ZC3H13*, *RBM15*, and *METTL3* expression.

CONCLUSIONS: Our new model of lncRNA-associated ferroptosis effectively predicted NSCLC prognoses.

Key Words:
Prognostic model, Ferroptosis-related, LncRNA, NSCLC.

Introduction

Lung cancer is a malignant tumor associated with one of the highest rates of incidence and a huge socioeconomic burden[1]. Non-small cell lung cancer (NSCLC) accounts for more than 85% of all cases[2] and is the most prevalent primary lung cancer subtype, with lung adenocarcinoma (LUAD) and squamous cell carcinoma (LUSC) being the predominant histological types[3,4]. Despite targeted therapy and immunotherapy breakthroughs, the lack of an early detection system for NSCLC reduces the 5-year survival rate to less than 30%. The risk of NSCLC recurrence and metastasis remains high even after treatment[5,6]. Effective clinical management strategies for NSCLC are limited by suboptimal preclinical models or lack of accurate biomarkers of early diagnosis. Thus, a comprehensive analysis of molecular mechanisms underlying NSCLC pathogenesis could

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facilitate the discovery of new treatment and evaluation strategies.

Ferroptosis is a newly discovered iron-dependent cell death mechanism, and is distinct from apoptosis, pyroptosis, autophagy, and necrosis. It is mediated by iron accumulation and lipid peroxidation, and is characterized by unique morphological, genetic, and biochemical traits[7]. Emerging evidence suggests that ferroptosis is closely related to the development of several human diseases, notably cancer[8,9]. Dysregulated iron metabolism is a risk factor for cancer and also promotes tumor growth. When compared with normal cells, cancer cells are over-reliant on iron for proliferation[10]. Indeed, activated ferroptosis may override the resistance to current chemotherapeutic agents, opening a new therapeutic frontier for cancer therapy. Some reports[11-13] have suggested that during NSCLC, ferroptosis is inhibited via multiple mechanisms, e.g., high P53RRA expression promotes ferroptosis in lung cancer cells[11], whereas CDGSH iron-sulfur domain 1 (CISD1)[12] and TP53[13] negatively regulate ferroptosis. However, whether ferroptosis-related genes (FRGs) are associated with NSCLC prognosis is unclear.

Long non-coding RNAs (lncRNAs) are RNAs that contain more than 200 bases. Although they do not code for any proteins, they are involved in tumorigenesis, progression, and metastasis by regulating gene expression at chromatin, transcriptional, and post-transcriptional levels, and are considered targets for cancer gene therapy[14,15]. In addition, some lncRNAs inhibit ferroptosis by acting as competing endogenous RNAs to inhibit oxidation in some cancers including lung cancer[16,17]. In recent years, in the literature were constructed cancer prognostic models by investigating the association between lncRNA and ferroptosis to facilitate patient prognoses. However, lncRNAs associations with ferroptosis during NSCLC and disease prognosis require systematic investigation.

In this study, we investigated FRG expression and ferroptosis-related lncRNAs during NSCLC based on The Cancer Genome Atlas (TCGA) database, and also investigated relationships between lncRNAs and NSCLC prognosis. Enriched functions and pathways between high- and low-risk subgroups were explored using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. We also developed a nomogram to assess disease prognosis based on risk scores (RS's) and clinical characteristics. Finally, we analyzed the role of ferroptosis-related mRNA, n6-methyladenosine (m6A), and immune responses for NSCLC prognosis.

Materials and Methods

Cell Lines and Cell Culture

A549, NCI-H1299, NCI-H1975, and BEAS-2B were obtained from American Type Culture Collection. Cells were cultured in DMEM (Gibco, Rockville, MD, USA). Supplemented with 5% fetal bovine serum (Gibco, Rockville, MD, USA) in a humidified incubator with 5% CO_2 at 37°C.

Data Collection

RNA-sequence data of 1,027 patients (110 normal and 1,128 tumor patients) were extracted from the TCGA-NSCLC database. Corresponding FRGs were downloaded from FerrDb[18], a comprehensive up-to-date database that provides a web-based link for ferroptosis markers, their regulatory molecules, and associated diseases. Overall, we identified 382 FRGs (driver: 150; marker: 123; and suppressor genes: 109). Relationships between lncRNAs, FRGs, and NSCLC were assessed using Pearson correlation. Correlations were considered significant if the correlation coefficient, |R2| > at $p < 0.001$ was 0.3. The clinicopathological data obtained from patients with NSCLC included, gender, age, tumor-node-metastasis (TNM) stage, survival status, and survival time. Significant differential lncRNA expression associated with ferroptosis was set as a false discovery rate (FDR) < 0.05 and $|\log_2 FC| \geq 1$.

We first explored the function of upregulated and downregulated ferroptosis-related differentially expressed genes (DEGs) and used GO annotations to evaluate biological pathways associated with DEGs. Also, using KEGG data, the R software *ggplot2* package was utilized to evaluate biological processes (BP) regulated by differentially expressed iron disease-related lncRNAs, molecular functions (MF) and cellular components (CC).

Prognostic lncRNA Features Associated with Ferroptosis

We used Lasso–penalized Cox regression and univariate Cox regression analysis to construct a ferroptosis-related lncRNA prognostic model, stratified using risk scores (RS) (coefficient

lncRNA1 × expressed lncRNA1) + (coefficient lncRNA2 × expressed lncRNA2) +···+ (coefficient lncRNA × expressed lncRNAn), while assessing RS associated with each patient diagnosed with NSCLC. RNAs were divided into low-risk (< median) and high-risk (≥ median) groups based on median scores.

Prediction Nomogram

We performed Gene Set Enrichment Analysis (GSEA) to define lncRNA signatures in KEGG and then searched the TCGA-NSCLC database. $p < 0.05$ and FDR q < 0.25 values were considered statistically significant. In turn, a nomogram integrating prognostic features was constructed to predict 1-, 3-, and 5-year overall survival (OS) rates of patients with NSCLC.

Quantitative RT-PCR Analysis

A549, NCI-H1299, NCI-H1975, and BEAS-2B cells were seeded into 6-well plates at a density of 6×10^5 cells/well. Total RNAs were extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA). The gDNA were removed using the TurboDNase kit. cDNA synthesis using PrimeScript RT reagents kit (TaKaRa, Shiga, Japan). Real-time PCR was performed using the SYBR Premix Ex Taq II reagents (TaKaRa, Shiga, Japan) according to the manufacturer's instructions. Genes expression level were normalized to GAPDH. Fold change of gene expression was expressed as $2^{-\Delta\Delta Ct}$. The primers used in this study were shown in Table I.

Table I. Primers used for RT-PCR.

Genes	Sequence (5′ to 3′)
SALRNA1-81F	TGCCATCTCACCCTCATACAT
SALRNA1-81R	GATAGTGGTGGGGACTGGTAG
AL365181.3-236F	CAAGGACGAGCGAAGTAGGAC
AL365181.3-236R	CCCCATTCTCCCTTCATCCAC
AC125807.2-190F	CAACCATAGCAGGTGGCAGA
AC125807.2-190R	AAGACGGGATGAGAACCACG
AL606489.1-90F	TTCGGACCGTTTCCTGAACT
AL606489.1-90R	TTTGAAGGCAGGAGTGGTCT
LINC02320-83F	AAGGTGTTGAAGCATAGACGC
LINC02320-83R	AAGAGAGCCCACGATGTTCC
AP002360.1-173F	CTTTGTTCCTGAAGTGCCGT
AP002360.1-173R	AACTCCGTAACCCGCAAATC
ENST-3418.1F	TTTGAGGTCTTTCCCGATGG
ENST-3418.1R	ACTGTCTTGTAGTTCCCACAGAT
AC099850.3F	AGGCTGGAGTGGCAGTGTT
AC099850.3R	CCTGGGAAACATAGCGAGACC
GAPDH-F	TACAGCAACAGGGTGGTGGAC
GAPDH-R	TGGGATAGGGCCTCTCTTGCT

Immunoassays and Gene Expression

Several immune cell databases such as TIMER, CIBERSORT, QUANTISEQ, MCPCOUNTER, XCELL, EPIC, and single-sample GSEA (ssGSEA) were compared based on ferroptosis-related lncRNA signatures to assess CCs or cell immune responses between high- and low-risk patient groups. Differences in immune responses were identified using heatmaps using different algorithms. In addition, ssGSEA was used to quantify tumor-infiltrating immune cell subsets between groups to assess immune functions. Multiple immune checkpoints were identified based on literature searches.

Statistical Analysis

Data analysis was performed using the R package (TaKaRa, Shiga, Japan), version 4.0.2. Normally and non-normally distributed variables were analyzed using unpaired Student's *t*-test and Wilcoxon test, respectively. Based on FDR, differentially expressed lncRNAs were identified using the Benjamini-Hochberg method. We compared ssGSEA-normalized NSCLC-DEGs to the genome using "Gene Set Variation Analysis" in the R package. The sensitivity and specificity of NSCLC prognostic features compared with other clinicopathological features were assessed using the receiver operator characteristic curve (ROC) method. Logistical regression analyses and heatmaps were used to evaluate relationships between ferroptosis-related lncRNAs and clinicopathological manifestations. Using ferroptosis-related lncRNA signatures, Kaplan-Meier survival analyses were used to evaluate prognoses of patients with NSCLC. $p < 0.05$ was considered statistically significant.

Results

Enrichment Analysis of FRGs

We identified 87 DEGs associated with ferroptosis (30 downregulated and 57 upregulated genes). BPs were involved in response to oxidative stress, metal ions, cofactor metabolic processes, and reactive oxygen species (ROS) metabolism. MFs mainly regulated apical components of the cell, basolateral plasma membrane, NADPH oxidase complexes, and apical plasma membranes.

CCs were involved in upregulated coenzyme binding, dioxygenase activity, antioxidant activity, oxidoreductase activity, heme-binding, incorporation of molecular oxygen, iron-ion binding, and oxidoreductase activity involving NAD(P)H (Figure 1A). KEGG analyses showed that overexpressed genes mainly involved microRNAs in cancer, lipids and atherosclerosis, ferroptosis, arachidonic acid metabolism, central carbon metabolism in cancer, HIF-1 signaling, glutathione metabolism, and NOD-like receptor signaling (Figure 1B).

Ferroptosis-Based lncRNA Prognostic Features

We identified 765 lncRNAs associated with ferroptosis. Using the Cox function in the R-survival package to perform lncRNA univariate regression analyses, 18 lncRNAs were significantly associated with ferroptosis (Figure 2) and were subsequently included in multivariate analysis. The results of multivariate analysis showed that eight ferroptosis-related lncRNAs (*SALRNA1, AL365181.3, AC026355.1, AC125807.2, AL606489.1, LINC02320,* and *AP002360.1, AC099850.3*) were independent prognostic factors for NSCLC. We also constructed a prognostic RS for lncRNAs (RS = *SALRNA1* × -0.718 + *AL365181.3* × 0.016 + *AC026355.1* × -0.068 + *AC125807.2* × 0.127 + *AL606489.1* × 0.178 + *LINC02320* × 0.205 + *AP002360.1* × -0.043 + *AC099850.3* × 0.024).

Survival Analysis Prediction and Multivariate Validation

We first evaluated the expression of these lncRNAs in NSCLC cell lines (A549, NCI-H1299, and NCI-H1975) and normal lung cell line (BEAS-2B). Consistent with the above results, the expression of *AC125807.2, AL365181.3, AL606489.1, LINC02320*, and *AC099850.3* were upregulated and *SALRNA1, AC026355.1*, and *AP002360.1* were downregulated in NSCLC cell lines (Figure 3). Using the median RS (0.997) as the cut-off, the RS were divided into high- and low-risk groups. Patients were ranked from low to high RS. Kaplan-Meier analysis showed that the expression of high-risk lncRNAs was associated with poorer survival ($p < 0.001$, Figure 4A). Univariate and multivariate (Figure 4B) Cox analyses showed that the RS [hazard ratio (HR): 1.367, 95% confidence interval (CI): 1.281-1.459] and tumor stage (HR: 1.489, 95% CI: 1.334-1.662) were both independent prognostic factors for OS in patients with NSCLC. The AUC of the RS was

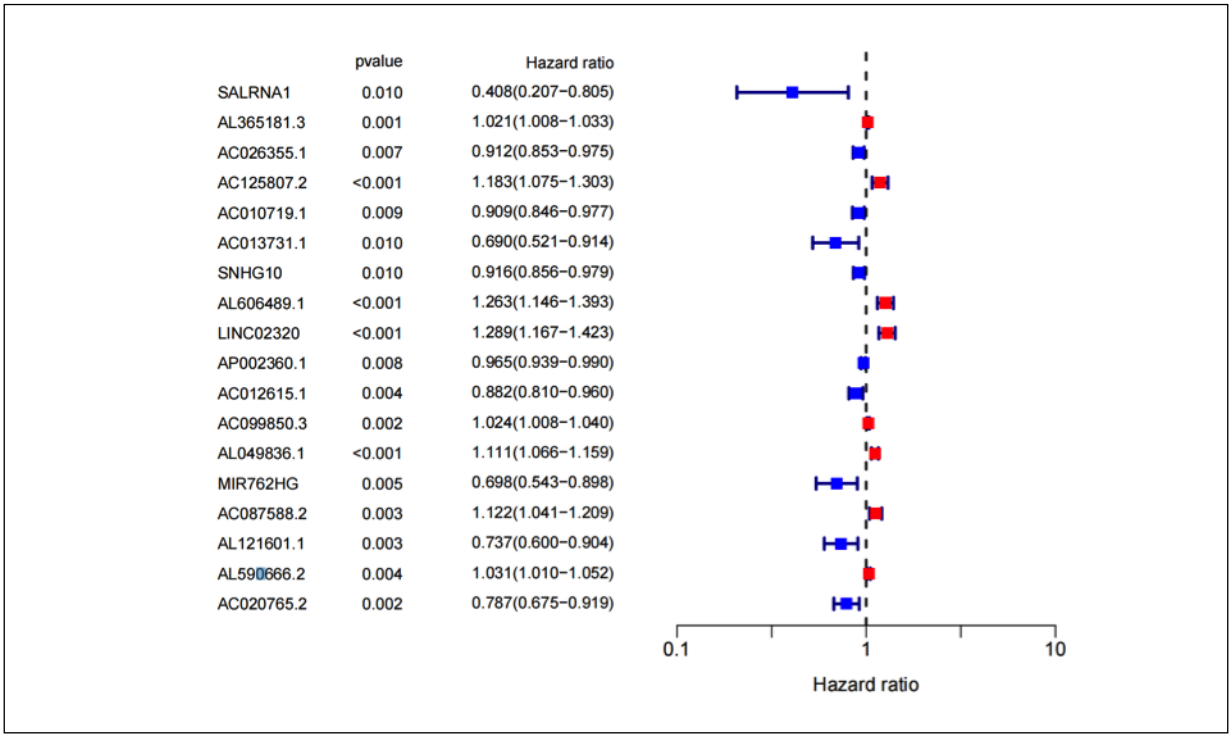

Figure 2. Univariate regression analysis results of 765 lncRNAs associated with non-small cell lung cancer.

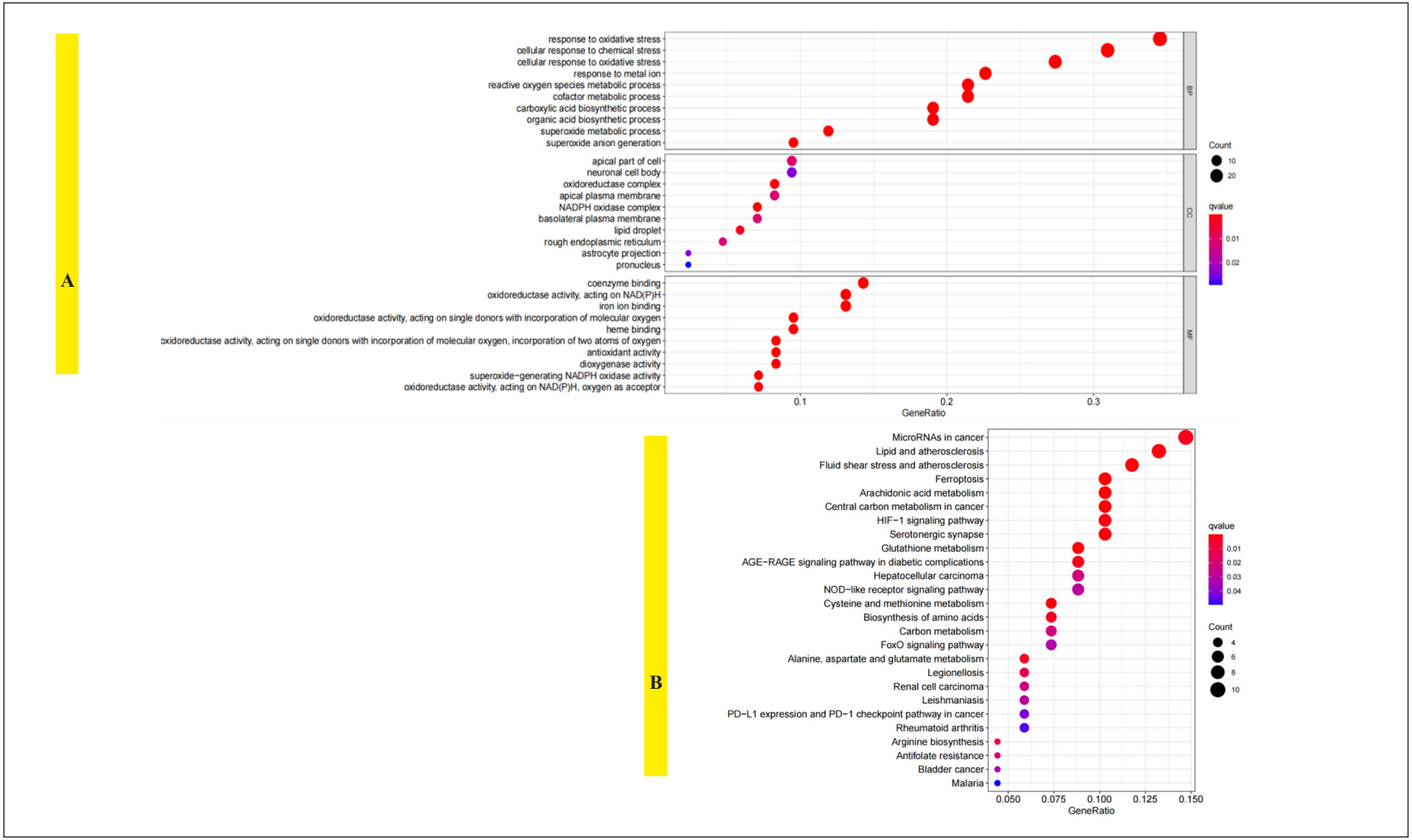

Figure 1. GO and KEGG analyses for ferroptosis-related differentially expressed genes. **A**, GO and (**B**) KEGG.

0.652 and was superior to traditional clinicopathological features used to predict NSCLC prognosis (Figure 4C). Using patients' risk-survival status map, we observed that the patients' RS were inversely related to the survival rate of patients with NSCLC. Heatmap analysis showed that most of the novel lncRNAs were positively associated with risk in our model; however, this requires further studies (Figure 4D). The AUC predicted RS values for 1-, 3-, and 5-year survival were 0.652, 0.653, and 0.670, respectively (Figure 4E).

Cytoscape was used to map the regulatory network between lncRNAs and mRNAs (Figure 5A). We also analyzed a heatmap showing the relationship between ferroptosis-related prognostic features of lncRNAs and clinicopathological manifestations (Figure 5B). The nomogram (Figure 6), combining clinicopathological and prognostic features of novel ferroptosis-related lncRNAs, was stable and accurate, with potential application for the clinical management of patients with NSCLC.

GSEA

GSEA was used to analyze pathways significantly enriched in 985 patients identified in TCGA including high- (492 patients) and low-risk groups (493 patients). We observed that the high RS associated with iron metabolism-related signaling pathways were strongly affected by immune-mediated and tumor-related pathways, such as small cell lung cancer, chemokine signaling, leukocyte transendothelial migration, NOD-like receptor signaling, prostate cancer, TOLL-like receptor signaling, T cell receptor signaling, ECM receptor interactions, T/B cell receptor signaling, FCγR-mediated phagocytosis, chronic myeloid leukemia, renal cell carcinoma, melanoma, and JAK-STAT signaling. Low RS for iron metabolism-related signals were strongly affected by oxidative phosphorylation pathways and fatty acid metabolism, such as oxidative phosphorylation, fatty acid metabolism, arachidonic acid metabolism, butyric acid metabolism, primary bile acid biosynthesis, and α-linolenic acid metabolism. Thus, our combined data reflect the expression profiles and biological properties of NSCLC.

Immunity and m6A-Related Gene Differences

An immune response heatmap based on TIMER, CIBERSORT, CIBERSORT-ABS, QUANTISEQ, MCPCOUNTER, XCELL, and EPIC

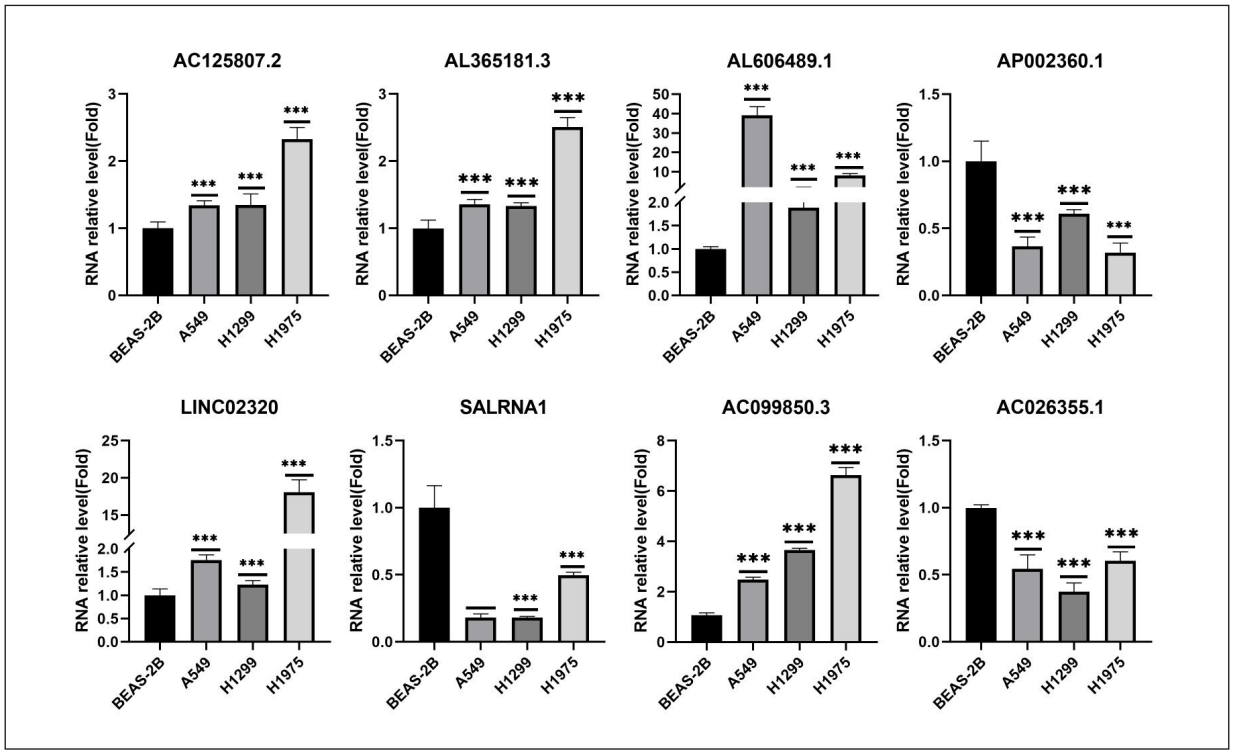

Figure 3. The expression levels of lncRNAs levels in normal lung cells (BEAS-2B) and NSCLC cells (A549, NCI-H1299, and NCI-H1975) were quantified by qRT-PCR. ***$p < 0.05$.

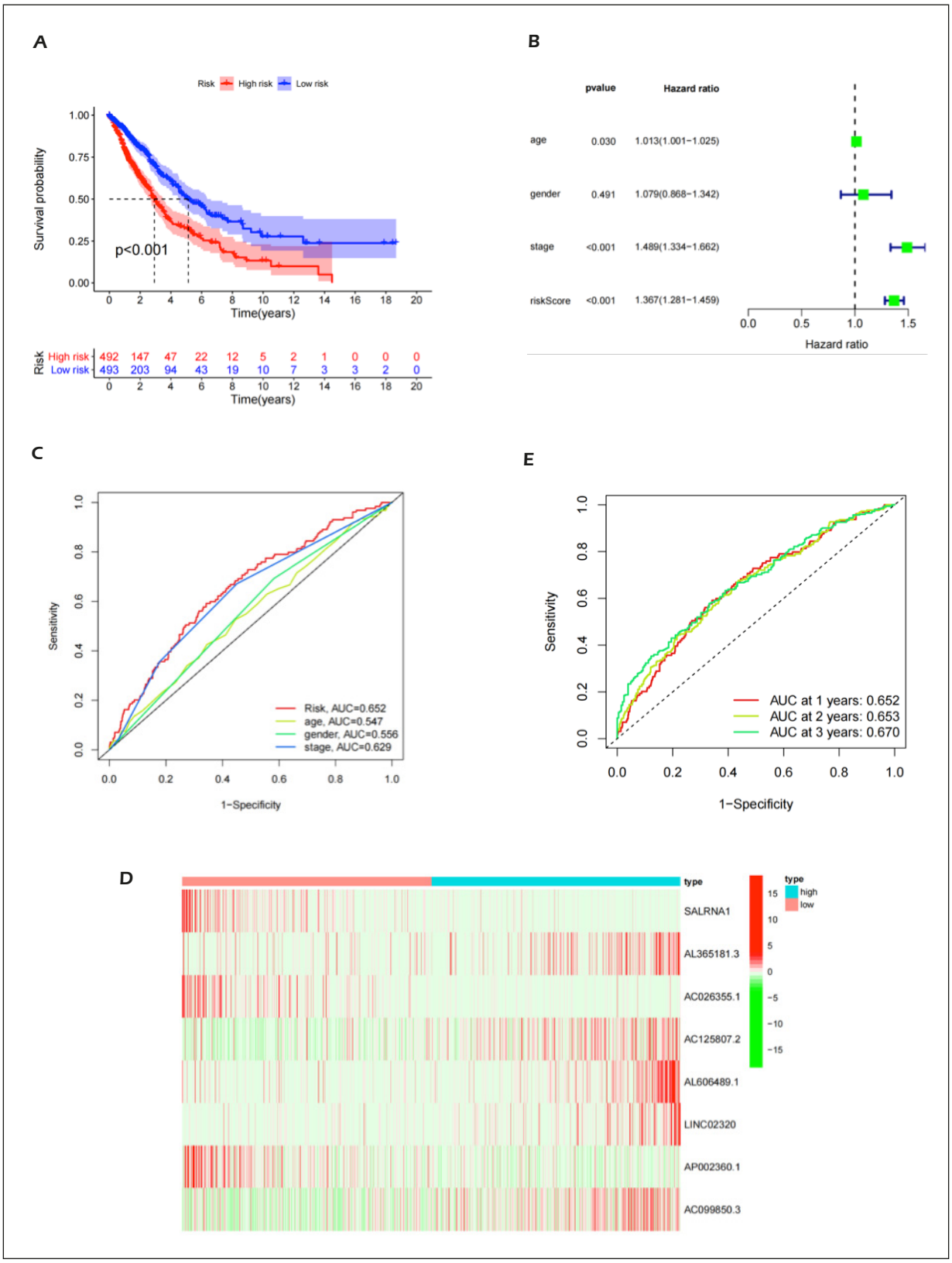

Figure 4. Ferroptosis-related lncRNAs signature based on TCGA. **A**, Kaplan-Meier curves result. **B**, Univariate and multivariate COX analysis for the expression of ferroptosis-related lncRNAs (univariate and multivariate). **C**, The AUC values of the risk factors. **D**, Risk survival status plot. **E**, The AUC of the for the prediction of 1, 3, 5-year survival rate of HNSCC.

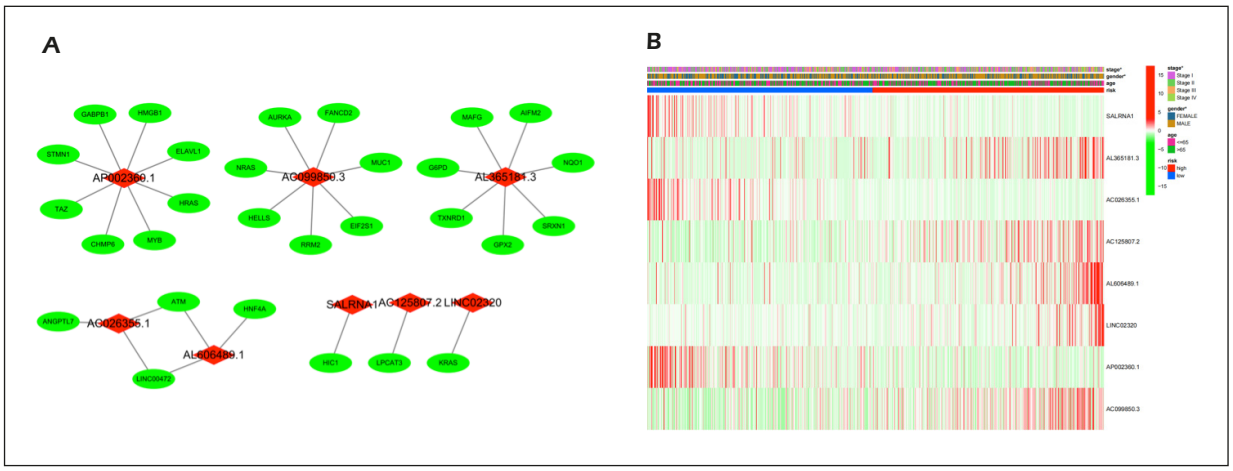

Figure 5. **A**, The relationship between the novel lncRNA and mRNA expression. **B**, Heatmap for ferroptosis-related lncRNAs prognostic signature and clinicopathological manifestations.

databases is shown (Figure 7). Correlation analysis between immune cell subpopulations and related functions based on ssGSEA TCGA-NSCLC data showed that APC co-suppression, APC co-stimulation, chemokine receptor (CCR), MHC class I, parainflammation, T cell co-inhibition, and checkpoints differed significantly between low- and high-risk groups (Figure 8A). As immune checkpoints play a key role in immunotherapy, and tumor immunotherapy is frequently used in clinical practice, we investigated the differences in immune checkpoints between groups. We found significant differences in CD70, TNFRSF8, CD276, CD274, PDCD1, VTCN1, ICOSLG, PDCD1LG2, and TNFRSF9 expression between patient groups (Figure 8B). A comparison of m6A-related mRNAs between high- and low-risk groups revealed significant differences in ZC3H13, RBM15, and METTL3 expression.

Discussion

Cell death is an important component of mammalian development and homeostasis and is tightly coupled to physiological and pathological status[19]. Ferroptosis is an iron-catalyzed form of regulated cell death, characterized biochemically by iron accumulation and lipid peroxidation[20]. Recent studies[21] reported that ferroptosis plays a key role in cancer development and treatment. It induces the death of malignant cells

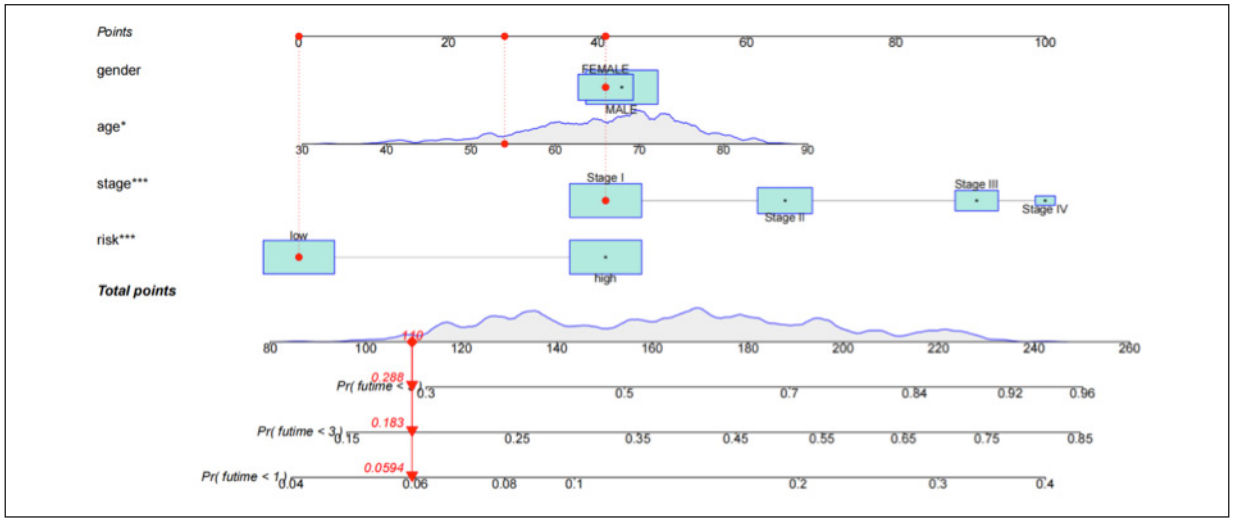

Figure 6. A nomogram for both clinic-pathological factors and prognostic ferroptosis-related lncRNAs

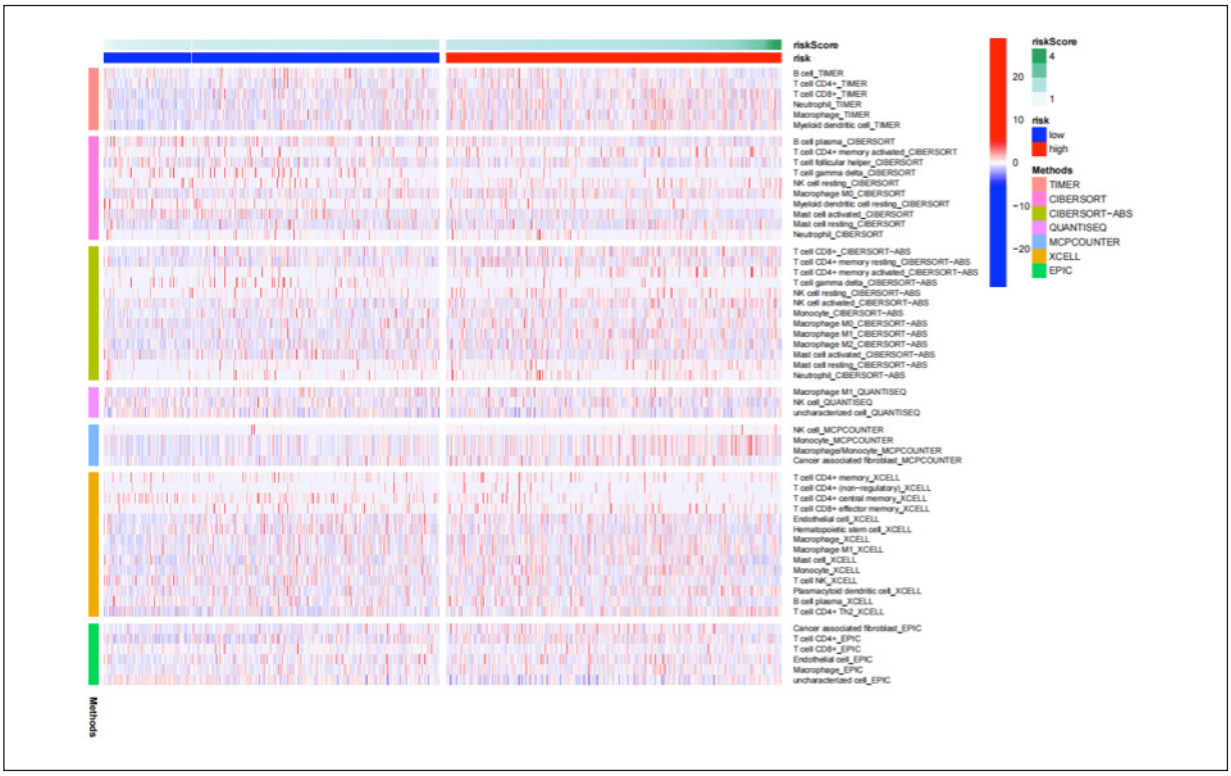

Figure 7. Heatmap for immune responses based on TIMER, CIBERSORT, CIBERSORT−ABS, QUANTISEQ, MCPCOUNTER, XCELL, EPIC algorithms among high and low risk group.

and inhibits tumor progression in several cancers, including NSCLC[22], pancreatic[23], breast[24] and hepatocellular cancers[25], and thus represents a new cancer treatment strategy. Additional studies highlight the critical role of lncRNAs in ferroptosis regulation[26]. However, the roles and specific signaling pathways of FRG-lncRNAs in NSCLC prognosis and immune responses remain unclear. Therefore, we identified potential biomarkers and possible therapeutic targets in the ferroptosis signaling pathway.

We found that 87 FRGs and 765 FRG-lncRNAs were differentially expressed between NSCLC and normal patients. A prognostic model was established based on 765 lncRNAs, and its efficacy was compared to a clinically characterized prognostic model. We also assessed the relationship between RS and NSCLC clinical characteristics. To this end, a nomogram was constructed to improve clinical decision-making and guide the development of treatment strategies.

We also inv estigated the correlations between immune cells, immune functions, immunosuppressant checkpoints, and RS to evaluate the potential role of FRG-lncRNAs in NSCLC immune response. Our findings suggest that FRG-lncRNAs play a potentially important role in NSCLC.

We also established an NSCLC prediction model based on eight FRG-lncRNAs: *SALRNA1, AL365181.3, AC026355.1, AC125807.2, AL606489.1, LINC02320, AP002360.1,* and *AC099850.3*. Based on ROC curve analysis, lncRNA signatures were moderately predictive of OS. Abdelmohsen et al[27] reported that the abundance of senescence-associated lncRNA (SAL-RNA) in senescent cells was low. The *SAL-RNA1* (XLOC_023166) delayed senescence, reduced the level of *SAL-RNA1* and increased the frequency of senescence traits, including amplifying cell morphology and increasing positive β-galactosidase activity and P53 levels. Yuan et al[28] demonstrated that *SAL-RNA1*-mediated-*SIRT1* signaling reduced cigarette smoke-induced senescence in AECII. We observed that *SAL-RNA1* acted as a protective factor in NSCLC, which may be related to its anti-aging mechanisms. However, the biological function of *SAL-RNA1* in ferroptosis and lung cancer remains unclear.

Similarly, the biological functions of *AL365181.3, LINC02320,* and *AP002360.1* in NSCLC also remain unclear. However, our study re-

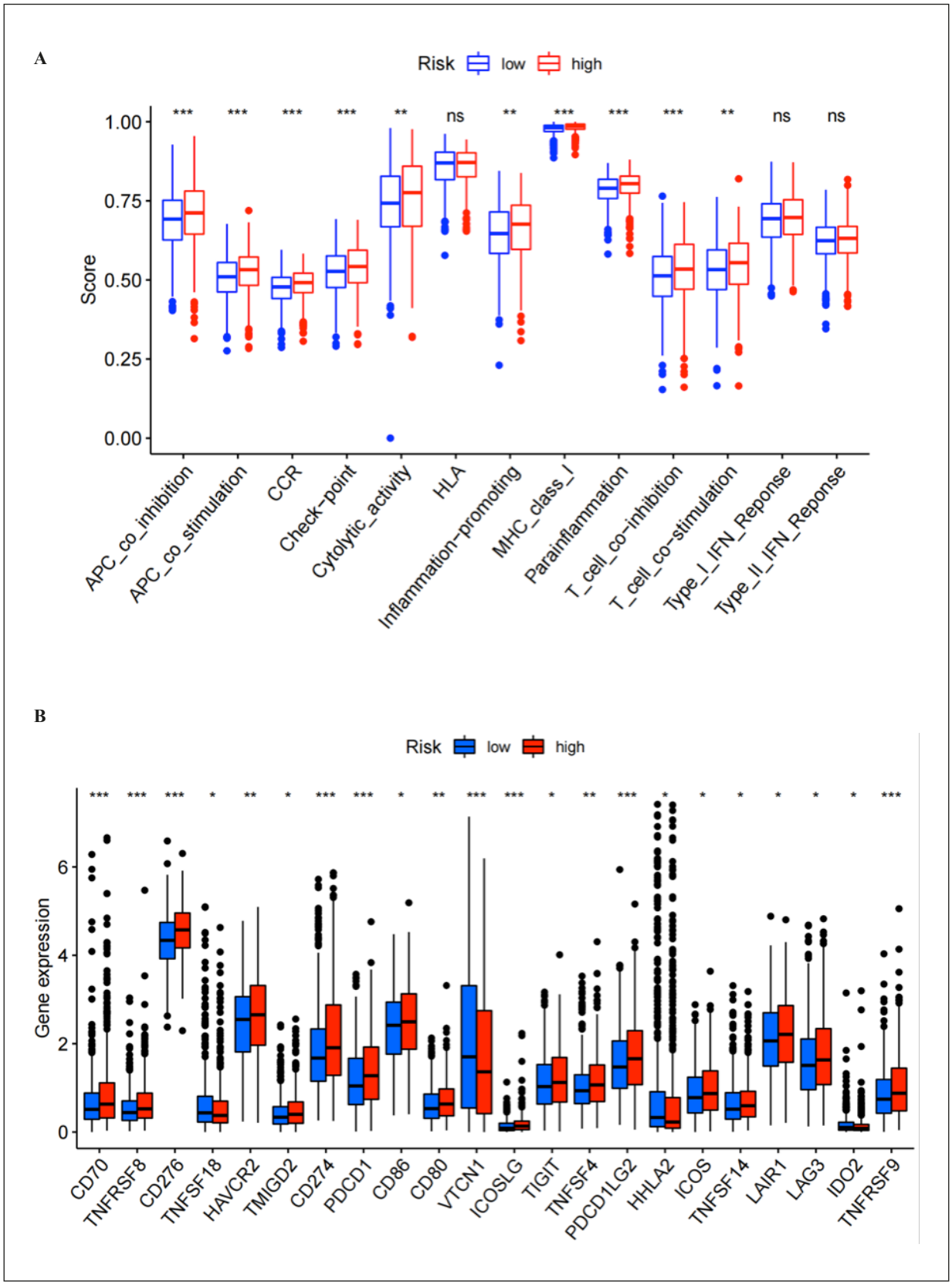

Figure 8. **A**, ssGSEA for the association between immune cell subpopulations and related functions (**B**). Expression of immune checkpoints among high and low NSCLCrisk groups. NSCLC risk group.

vealed the correlations between these genes and NSCLC prognosis. Wu et al[29] constructed a lncRNA-related prognostic model of LUAD and reported that *AL365181.2* was a risk factor for NSCLC, consistent with our findings. Equally, several studies reported that *AC026355.1* was a protective factor in LUAD[30,31] and played a key immunoregulatory role in the disease[32], consistent with our findings. Hou et al[33,34] confirmed that *AC125807.2* was an independent risk factor for LUAD and a potential prognostic marker for the disease, but its specific mechanisms are unclear and warrant further study. Multiple prognostic models of LUAD identified *AL606489.1* as a prognostic risk factor, consistent with our data[35-37]. Wu et al[38] showed that *AC099850.3* promoted liver cell migration and proliferation *in vitro*, while *AC099850.3* promoted the expression of cell cycle molecules, BUB1, CDK1, PLK1, and TTK[38]. BUB1 induces hepatocellular carcinoma (HCC) by activating mTORC1-related proteins[39]. PLK1 and CDK1 are important players in the abnormal cell cycle of HCC cells[40]. TTK activates AKT signaling and promotes HCC cell progression[41]. Western blotting analysis revealed CD155 and PD-L1 expression in the interference group. Also, CD155 and PD-L1 deletion in host and tumor cells led to a greater inhibition of tumor growth and metastasis[42,43]. These results suggest that *AC099850.3* was related to tumor progression and may, in part, explain some of the immune mechanisms seen in NSCLC. However, further studies are warranted.

This is the first study to analyze the relationship between ferroptosis-related lncRNA RS and tumor immune microenvironment. Notably, the complex interactions between tumor cells and the tumor microenvironment not only play a key role during tumor development, but also significantly affect immunotherapy efficacy and OS[44,45]. We used seven immune cell databases (TIMER, CIBERSORT, CIBERSORT−ABS, QUANTISEQ, MCPCOUNTER, XCELL, and EPIC) to analyze NSCLC associations with ferroptosis-related lncRNAs and showed that patients in the high-risk group had higher proportions of B cells, activated memory CD4 T cells, CD8+ killer T cells, M0 macrophages, M1 macrophages, and natural killer cells.

The role of ferroptosis-related lncRNAs in regulating tumor immune infiltration was identified. We correlated the RS and immune infiltration mechanisms in NSCLC. The findings suggest that these ferroptosis-related lncRNAs act as targets for combined therapy with immune checkpoint inhibitors.

In recent years, several studies[46,47] suggested that ferroptosis not only played an important role in tumor occurrence and development, but also affected tumor immunotherapy. Ferroptosis induction enhances tumor immunotherapy efficacy. Based on the pivotal role of checkpoint inhibitor immunotherapy during NSCLC, we compared the expression of ICGs between both groups. CD70, TNFRSF8, CD276, CD274, PDCD1, VTCN1, ICOSLG, PDCD1LG2, and TNFRSF9 gene expression differed significantly between groups of patients with NSCLC and suggested that FRG-lncRNAs regulated NSCLC development and progression by modulating immune responses and played a key role in immunotherapy resistance to NSCLC. Interestingly, PD-1 was highly expressed in the high-risk group, suggesting that these patients benefit from increased anti-PD-1 immunotherapy, thus providing new insights into tumor immunotherapy.

Limitations

Our study has several limitations. First, while ferroptosis is a new form of cell death with the potential to provide new tumor therapies, the relationship between ferroptosis and other cell death mechanisms and host immunogenicity remain unknown. Secondly, we used retrospective data available from public databases to construct our risk model, suggesting the need for prospective studies to confirm the reliability of our predictive model. Third, the risk model was only related to FRGs. The mutation status of oncogenic drivers such as EGFR and ALK was not included, suggesting that our model requires revision to accommodate this gene cohort. Finally, our findings were theoretical and based on risk models and immune responses of ferroptosis-related lncRNAs. The underlying molecular mechanisms require comprehensive investigation experimentally.

Conclusions

Ferroptosis induction enhances tumor immunotherapy efficacy. Based on the pivotal role of checkpoint inhibitor immunotherapy during NSCLC, we compared the expression of ICGs between both groups. CD70, TNFRSF8, CD276, CD274, PDCD1, VTCN1, ICOSLG, PDCD1LG2,

and TNFRSF9 gene expression differed significantly between groups of patients with NSCLC and suggested that FRG-lncRNAs regulated NSCLC development and progression by modulating immune responses and played a key role in immunotherapy resistance to NSCLC. Specific ferroptosis-associated lncRNAs can be used to predict the prognosis of NSCLC.

Conflict of Interest
The authors declare that they have no competing interests.

Ethics Approval
Ethics committee approval was not required in this study since all the expression, clinical information, and prognosis data of patients were obtained only from a public database. We are strictly in accordance with the declaration of Helsinki and the data access policies and publication guidelines of the TCGA database.

Informed Consent
Not applicable.

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Authors' Contribution
Weiwei Yi and Xiaoqing Guo performed the conception and design of study; Bin Liang contributed to the analysis of data and wrote the manuscript; Yu Xu performed the data acquisition; Peng Song contributed to the paper revision.

Availability of Data and Materials
The data presented and supporting the findings of this study are available upon reasonable request from the corresponding author (P.S).

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