Potential Biomarkers for Predicting the Overall Survival of Lung squamous cell carcinoma: A analysis of Ferroptosis-Related lncRNAs

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Research

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Abstract

Background

In 502 Lung squamous cell carcinoma (LUSC) samples from The Cancer Genome Atlas (TCGA) datasets, the predictive significance of ferroptosis-related long non-coding RNAs (lncRNAs) was investigated. In LUSC, we meant to express how ferroptosis-associated lncRNAs interact with immune cell infiltration.

Methods

Gene expression enrichment was investigated using gene set enrichment analysis in the Kyoto Encyclopedia of Genes and Genomes. The prognostic model was constructed using Lasso regression. To better understand immune cell infiltration in different risk groups and its relationship to clinical outcome, researchers analyzed by modifications in the tumor microenvironment (TME) and immunological association. The expression of lncRNA was intimately connected to that of ferroptosis, according to co-expression analyses. Ferroptosis-related lncRNAs were shown to be partially overexpressed in high-risk patients in the absence of additional clinical signs, suggesting that they may be incorporated into a prediction model to predict LUSC prognosis. GSEA revealed the immunological and tumor-related pathways in the low-risk group.

Results

According to TCGA, CCR and inflammation-promoting genes were considered to be significantly different between the low-risk and high-risk groups. The expression of C10orf55, AC016924.1, AL161431.1, LUCAT1, AC104248.1, and MIR3945HG were likewise different in the two risk groups.

Conclusion

lncRNAs linked to ferroptosis are connected to the occurrence and development of LUSC. With the use of matching prognostic models, the prognosis of LUSC patients can be predicted. In LUSC, ferroptosis-related lncRNAs and immune cell infiltration in the TME might be novel therapeutic targets that should be investigated further.

1 Background

Lung cancer is among the most frequent cancers in the world, killing about 500,000 people in China each year¹. The most frequent kind of lung cancer is lung squamous cell carcinoma (LUSC), which accounts for 40–51% of all primary lung malignancy¹. The prevalence of LUSC is strongly related to age, gender, and smoking. LUSC developed slowly, metastasized late, was more likely to be surgically respected in the

middle and early stages, and had a higher 5-year survival rate^[1]. Small cell undifferentiated carcinoma was more susceptible to radiotherapy and chemotherapy than LUSC. Recent developments in molecular research and the development of new medications targeting molecular anomalies have largely driven advances in the treatment of LUSC. Existing therapy targets, on the other hand, are susceptible to resistance^[1]. Therefore, novel predictive biomarkers for the diagnosis, prognosis, and therapy of LUSC are so urgently needed.

lncRNAs are RNA molecules that have a high degree of expression selectivity. Several studies have discovered that lncRNAs have a variety of biological roles, including gene control, cancer, development, and even metastasis regulation^[1]. Iron droop in tumor cells has received a lot of interest in recent years as a novel cell death that can help tumor cells escape therapeutic resistance^[1]. In contrast to apoptosis and autophagy, iron failure, which is an iron dependent and reactive oxygen species (ROS)-dependent cell death, can be used to treat a variety of disorders. Cancer cells are more iron dependent than normal cells and rely on iron excessively to proliferate. Hence an imbalance in iron metabolism may hasten tumor growth^[1]. There is no question that activating the Ferroptosis pathway could overcome resistance to present chemotherapeutic drugs and expand the boundaries of cancer therapy^[1]. The relationship between lncRNA and immune cell infiltration in ironophilic cell illness, on the other hand, is still unknown. Depending on research, lncRNA can regulate iron droop and control iron death and apoptosis, whereas silencing lncRNA can greatly reduce iron droop and regulate inflammation and lipid peroxidation^[1]. Nonetheless, sequencing investigations of aberrant lncRNA expression and its relationship to overall survival (OS) in iron-addicted LUSC patients are uncommon.

Immune checkpoint-associated gene profiles in LUSC patients may be beneficial in detecting therapy response, assessing risk, and predicting survival^[1]. Despite the fact that little study has been conducted on the association between iron-cytopathic-associated lncRNAs and immune cell infiltration in LUSC, it is critical to explore immune cell infiltration in TME and its relationship with clinicopathological characteristics of LUSC tumors. There is limited research on the causes and mechanisms of aberrant lncRNA expression and iron droop in LUSC at the moment. To further understand the lncRNA-related pathways that alter the prognosis of LUSC patients, a transcription map of lncRNA expression and ferroptosis changes in LUSC patients is required. Furthermore, immune checkpoint-associated gene profiles can be used as predictors of therapeutic response to LUSC patients to assess risk and predict overall survival.

The purpose of this work was to identify ferroptosis-related lncRNAs whose expression is linked to LUSC patients' prognosis in order to develop a predictive model for LUSC prognosis prediction. To aid in the identification of novel LUSC therapeutic targets and pharmacological options by better understanding the infiltration of ferroptosis-related lncRNAs and their associated immune cells in TME.

Figure 1. Framework based on an integration strategy of ferroptosis-related lncRNAs.

2 Marerials And Methods

2.1 Datasets and ferroptosis-related Genes

Using Genomic Data Commons Data Portal (<https://portal.gdc.cancer.gov/>)^[1], we collected LUSC gene expression patterns and clinical data from the Cancer Genome Atlas (TCGA). The strategy is to investigate at gene expression profiles (Cases: bronchus and lung, TCGA and TCGA-LUSC; Files: transcriptome profiling and Gene Expression Quantification and HTSeq-FPKM). Information on clinical research (Cases: bronchus and lung, TCGA and TCGA-LUSC; Files: clinical and bcr xml). The expression patterns of 502 cases of LUSC and 49 cases of normal tissues were enrolled in the TCGA shared database on October 5, 2021. Table 1 summarizes the clinical features of the patients. In addition, corresponding ferroptosis-related genes were downloaded from FerrDb^[2], a web-based consortium that provided a comprehensive and up-to-date database for ferroptosis markers, their regulatory molecules and associated diseases. We identified 382 ferroptosis-related genes (driver: 150; suppressor: 109; marker: 123) in total (Table S1).

Table 1
The clinical characteristics of patients in the TCGA dataset.

Variable	Number of samples
Gender	
Male/Female	373/131
Age at diagnosis	
≤65/>65	190/305
Grade	
G1/G2/G3/G4/NA	Unknow
Stage	
I/II/III/IV/NA	245/163/85/7/4
T	
T1/T2/T3/T4	114/295/71/24
M	
M0/M1/NA	414/7/83
N	
N0/N1/N2/N3/NA	320/133/40/5/6

2.2 Annotation of IncRNAs

For annotation of the lncRNAs in the TCGA dataset, Genome Reference Consortium Human Build 38 (GRCh38) lncRNA annotation file was obtained from the GENCODE website⁴. With the help of perl software (<https://www.perl.org/>), the transcriptome data and human configuration files were matched and sorted, and the appropriate mRNA and lncRNA gene expression data were obtained. The gene IDs were translated to gene names using information from the ensemble database (<http://asia.ensembl.org/info/data/index.html>). The R4.1.0 Limma package was used to extract ferroptosis-related gene expression data, which was based on the gene expression matrix of ferroptosis-related lncRNA gene expression profile data collected before.

2.3 Identification of ferroptosis-related lncRNAs

The relationship between ferroptosis-related lncRNAs and LUSC was investigated using Pearson correlation. After removing the normal samples and using $p < 0.001$ and $\text{corFilter} = 0.4$ as screening criteria, the Limma package's correlation test was performed to evaluate the expression of ferroptosis-related lncRNA. Co-expression analysis was utilized to look at the relationship between ferroptosis-related gene expression and lncRNAs. The clinical-pathological information acquired from LUSC patients included gender, age, stage, grade, TMN, survival status, and survival time. To determine whether there was a significant difference in expression of ferroptosis-related lncRNAs, $\text{FDR} < 0.05$ and $|\log_2\text{FC}| \geq 1$ were utilized. First, we investigated into the function of ferroptosis-related differentially expressed genes that were both upregulated and downregulated (DEGs). The biological pathways connected with the DEGs were then analysed using Gene Ontology (GO). Biological processes (BP), molecular functions (MF), and cellular components (CC) regulated by the differently expressed ferroptosis-related lncRNAs were further analyzed using R software, clusterProfiler, org.Hs.eg.db, enrichplot, and ggplot2 package based on Kyoto Encyclopedia of Genes and Genomes (KEGG) data.

2.4 Development of the ferroptosis-related lncRNAs prognostic signature

To build a prognostic model, Ferroptosis-related lncRNAs signature was constructed using Lasso-penalized Cox regression and Univariate Cox regression analysis, stratified by risk score ($\text{Coefficient lncRNA}_1 \times \text{expression of lncRNA}_1 + (\text{Coefficient lncRNA}_2 \times \text{expression of lncRNA}_2) + \dots + (\text{Coefficient lncRNA}_n \times \text{expression lncRNA}_n)$). Each LUSC patient's associated risk score was further evaluated. Based on the median score, the RNAs were divided into three subgroups: low-risk ($<$ median number) and high-risk ($\geq$ median number). In Lasso regression, the low-risk (50%) and high-risk (50%) groups were identified, and the corresponding plots were obtained. The confidence interval and risk ratio were calculated after visualization, and the forest diagram was constructed. The high-risk and low-risk groups' survival curves were constructed and compared. We utilized the timeROC software to design a comparable receiver-operating characteristics (ROC) curve to examine the accuracy of our model for predicting survival in LUSC. The risk and survival status of ferroptosis-related lncRNAs was investigated in connection to the risk curve, which was generated using the risk score. An independent prognosis analysis was performed to check whether our model was unaffected by other clinical prognostic factors

that influence the patients' outcome. The researchers used multivariate and univariate models to calculate hazard ratios. To determine the association between clinical characteristics and our prediction risk model, as well as to distinguish between high-risk and low-risk ferroptosis-related cases. Risk and clinical correlation analyses were completed. Heatmap and limma packages were used to construct the Heatmap. To further demonstrate the correctness of our model, Decision Curve Analysis (DCA) was constructed.

2.5 GSEA enrichment analyses and the predictive nomogram

GSEA (<https://www.gsea-msigdb.org/gsea/index.jsp>) was used to discover variations in linked functions and pathways in diverse samples, and data was imported using the PERL programming language. Associated score and graphs were used to see if the functions and routes in various Risk groups were dynamic(c2.cp.kegg.v.7.2.symbols.gmt,Risk.cls#h versus l). Depending on it was a high-risk cluster of prognosis-related lncRNAs, each sample was labeled as 'H' or 'L'. The number of permutations, no collapse, and phenotype were set to 1000, no collapse, and phenotypic, respectively. The gene list was sorted in 'real' mode, with the order of the genes in 'descending' mode. The 'Signal2Noise' measure was utilized to rank the genes. The normalization method was 'meandiv,' and the difference was statistically significant with a FDR<0.05. A nomogram was constructed integrating the prognostic signatures, for predictive of 1, 2 and 3 year OS of LUSC patients.

2.6 Immunity analysis and gene expression

Simultaneously, the CIBERSORT^[1], ESTIMATE^[2], MCPcounter^[3], single-sample gene set enrichment analysis (ssGSEA)^[4], and TIMER^[5] algorithms were compared to evaluate cellular components or cell immune responses between high and low risk groups based on ferroptosis-related lncRNA signatures. A Heatmap was utilized to discover changes in immune response under different algorithms. In addition, ssGSEA was utilized to compare and quantify the tumor-infiltrating immune cell subgroups in both groups, as well as their immunological function. Previous literature was designed to identify a possible immunological roadblock.

2.6 Statistical analysis

The data was analyzed using Bioconductor programs in R software version 4.1.0. To investigate normally and non-normally distributed variables, the Wilcoxon test and the unpaired student's t-test were utilized. The Benjamini-Hochberg technique was used to determine the variable expressed lncRNAs based on FDR. Utilizing "GSVA" and ssGSEA-normalized LUSC DEGs, the LUSC DEGs were compared to a genome (R-package). The sensitivity and specificity of the LUSC generate prognostic signals in comparison to other clinicopathological factors were evaluated using the operating characteristic curve (ROC) and decision curve analysis (DCA). The connection between ferroptosis-related lncRNAs and clinicopathological symptoms was investigated using logistic regression analysis and a heatmap graph. Based on the ferroptosis-related lncRNAs signature, the Kaplan-Meier survival analysis was used to estimate the survival of LUSC patients. For each analysis, statistical significance was identified as P<0.05.

3 Results

The purpose of this study was to illustrate how immune cell infiltration and ferroptosis-related lncRNAs influence LUSC. We identified 102 ferroptosis-related DEGs and 8 risk ferroptosis-related lncRNAs based on expression differences between tumor and normal tissues. GSEA was performed to discover latent signaling pathways that could be implicated in the development and progression of LUSC, and lasso regression was used to generate a suitable prognostic model.

3.1 Enrichment Analysis of ferroptosis-related genes

We discovered 102 DEGs linked to ferroptosis (35 downregulated and 67 upregulated; Table S2). 655 core targets were discovered by GO enrichment analysis, including MF, CC, and BP. The MF mainly involves antioxidant activity (GO:0016209), heme binding (GO:0020037), iron ion binding (GO:0005506), oxidoreductase activity, acting on NAD(P)H (GO:0016651). The CC mainly involves apical part of cell (GO:0045177), apical plasma membrane (GO:0016324), basal plasma membrane (GO:0009925), basal part of cell (GO:0045178). The BP mainly involves response to extracellular stimulus (GO:0009991), response to nutrient levels (GO:0031667), intrinsic apoptotic signaling pathway (GO:0097193), neuron death (GO:0070997). In addition, the main signaling pathways were identified by KEGG enrichment analysis, revealed the over-expressed genes were mainly involved in Lipid and atherosclerosis (hsa05417), HIF-1 signaling pathway (hsa04066), Chemical carcinogenesis-reactive oxygen species (hsa05208), Ferroptosis (hsa04216), MicroRNAs in cancer (hsa05206) (Figure 2 and Table S3).

Figure 2. GO and KEGG analyses for ferroptosis-related differentially expressed genes. (a) GO (b) KEGG.

3.2 The ferroptosis-based lncRNAs prognostic signature

505 ferroptosis-related lncRNAs were discovered (Table S4). 8 significant ferroptosis-related lncRNAs were discovered in the univariate COX study, and these were included in the multivariate COX analysis. Overall, 8 different lncRNAs (C10orf55, AC016924.1, AL161431.1, AP006545.2, LUCAT1, AC104248.1, AL122125.1, MIR3945HG) were discovered to be independent LUSC prognostic indicators (Figure 3) (Table S5). As a consequence, we calculated risk scores for the lncRNAs and constructed a prognostic signature.

Figure 3. Forest plot of significant lncRNAs.

3.3 Survival results and multivariate examination

Depending on Kaplan-Meier analyses, the expression of high-risk lncRNA signatures was associated with poorer survival ($P < 0.001$, Figure 4a). Meanwhile, the signature lncRNAs' AUC was 0.658, indicating that they outperformed standard clinicopathological characteristics in predicting LUSC prognosis (Figure 4b-c). We discovered that the patient's risk score was inversely proportional to the survival of LUSC patients using a patient's risk survival status plot. Surprisingly, the majority of the novel lncRNAs identified in this research exhibited a negative relationship with our risk model, indicating that more research is needed

(Figure 4d). For 1, 2, and 3 year survival rates, the AUC predictive value of the unique lncRNAs signature was 0.658, 0.693, and 0.687, respectively (Figures 4e). Risk-related heatmap: ferroptosis-related lncRNAs that such as C10orf55, AC016924.1, AL161431.1, LUCAT1, AC104248.1, MIR3945HG, were highly expressed in a high-risk group, which means all of them might be detrimental to the prognosis of LUSC patients (Figures 4f). COX analysis revealed that lncRNA signature (HR: 1.434, 95CI: 1.315-1.565), age (HR: 1.022, 95CI: 1.005-1.039), and tumor stage (HR: 1.242, 95CI: 1.050-1.470) were mostly independent prognostic variables for LUSC patients' OS (Figure 5a-b). Figure 5c demonstrates the link between lncRNA and mRNA. The heatmap for the prognosis signature of ferroptosis-related lncRNAs and clinicopathological manifestations was also evaluated (Figure 6). The hybrid nomogram (Figure 7) integrating clinicopathological features and the novel ferroptosis-related lncRNAs prognostic signature was stable and accurate, and hence may be employed in LUSC patient care.

Figure 4. Ferroptosis-related lncRNAs signature. (a) Kaplan-Meier curves result, (b). The AUC values of the risk factors, (c). The DCA of the risk factors. (d). Risk survival status plot, (e). The AUC of the for the prediction of 1, 2, 3-year survival rate of LUSC, (f) Heatmap of different lncRNAs.

Figure 5. COX analysis for the expression of ferroptosis-related lncRNAs, both univariate and multivariate. (a). univariate, (b). multivariate, (c). The relationship between the novel lncRNA and mRNA expression.

Figure 6. Prognostic hallmark and clinicopathological symptoms of ferroptosis-related lncRNAs in a heatmap.

Figure 7: A nomogram for prognostic ferroptosis-related lncRNAs as well as clinic-pathological variables.

3.4 Gene set enrichment analyses

According to gene set enrichment analyses (GSEA), the majority of the novel ferroptosis-related lncRNAs prognostic signature regulated immune and tumor-related pathways such as graft versus host disease, allograft rejection, asthma, type i diabetes mellitus, nod like receptor signaling pathway, chemokine signaling pathway, jak stat signaling pathway etc. The top 6 enriched functions or pathways for each cluster are shown, (Figure 8) and (Table S6). FDR q-value and FWER p-value were both <0.05. As a consequence, the 'NOD LIKE RECEPTOR SIGNALING PATHWAY' was the most enriched, and some of the genes were positively correlated with H or L.

Figure 8. Gene set enrichment analyses for ferroptosis-related lncRNAs.

3.5 Immunity and gene expression

Figure 9 demonstrates a heatmap of immunological responses generated using the CIBERSORT, ESTIMATE, MCP counter, single-sample gene set enrichment analysis (ssGSEA), and TIMER algorithms. Based on ssGSEA of TCGA-LUSC data, correlation analysis revealed that CCR, Inflammation-promoting, and other immune cell subpopulations and related functions were significantly different between the low-risk and high-risk groups (Figure 10a). Given checkpoint inhibitor-based immunotherapies are just as important, we investigated into the differences in immune checkpoint expression between the two groups.

Between the two groups of patients, we discovered a significant discrepancy in the expression of TMIGD2, TNFRSF4, CD244, NRP1, CD276, and other genes (Figure 10b). The expression of YTHDF1, METTL3, FTO, HNRNPC, YTHDC1 were meaningful when ferroptosis-related mRNA expression was compared between the high and low risk groups (Figure 11).

Figure 9. CIBERSORT, ESTIMATE, MCPcounter, ssGSEA, and TIMER algorithms were used to construct a heatmap for immune responses in high and low risk groups.

Figure 10. (a). ssGSEA for the association between immune cell subpopulations and related functions (b). Immune checkpoint expression in high and low LUSC risk groups.

Figure 11. The expression of ferroptosis-related genes in LUSC risk groups with high and low LUSC risk.

4 Discussion

Treating LUSC is a complex psychological challenge due to its advanced stage and poor prognosis^[1]. Diagnostic biomarkers and treatment targets for LUSC should constantly be highlighted at the molecular level. According to previous research, ferroptosis is implicated in the pathological cell death associated with degenerative illnesses, and it can also overcome chemotherapy resistance in malignant cells and increase the removal of defective cells^[2]. Ferroptosis has the potential to operate as a tumor suppressor, making it a viable cancer treatment option^[3]. Despite this, it's unclear how it affects LUSC development through modulating lncRNA. The involvement of immune infiltrating cells in the TME and immune checkpoint inhibitors in the prognosis of LUSC was investigated by this researcher. The findings of this study resulted in the discovery of a promising biomarker and therapeutic target.

We retrieved ferroptosis-related gene expression data and differentiated between mRNA and lncRNA in this study. Co-expression analysis was utilized to look at the relationship between ferroptosis-related gene expression and lncRNAs. Using the co-expression network plot, we observed phenomena in which numerous lncRNAs were associated with ferroptosis-related genes in LUSC. After that, we discovered 102 DEGs associated with ferroptosis. KEGG analyses further revealed the genes mainly participated in Chemical carcinogenesis-reactive oxygen species, Ferroptosis, MicroRNAs in cancer, HIF-1 signaling pathway, NOD-like receptor signaling pathway. A growing body of research reveals that miRNA and lncRNA are important regulators of ferroptosis. By reducing iron absorption, Nrf2 lowers the production of reactive oxygen species (ROS). As a result, miRNA inhibits ferroptosis via regulating the expression of Nrf2. Meanwhile, miRNA is engaged in iron transport, storage, usage, and absorption control^[4]. The interplay of MTOR and GPX4 signaling regulates autophagy-dependent ferroptotic cancer cell death^[5]. HIF-1 α is highly expressed in cancer-associated fibroblasts (CAFs), and HIF-1 α -expressed fibroblasts activate the NF- κ B signaling pathway, which promotes lung cancer tumor growth^[6]. Yana Zhang^[7] believes that HIF-1 α is a critical factor of CAFs in lung cancer, and that targeting HIF-1 α -expressed CAFs could be a future anticancer treatment. Visibility. In LUSC, ferroptosis is crucial.

To study their possible activities in LUSC, the ferroptosis-associated lncRNAs were split into two categories: high-risk and low-risk. Using data on prognosis-related lncRNAs, the confidence interval and the hazard ratio were calculated. In a university Cox regression study, ferroptosis-related lncRNAs appeared to be strongly correlated with LUSC prognosis. This study identified eight ferroptosis-related lncRNAs that have been linked to prognosis and show altered expression in high-risk and low-risk patients. Some lncRNAs were considered to be overexpressed in high-risk people, while others were found to be overexpressed in low-risk people ($P < 0.05$). We looked into the role of ferroptosis-related lncRNAs in LUSC in more detail. The predictive value of ferroptosis-related lncRNAs was measured using a survival analysis based on lncRNA subtypes. Low-risk lncRNAs were involved in a better prognosis than high-risk lncRNAs. According to the ferroptosis-related lncRNAs risk score, C10orf55, AC016924.1, AL161431.1, LUCAT1, AC104248.1, and MIR3945HG were significantly expressed in the high-risk group, demonstrating that they are LUSC oncogenes. Furthermore, the above-mentioned ferroptosis-related lncRNAs could be invoked as a therapeutic target for LUSC. In the LUSC study, lncRNAs were also related to patient outcomes. Only a little amount of study has been done on lncRNA changes connected to ferroptosis. More research is needed in order to fully understand the process of ferroptosis-related lncRNA modification and identification, as well as to corroborate our findings.

We also investigated and computed the infiltration of different immune cells in the samples to find out what function immune cell infiltration and the TME play in LUSC. CCR, HLA, Inflammation-promoting, Parainflammation, T cell co-inhibition, T cell co-stimulation, Type I IFN Response, Type II IFN Response considerably infiltrated tumor tissues in high-risk individuals, according to research of immune cell infiltration discrepancies. As a result, these cells' invasion of the TME may have a negative effect on LUSC patients' prognosis. Ferroptosis and immune checkpoint inhibitors (ICIs) work together to improve anticancer efficacy in ICI-resistant cancers^[1]. TMIGD2, TNFRSF4, CD244, NRP1, CD276, ICOS, CD80, and other checkpoint genes were considered to be highly expressed in our study, suggesting that they could become ICIs in LUSC. The relationship between ICI and ferroptosis has received very little attention. P53, ATF3/4, SLC7A11, ACSL4, and the BECN1 pathway are among the latest ferroptosis-regulating factors found in recent years. Surprisingly, lncRNA is connected to the regulation of these factors' expression^[2]. Despite the fact that research on ferroptosis-related lncRNA and LUSC is limited. We might conclude that changes in ferroptosis-related lncRNAs are associated with the onset and progression of LUSC based on the information presented above.

In GSEA, the nod like receptor-signaling pathway was found to be the most significantly enriched pathway. NOD-like receptors are involved in inflammatory responses that exacerbate the occurrence and development of lung remodeling. when there is a hypoxia plateau^[3]. Ferroptosis-related lncRNAs may regulate LUSC cell migration and proliferation through modulating the NOD LIKE RECEPTOR SIGNALING PATHWAY, based on the aforementioned properties. The low-risk subtype surpassed the high-risk subtype in terms of survival. The low-risk subtype showed a high survival rate than the high-risk subtype, according to the ferroptosis-related lncRNA prognostic model. Furthermore, our model is under a high level of accuracy when it comes to forecasting LUSC patient survival. Increases in risk score are

connected to higher death rates and a higher high-risk ratio. Our model had no effect on other clinical prognostic variables that could influence patient outcomes. The principle could be applicable to a variety of clinical situations. Ferroptosis-related lncRNAs seem to be viable biomarkers for predicting LUSC patient outcomes, based on our findings and data from the literature.

Even while our research provides some theoretical foundations and research recommendations, there are still limitations. Using the TCGA dataset, we first constructed and validated a ferroptosis-related lncRNA prediction signature. We were unable to obtain sufficient external data from other public sources in order to evaluate the model's reliability. Second, only the signature's eight ferroptosis-related lncRNAs were subjected to preliminary expression studies. Regardless, no more functional or mechanistic research was conducted. Finally, no studies in LUSC have been undertaken to confirm the relationship between prognostic lncRNAs and ferroptosis. However, we shall conduct extra investigation in order to completely appreciate the aforementioned facts.

5 Conclusions

In conclusion, we looked for prognosis-related ferroptosis-related lncRNAs by analyzing the expression patterns and clinical data of LUSC samples from the TCGA database. As part of the ferroptosis regulation, 8 ferroptosis-related predictive lncRNAs were discovered in 502 LUSC patients. For LUSC, it has a significant predictive value. Our findings contribute to the understanding of ferroptosis-related lncRNAs and immune cell infiltration in the TME, possibly paving the way for novel therapeutic targets and prognostic indicators in the future. It is desirable for our findings will benefit identify ferroptosis-related lncRNA that stimulates LUSC growth, allowing us to understand more about their possible function in the development and progression of LUSC tumors.

Abbreviations

LUSC	Lung squamous cell carcinoma	TCGA	The Cancer Genome Atlas
GO	Gene Ontology	lncRNAs	long non-coding RNAs
AUC	areas under the curve	BP	Biological processes
MF	molecular functions	CC	cellular components
KEGG	Kyoto Encyclopedia of Genes and Genomes	DEGs	differentially expressed genes
ROC	receiver-operating characteristics	DCA	Decision Curve Analysis
GSEA	gene set enrichment analyses	TME	tumor microenvironment
ICIs	immune checkpoint inhibitors	OS	overall survival

Declarations

Data availability

Patients who have provided informed consent for the use of their data have been included in the TCGA database, which is a public database. Users can freely obtain and publish appropriate articles based on the relevant data. Our study has no ethical difficulties or conflicts of interest because it is built on open-source data.

Ethics approval and consent to participation

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

Consent for publication

All authors have read and approved this manuscript to be considered for publication.

Competing interests

The authors declare no competing financial interests.

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Author Contributions

Zixuan Wu drafted and revised the manuscript. Xuyan Huang and Minjie Cai are in charge of data collection. Peidong Huang conceived and designed this article, in charge of syntax modification and

revise of the manuscript. Zunhui Guan revised the manuscript. All the authors have read and agreed to the final version manuscript.

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Figures

Figure 1

Framework based on an integration strategy of ferroptosis-related lncRNAs.

Figure 2

GO and KEGG analyses for ferroptosis-related differentially expressed genes. (a) GO (b) KEGG.

	pvalue	Hazard ratio
C10orf55	<0.001	1.330(1.166–1.517)
MIR22HG	0.010	1.037(1.009–1.067)
LINC01322	0.008	1.186(1.045–1.345)
AC016924.1	0.002	2.041(1.298–3.208)
AC011511.5	0.022	1.287(1.037–1.598)
AL136369.1	0.035	1.521(1.030–2.247)
AL161431.1	0.040	1.005(1.000–1.009)
AC007823.1	0.045	0.652(0.429–0.991)
LINC02345	0.034	1.193(1.014–1.404)
LINC02178	0.044	1.016(1.000–1.031)
SFTA1P	0.030	1.015(1.001–1.028)
AL606469.1	0.011	1.595(1.111–2.289)
LRRK2-DT	0.047	1.097(1.001–1.202)
LANCL1-AS1	0.023	1.822(1.088–3.054)
AP006545.2	0.015	0.713(0.543–0.938)
LUCAT1	0.015	1.105(1.020–1.197)
MYOSLID	0.029	1.038(1.004–1.073)
ARHGEF2-AS1	0.041	1.663(1.020–2.713)
AC104248.1	0.013	1.179(1.036–1.342)
AL357054.4	0.042	1.406(1.012–1.953)
AL122125.1	0.020	0.761(0.605–0.958)
AP001189.1	0.025	1.415(1.045–1.915)
AP001189.3	0.005	1.387(1.105–1.742)
LASTR	0.006	1.034(1.010–1.059)
LINC02555	0.006	1.438(1.108–1.867)
MIR3945HG	<0.001	1.634(1.251–2.135)
AL138756.1	0.048	1.434(1.002–2.052)
MIR762HG	0.029	0.708(0.520–0.965)
AC019080.1	0.038	0.932(0.871–0.996)

Figure 3

Forest plot of significant lncRNAs.

Figure 4

Ferroptosis-related lncRNAs signature. (a) Kaplan-Meier curves result, (b). The AUC values of the risk factors, (c). The DCA of the risk factors. (d). Risk survival status plot, (e). The AUC of the for the prediction of 1, 2, 3-year survival rate of LUSC, (f) Heatmap of different lncRNAs.

Figure 5

COX analysis for the expression of ferroptosis-related lncRNAs, both univariate and multivariate. (a). univariate, (b). multivariate, (c). The relationship between the novel lncRNA and mRNA expression.

Figure 6

Prognostic hallmark and clinicopathological symptoms of ferroptosis-related lncRNAs in a heatmap.

Figure 7

A nomogram for prognostic ferroptosis-related lncRNAs as well as clinic-pathological variables.

Figure 8

Gene set enrichment analyses for ferroptosis-related lncRNAs.

Figure 9

CIBERSORT, ESTIMATE, MCPcounter, ssGSEA, and TIMER algorithms were used to construct a heatmap for immune responses in high and low risk groups.

Figure 10

(a). ssGSEA for the association between immune cell subpopulations and related functions (b). Immune checkpoint expression in high and low LUSC risk groups.

Figure 11

The expression of ferroptosis-related genes in LUSC risk groups with high and low LUSC risk.

Supplementary Files

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