ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

A Ferroptosis-Related lncRNAs Signature Predicts Prognosis for Lung Adenocarcinoma

Fangfen Li, Liangxiu Wu, Shengyi Lian, Ruanruan Yang, Jinzhong Li, Yanli Li and Sen Yang, Xiaobing Gong*

Department of General Practice, The First Affiliated Hospital of Jinan University, Guangzhou 510630, P.R. China.

*Corresponding Author

Abstract

Lung adenocarcinoma (LUAD) is the most common type of nonsmall cell lung cancer. Ferroptosis, a regulated cell death which is driven by the iron-dependent peroxidation of lipids, plays an important role in cancer. Long non-coding RNAs (lncRNAs) are increasingly recognized as key mediators of ferroptosis metabolism. This study aimed to explore the potential prognostic value of ferroptosis-related lncRNAs and risk score in Lung adenocarcinoma. In this study, we obtained RNA sequencing (RNA-seq) data and corresponding clinical information of LUAD patients from The Cancer Genome Atlas (TCGA), and ferroptosis-related genes were retrieved from the FerrDb database. Receiver operating characteristic (ROC) time curve analysis and univariate and multivariate Cox regression analysis to determine the best prognostic lncRNA, and its prognostic value was further tested in the validation cohort. A 13 gene signature was found from the training cohort and validated by survival analysis and Cox regression models, revealing its independent prognosis value in LUAD. Demonstrated the ability of stratification to divide patients into high-risk groups and low-risk groups, with significantly different survival outcomes. In addition, this signature was identified as an independent prognostic factor and was significantly related to the overall survival (OS) of LUAD. In conclusion, we identified a novel ferroptosis-related lncRNA signature which could precisely predict the prognosis of LUAD patients. Ferroptosis-related lncRNAs may have a potential role in the process of anti-tumor immunity and serve as therapeutic targets for lung cancer.

Keywords

Lung Adenocarcinoma; Ferroptosis; lncRNAs; Prognosis.

1. Introduction

Lung cancer is considered to be the leading cause of cancer-related deaths worldwide [1]. LUAD is the most common type of nonsmall cell lung cancer [2]. Despite great efforts having been made in developing novel treatments but still received a poor prognosis with 5-year survival rates vary from 4% to 17% [3]. Most patients with lung adenocarcinoma have advanced to advanced stage (stage IIIB or IV) when they are diagnosed, and miss the best period of surgical treatment. Therefore, systemic chemotherapy has become one of the current effective and standard treatments, but it has little effect on improving the survival rate of patients [4]. Individualized treatment guided by genetic testing results has become the main treatment for LUAD. Due to the high mutation load and complex tumor microenvironment, there is an urgent need to improve the diagnosis and treatment of LUAD, and to determine new prognostic biomarkers and therapeutic targets for LUAD.

ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

Ferroptosis, a regulated cell death which is driven by the iron-dependent peroxidation of lipids, plays an important role in cancer [5]. In recent years, studies have demonstrated that ferroptosis has different effects on different stages of tumor, and promote tumor growth in the early stage and participate in the formation of tumor drug resistance. This iron dependency can make cancer cells more vulnerable to iron-catalyzed necrosis, referred to as ferroptosis. Its inducers can induce such drug-resistant tumor cells to bypass the apoptosis pathway and directly cause ferroptosis [6, 7]. The ferroptosis has been identified to suppress tumor growth and the progression, and as a result, the induction of ferroptosis has emerged as a promising anti-cancer treatment [8, 9].

Long non-coding RNAs (lncRNAs) are increasingly recognized as key mediators of ferroptosis metabolism. LncRNAs are participated in various biological purposes, such as immune, metabolism, infection, and so on [10]. LncRNAs have been shown to function as master regulators in various disease processes including cancer [7]. The latest research shows that lncRNA expression is frequently dysregulated in cancer, and specific lncRNAs are correlated with cancer recurrence, metastasis, and poor prognosis in different kinds of cancer, including LUAD [11]. Recently, the study found that the cytosolic lncRNA P53RRA is downregulated in cancers and functions as a tumor suppressor by inhibiting cancer progression [12]. At present, the mechanism of ferroptosis-related lncRNAs in lung cancer progression has not been clarified, and their important significance in lung cancer treatment and prognosis needs to be further elucidated. Therefore, we speculate whether ferroptosis is related to the prognosis of LUAD and the possible involvement of ferroptosis-related lncRNAs.

2. Materials and Methods

2.1. Data Resources and Pretreatment

TCGA database is a public platform with more than 30 cancer types and clinicopathological information for at least 11,000 patients. It has been widely used by a large number of researchers to explore the genetic basis of tumors through high-throughput sequencing. The RNA sequencing (RNA-seq) data and corresponding clinical information of patients with LUAD were downloaded from The Cancer Genome Atlas (TCGA) database [13], including age, gender, pathological stage and histological grade, and ferroptosis-related genes were retrieved from the FerrDb database [14]. The prognostic signature was developed using the overlapped prognostic genes based on a risk score method. The present study was in compliance with the publication guidelines provided by TCGA, and the data obtained from TCGA did not require approval from an ethics committee.

2.2. Construction of the LncRNA-mRNA Co-Expression Network

In order to demonstrate the correlation between the ferroptosis-related lncRNAs and their corresponding mRNAs, the LncRNA-mRNA co-expression network was constructed. Extracted ferroptosis-related lncRNAs that are significantly related to co-expression and ferroptosis genes to construct a regulatory network, and used Cytoscape software (version 3.8.0) to display ferroptosis genes and ferroptosis-related lncRNA regulatory networks [15].

2.3. Functional Enrichment Analysis

After determining the lncRNA signature associated with the prognosis of LUAD, the gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways are used by the "clusterProfiler" R package to evaluate the biological effects of prognostic candidates. Gene ontology (GO) analysis of genes differentially expressed between the high-risk group and the low-risk group was performed to determine the biological processes, molecular functions, and cellular components associated with the ferroptosis-related lncRNAs signature. The Kyoto

ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

Encyclopedia of Genes and Genomes (KEGG) pathway analysis was carried out to determine the signaling pathways related to the characteristics of ferroptosis-related lncRNAs signature.

2.4. Independent Prognostic Analysis

Univariate and multivariate Cox regression analysis were performed to assess whether the prognostic model was independent of other traditional clinical characteristics (including age, gender, and TNM stage) in predicting OS in LUAD patients. Independent prognostic analysis was performed on the TCGA cohort, and get statistically significant parameters from it($P<0.05$). Comparing survival time and survival status, if pvalue <0.05 in the single factor and multi-factor analysis, it means that this factor can be independent of other factors and serve as an independent prognostic factor.

2.5. Validation of the Risk Score

Integrate the prognostic model with clinical traits and draw a comprehensive curve. Construct a prognostic model for the ferroptosis-related lncRNA, and obtain the formula of the model. For each sample, there is the expression level of lncRNA. According to the formula of the prognostic model, The computational formula was as follows:

$$\text{Risk Score} = e^{\text{sum (each lncRNA's expression} \times \text{corresponding regression coefficient)}}. \quad [16]$$

Use the "survival" R package and the "survivalROC" R package to perform time-dependent ROC curve analysis to evaluate the prediction accuracy of ferroptosis-related lncRNAs signature. By comparing the area under the time-dependent receiver operating characteristics, the larger the area under the curve, the higher the accuracy of the prognostic model to predict the patient's survival.

3. Statistical Analysis

3.1. The Clinical characteristics of patients with Prognostic analysis on TCGA database

Downloaded the expression data and clinical information of from TCGA. Quantitative gene expression data of a total of 594 samples, including 535 tumor samples and 59 normal samples. 535 tumor genes and 59 normal genes were obtained respectively, including age, gender, pathological stage, Among them, there are 241 patients younger than or equal to 65 years old, 262 patients older than 65 years old, and 32 unknown ones, which need to be excluded. There are 242 male patients and 280 female patients, see Table 1. Univariate Cox regression analysis revealed that among the ferroptosis-related genes, 37 were signifcantly correlated to the prognosis of LUAD ($p < 0.05$). In addition, the lncRNAs with the 25 highest and 12 lowest hazard ratios (HRs) were reported in Figure 1.

Table 1. The baseline characteristics of the patients in TCGA cohorts.

Age		Gender		Stage			
<=65y	>65y	Male	Female	I	II	III	IV
n=241	n=262	n=242	n=280	n=279	n=124	n=85	n=26

3.2. Construction of the LncRNA-mRNA Co-Expression Network

First, by analyzing the RNA-seq data of LUAD patients, 2051 lncRNAs were identified, of which 949 lncRNAs were differentially expressed between normal tissues and tumor tissues. In order to identify the ferroptosis-related lncRNAs, we downloaded 382 ferroptosis-related genes from the ferroptosis database. It was found that the expression of 209 ferroptosis-related lncRNAs ($p< 0.001$) was correlated with the expression of ferroptosis-related genes. Finally, we found 13 best predictors related to the prognosis of LUAD. In order to explore the potential role of 13

ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

ferroptosis-related lncRNAs in LUAD, a co-expression network of lncRNA-mRNA was constructed using Cytoscape, and LncRNA and mRNA in the network are represented by diamonds and rounded rectangles, respectively, see Figure 2.

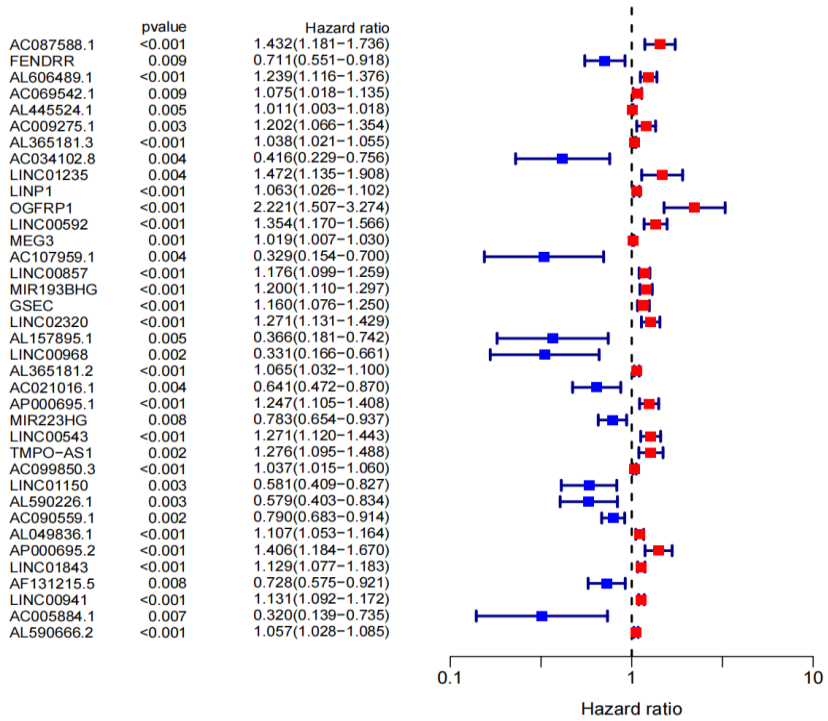

Figure 1. Indicates the HR (95% Cl) and p-value of selected lncRNAs by univariate Cox proportional hazards. Blue dots represent protective factors, and red dots represent risk factors.

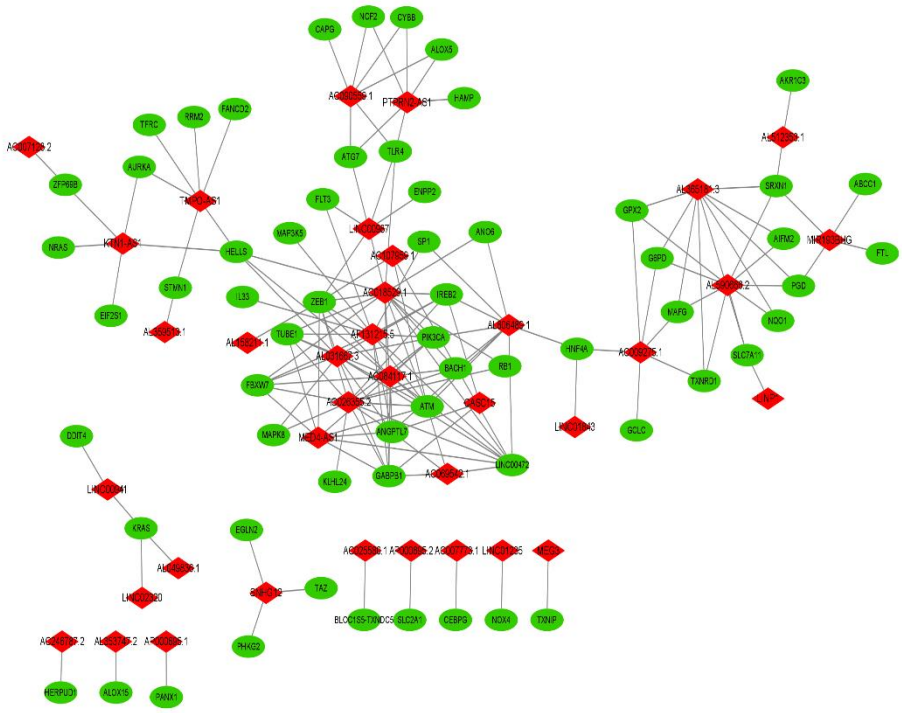

Figure 2. The LncRNA-mRNA co-expression network of lung adenocarcinoma.

ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

3.3. Functional Enrichment Analyses

GO functional enrichment analyses, and KEGG pathway enrichment analyses were performed on the lncRNAs between the high- and low-risk groups. From the bar plot and bubble plot, the KEGG analysis results show that the 13 prognostic lncRNAs are mainly enriched in Ferroptosis pathway, HIF−1 signaling pathways, and Cysteine and methionine metabolism pathways, see Figure 3. In addition, some pathways and functions closely related to ferroptosis metabolism have also been identified, which provides a reference for further research.

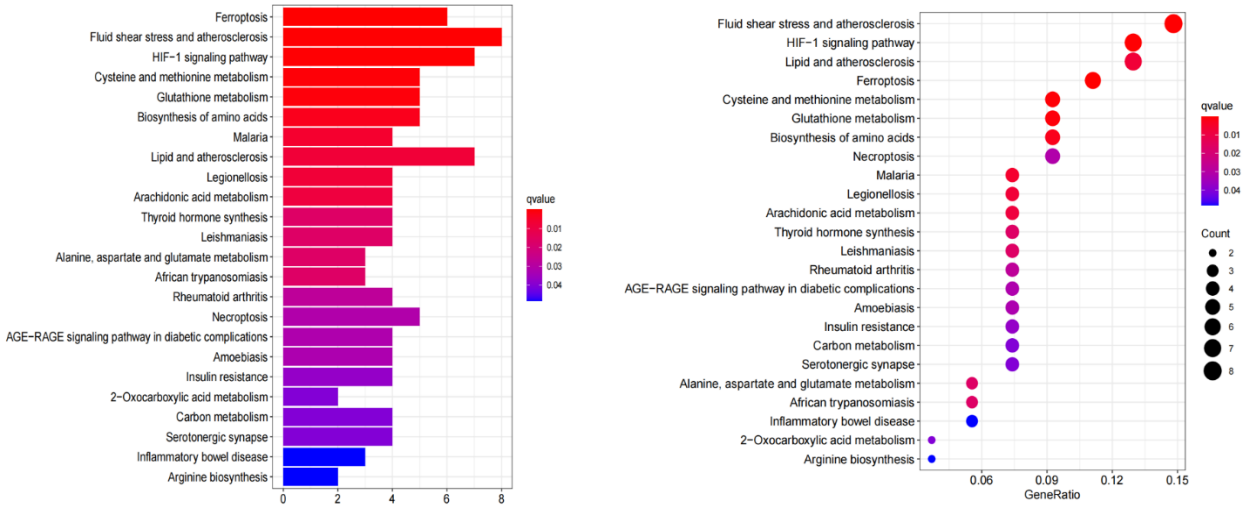

Figure 3. The bar plot and bubble plot of significant KEGG pathways based on the TCGA database.

3.4. Independent Prognostic Analysis

The univariate and multivariate Cox regression analyses were performed to evaluate whether clinical parameters and the risk score are independent prognostic factors of overall survival (OS). It can be seen from the figure 4(a,b) that stage and risk score have been proved to be independent factors of LUAD in the TCGA cohort (HR>1, p<0.05).

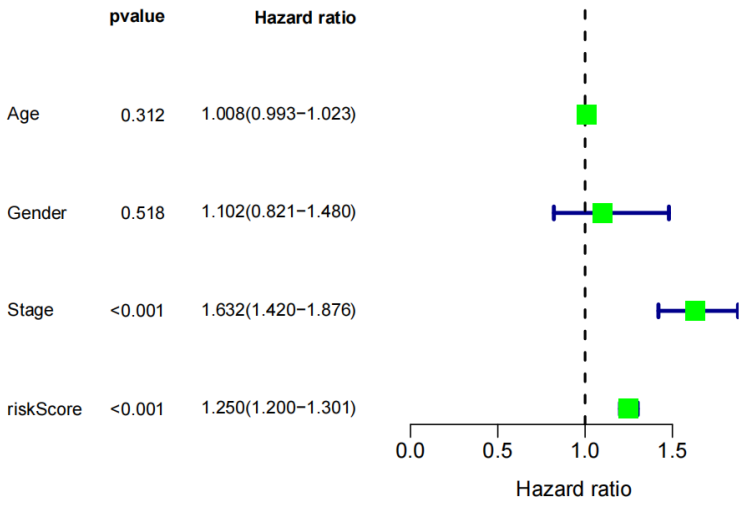

Figure 4a. Univariate COX regression analysis of the prognosis of LUAD patients based on the TCGA database.

ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

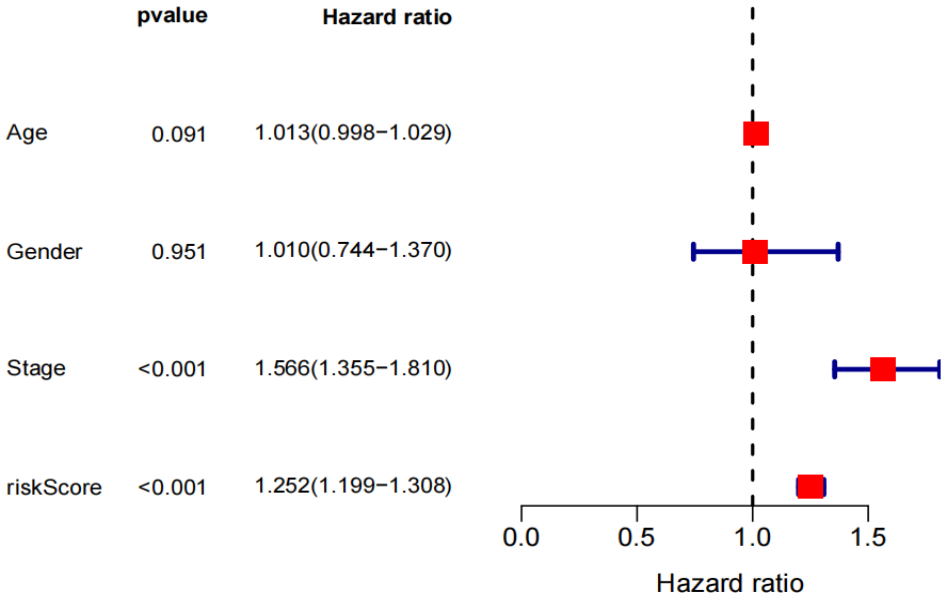

Figure 4b. Multivariate COX regression analysis of the prognosis of LUAD patients based on the TCGA database.

3.5. Validation of the Risk Score

LUAD patients in TCGA are divided into high-risk groups and low-risk groups according to the median risk score. It showed that there was a significant difference in overall survival between the two groups ($p<0.01$). Patients in the high-risk group are more likely to die earlier than those in the low-risk group, see Figure 5a.

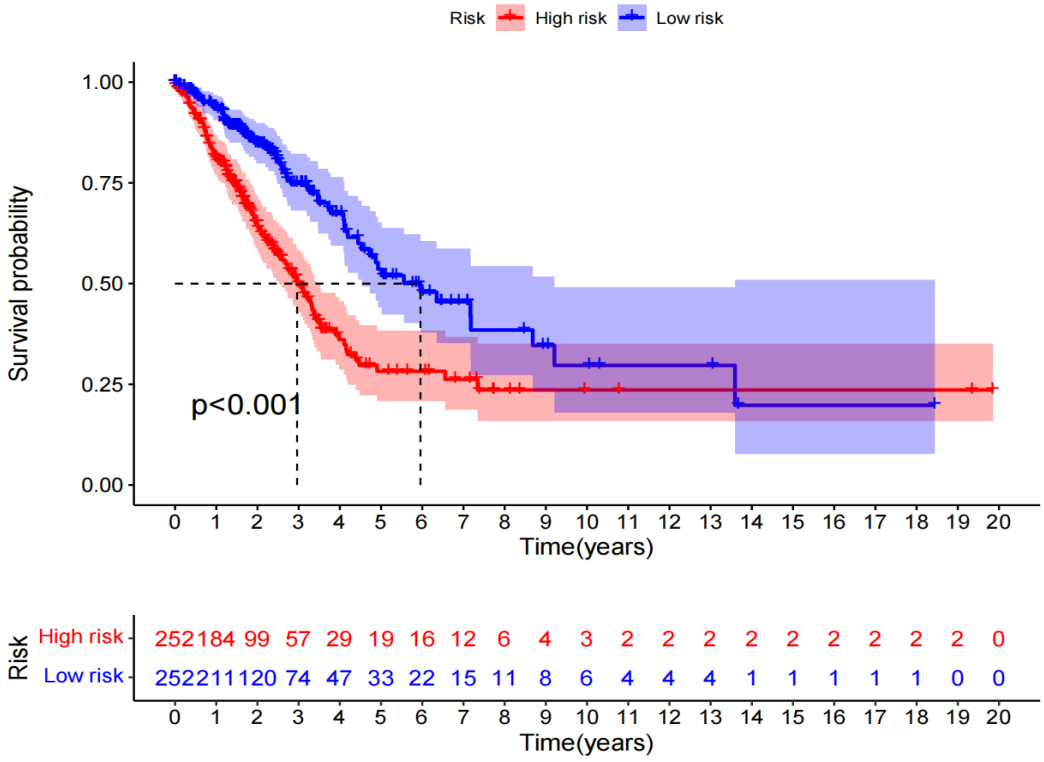

Figure 5a. Survival plot of comparison the prognosis of lung adenocarcinoma patients between the low-risk group and the high-risk group based on the TCGA database.

ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

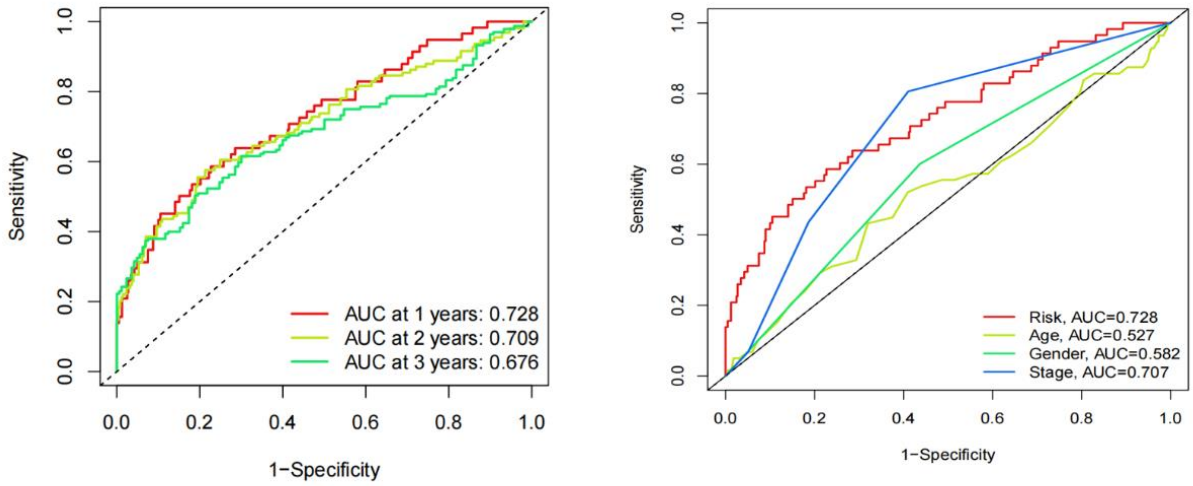

Figure 5b. Prognostic analysis of the risk score in the TCGA database.

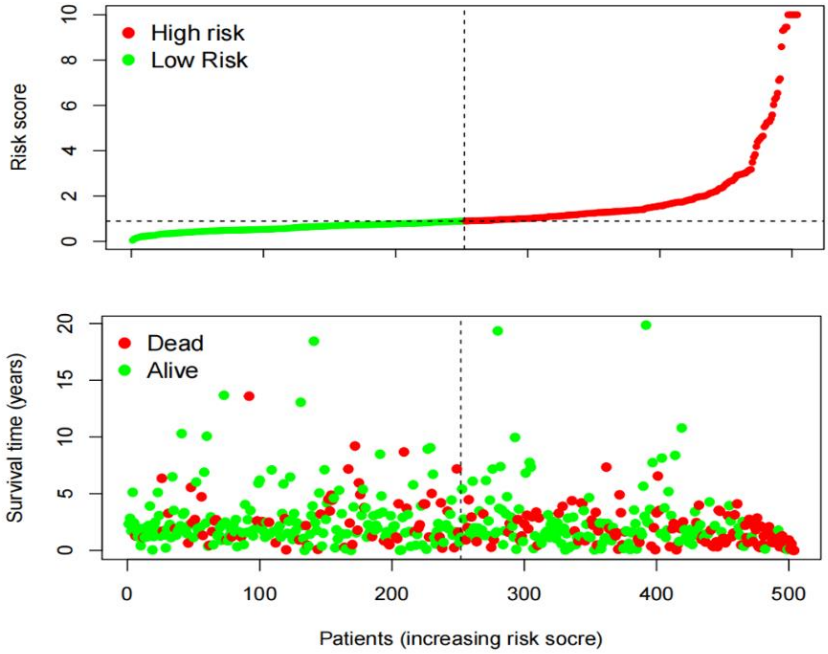

Figure 5c. Survival difference between high-risk and low-risk groups.

As shown in Figure 5b, the areas under the time-dependent ROC of the TCGA cohort are 0.728, 0.709, and 0.676 for 1-, 2-, and 3-years survival, and the prognostic model with other comparing the clinical characteristics, it can be found that the areas under the time-dependent ROC of the TCGA cohort are 0.728, 0.527, 0.582 and 0.707 for Risk, Age, Gender and Stage, respectively.

As shown in Figure 5c, the abscissa is the sorted LUAD patients, and the risk of patients increases from left to right. The ordinate of the figure above is the patient's risk score, and the ordinate is ascending from left to right. According to the median value of the patient's risk value, patients can be divided into high and low risk groups. Green represents the low-risk group, and the high-risk group is represented by red. The ordinate in the figure below represents survival time, and the green dots represent surviving patients. The red dots represent the patients who died. It can be observed from the figure 5c that as the risk of the patient increases, the number

ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

of patients who die also increases. The patients in the high-risk group had a higher probability of death earlier than patients in the low-risk group.

4. Discussion

Lung adenocarcinoma is a multifactorial disease with high heterogeneity and mixed genetic factors [2]. For patients with different advanced lung adenocarcinomas, a variety of combination therapies are gradually being explored, and clear and specific molecular markers are used to predict the response of immunotherapy or the prognosis of patients is extremely urgent [17], next-generation sequencing is important for LUAD prognosis prediction [18].

LncRNA is involved in regulation of ferroptosis. Increasing evidence has revealed that LncRNA plays a key role in the ferroptosis of cancer cells [24], cytoplasmic lncRNA P53RRA is downregulated and interacts with Ras-GTPase activating protein binding protein 1 (G3BP1) to transfer p53 from the G3BP1 complex, resulting in p53 retention in the nucleus, leading to cell cycle arrest, ferroptosis, and apoptosis [19,23,27]. Previous study has showed that MAFG-AS1 was upregulated in LUAD samples and related with patients' prognosis. In addition, downregulating MAFG-AS1 could repress cell proliferation and promote cell apoptosis, which supplemented the impact of MAFG-AS1 on the proliferation in lung cancer [20], MIR6852 inhibited cell growth by promoting ferroptosis, and DANCR might be an oncogenic lncRNA that regulates mTOR expression through directly binding to miR-496 [21,22]. These results indicate that ferroptosis is closely related to anti-tumor immunity, which is consistent with our hypothesis.

This study comprehensively studied the relationship between lncRNAs and ferroptosis-related genes in lung adenocarcinoma, and screened lncRNAs related to ferroptosis. According to the risk score, the genes identified between the high-risk group and the low-risk group were subgrouped to analyze their survival rates, biological functions and main enrichment pathways. In the constructed prediction model, the best predictors associated with LUAD prognosis, including AL606489.1, AC069542.1, AL365181.3, AC034102.8, LINC01235, MEG3, MIR193BHG, LINC02320, LINC00543, AC090559.1, AL049836.1, AF131215.5, LINC00941, which are closely related to the ferroptosis process. It was verified by Kaplan-Meier and Cox regression analysis, revealing its independent prognostic value in LUAD, and ROC analysis was performed, which confirmed that this feature has a strong predictive power for the prognosis of LUAD [25,26]. Demonstrated the ability of stratification to divide patients into high-risk groups and low-risk groups, with different survival outcomes, obviously, the low-risk group has a significantly higher survival rate, the KEGG also analysis results show that the 13 prognostic lncRNAs are mainly enriched in Ferroptosis pathway, HIF−1 signaling pathways, and Cysteine and methionine metabolism pathways. In addition, stage was identified as an independent prognostic factor and was significantly related to the overall survival (OS) of LUAD. Therefore, we can conclude that these can be clarified a novel ferroptosis-related lncRNA signature which could precisely predict the prognosis of LUAD patients, our findings provide a theoretical reference for the underlying mechanism of ferroptosis in lung cancer patients.

However, our research has some limitations, our research is based on the TCGA public database. This prognostic model of ferroptosis-related lncRNAs needs to be further validated with prospective, multi-center, real-world data.

5. Conclusion

This study identified a novel ferroptosis-related lncRNA signature which could precisely predict the prognosis of LUAD patients. Ferroptosis-related lncRNAs may have a potential role in the process of anti-tumor immunity and serve as therapeutic targets for lung cancer.

ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

Acknowledgments

The authors gratefully acknowledge contributions from the TCGA network.

References

[1] Siegel RL, Miller KD, Fuchs HE, Jemal A: Cancer Statistics, 2021. CA Cancer J Clin, Vol. 71 (2021) No.1, p.7–33.

[2] Keith M Kerr: Pulmonary adenocarcinomas: classification and reporting. Histopathology, Vol. 54 (2009) No.1, p.12-27.

[3] Fred R Hirsch, Giorgio V Scagliotti and Luis Paz-Ares: Lung cancer: current therapies and new targeted treatments. Lancet, Vol. 389 (2017) No.10066, p.299-311.

[4] Keiju Aokage, Junji Yoshida and Tomoyuki Hishida: Limited resection for early-stage non-small cell lung cancer as function-preserving radica surgery: a review. Japanese Journal of Clinical Oncology, Vol. 47 (2017) No.1, p.7-11.

[5] Scott J. Dixon, Kathryn M. Lemberg: Ferroptosis: An Iron-Dependent Form of Nonapoptotic Cell Death. Cell, Vol. 149 (2012) No.5, p.1060-1072.

[6] Vasanthi S. Viswanathan, Matthew J. Ryan: Dependency of a therapy-resistant state of cancer cells on a lipid peroxidase pathway. Nature, Vol. 547 (2017) No.7664, p.453-457.

[7] Guodong Yang a, Xiaozhao Lu: LncRNA: A link between RNA and cancer. Biochimica et Biophysica Acta, Vol. 1839 (2014) No.11, p.1097-1109.

[8] Xiaojun Xia, Xiaoping Fan: The Relationship between Ferroptosis and Tumors: A Novel Landscape for Therapeutic Approach. Current Gene Therapy, Vol. 19 (2019) No.2, p.117-124.

[9] Behrouz Hassannia, Peter Vandenabeele, and Tom Vanden Berghe: Targeting Ferroptosis to Iron Out Cancer. Cancer Cell, Vol. 35 (2019) No.6, p.830-849.

[10] Sonali Jathar, Vikram Kumar: Technological Developments in lncRNA Biology. Long Non Coding RNA Biology, Vol. 1008 (2017), p.283-323.

[11] Weijia Xie, Shuai Yuan: Long noncoding and circular RNAs in lung cancer: advances and perspectives. Epigenomics, Vol. 8 (2016) No.9, p.1275-1287.

[12] Chao Mao, Xiang Wang, Yating Liu: A G3BP1-Interacting lncRNA Promotes Ferroptosis and Apoptosis in Cancer via Nuclear Sequestration of p53. Cancer Research, Vol. 78 (2018) No.13, p.3483-3496.

[13] Information on: https://portal.gdc.cancer.gov/

[14] Information on: https://www.zhounan.org/ferrdb/

[15] Information on: http://www.cytoscape.org/

[16] Kaiming Zhang, Liqin Ping: A Ferroptosis-Related lncRNAs Signature Predicts Prognosis and Immune Microenvironment for Breast Cancer. Frontiers In Molecular Biosciences, Vol. 8 (2021), p.1-19.

[17] Aimin Jiang, Na Liu: Identification and validation of an autophagy-related long non-coding RNA signature as a prognostic biomarker for patients with lung adenocarcinoma. Journal of Thoracic Disease, Vol 13 (2021) No.2, p.720-734.

[18] Kosvyra A, Maramis C, Chouvarda I: Developing an integrated genomic profle for cancer patients with the use of NGS data. ESJ. Vol. 3 (2019) No.3, p.157–167.

[19] Yuan Sui, Guangyao Lin: LncRNA MAFG-AS1 boosts the proliferation of lung adenocarcinoma cells via regulating miR-744-5p/MAFG axis. European Journal of Pharmacology, Vol. 859 (2019), p.1-8.

[20] Qing-chun Lu a, Zhuang-hua Rui: LncRNA-DANCR contributes to lung adenocarcinoma progression by sponging miR-496 to modulate mTOR expression. J. Cell. Mol. Med, Vol 22 (2018) No 3, p.1527-1537.

ISSN: 2710-0588 DOI: 10.29556/FSE.202108_1(5).0009

[21] Min Wang, Chao Mao: Long noncoding RNA LINC00336 inhibits ferroptosis in lung cancer by functioning as a competing endogenous RNA. Cell Death & Differentiation, Vol. 26 (2019) No.11, p.2329-2343.

[22] Bumin Xie, Yuan Guo: Molecular mechanism of cell ferroptosis and research progress in regulation of ferroptosis by noncoding RNAs in tumor cells. Cell Death Discovery, Vol. 7 (2021) No.1, p.1-10.

[23] Anran Zhang, Jinpo Yang: Development and Validation of a Robust Ferroptosis-Related Prognostic Signature in Lung Adenocarcinoma. Frontiers In Cell and Developmental Biology, Vol. 9 (2021), p.1-21.

[24] Jie Yao, Xiao Chen: Characterization of a ferroptosis and iron-metabolism related lncRNA signature in lung adenocarcinoma. Cancer Cell International, Vol. 21 (2021) No.1, p.340.

[25] Jili Hou, Cheng Yao: Potential Prognostic Biomarkers of Lung Adenocarcinoma Based on Bioinformatic Analysis. BioMed Research International, Vol. 2021 (2021), p.1-14.

[26] Pancheng Wu, Yi Zheng: Development and validation of a robust immune-related prognostic signature in early-stage lung adenocarcinoma. Journal of Translational Medicine, Vol. 18 (2020) No.1, p.380.

[27] Le Lia, Yong-Xian Chen: The crosstalk between RNA m6A epitranscriptome and TGFβ signaling pathway contributes to the arrest of cell cycle. Gene, Vol, 738 (2020) No.1, p.1-7.