Ferroptosis-Related lncRNAs Are Prognostic Biomarker of Overall Survival in Pancreatic Cancer Patients

Dongjie Chen

Central South University Third Xiangya Hospital

Wenzhe Gao

Central South University Third Xiangya Hospital

Longjun Zang

Central South University Third Xiangya Hospital

Xianlin Zhang

Three Gorges University Renhe Hospital

Zheng Li

Three Gorges University Renhe Hospital

Hongwei Zhu (✉ zhw_0509@csu.edu.cn)

Central South University Third Xiangya Hospital <https://orcid.org/0000-0001-8620-3842>

Xiao Yu

Central South University Third Xiangya Hospital

Primary research

Keywords: pancreatic adenocarcinoma, ferroptosis, long non-coding RNA, prognostic biomarker, ceRNA network

Posted Date: May 5th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-452472/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at Frontiers in Cell and Developmental Biology on February 10th, 2022. See the published version at <https://doi.org/10.3389/fcell.2022.819724>.

Abstract

Background: Pancreatic cancer (PC) is one of the most lethal malignancies, the mortality and morbidity of which have been increasing over the past decade. Ferroptosis, a newly identified iron-dependent pattern of cell death, can be induced by iron chelators and small lipophilic antioxidants. Nonetheless, the prognostic significance of ferroptosis-related lncRNAs in PC remains to be explicated.

Methods: We obtained the lncRNA expression matrix and clinicopathological information of PC patients from The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) datasets in the current study. Pearson correlation analysis was conducted to delve into the ferroptosis-related lncRNAs, and univariate Cox analysis was implemented to examine the prognostic values in PC patients. The least absolute shrinkage and selection operator Cox (LASSO-Cox) analysis was performed to establish a ferroptosis-related lncRNA prognostic marker (Fe-LPM). Furthermore, we adopted a multivariate Cox regression to establish a nomogram. Gene set enrichment analysis (GSEA) and a competing endogenous RNA (ceRNA) network were also created.

Results: The eight Fe-LPM (including SLC16A1-AS1, SETBP1-DT, ZNF93-AS1, SLC25A5-AS1, AC073896.2, LINC00242, PXN-AS1 and AC036176) was confirmed, and displayed a sturdy prognostic capability in the training and validation dataset. We established a nomogram based on risk score, age, pathological T stage and primary therapy outcome. GSEA revealed that several ferroptosis-related pathways are enriched in low-risk subgroups. A ceRNA network (including 4 lncRNAs, 27 miRNAs and 57 mRNAs) was also constructed to provide us some clues for finding the potential functions of these ferroptosis-related lncRNAs in PC.

Conclusion: The eight Fe-LPM can be utilized for anticipating the overall survival (OS) of PC patients, which are meaningful to guiding clinical strategies of PC.

Background

Pancreatic cancer (PC) is one kind of the most devastating carcinoma all over the world [1]. Although the therapeutic strategies of PC have been developed, widespread metastases and drug resistance remain to be challenges for clinicians. What's more, the overlapping symptoms and long latency period hampered the early diagnosis of PC. These factors result in PC having a typically poor prognosis [2]. Hence, it is imperative to hunt for novel clinical regimens for patients with PC [3, 4].

Ferroptosis, a unique type of cell death distinct from other regulated cell death (RCD), plays a pivotal role in iron metabolism and lipid peroxidation [5, 6]. Since its first found in 2012, numerous studies have revealed that ferroptosis can regulate pathogenesis of various human malignancies [7]. For instance, GPX4 overexpression or ACSL4 depletion in glioblastoma cells could promote tumor necrosis via neutrophil-induced ferroptosis [8], and CAFs secrete exosomal miR-522 to inhibit ferroptosis in gastric cancer cells by targeting ALOX15 and blocking lipid-ROS accumulation [9]. Lately, a number of

researchers have elucidated that the dysfunction of ferroptosis regulators is tightly associated with the progression of PC [10].

Long noncoding RNA (lncRNA) is a class of non-coding RNA whose length exceeds 200 nucleotides. With the deep recognition of lncRNAs, it is also believed that the aberrant expression and mutation of lncRNAs serve a crucial part in the malignant proliferation of PC [11, 12]. For example, PLACT1 is previously reported to be an E2F1-mediated lncRNA promoting pancreatic ductal adenocarcinoma (PDAC) tumorigenicity and metastatic potential [13]; THAP9-AS1 is considered as a potential biomarker to play an important role in PDAC growth via enhancing YAP signaling [14], and overexpression of GSTM3TV2 could strengthen the chemoresistance in PC [15]. However, few studies have focused on the mechanisms of how ferroptosis regulates the development and advance in lncRNA-mediated PC. On that account, fathoming out the relationship between ferroptosis of lncRNA and PC development is urgently needed so as to detect novel prognostic signatures that can behave as effective targets for treatment.

In our study, based on the TCGA and ICGC cohorts of PDAC patients, 23 prognostic ferroptosis-related lncRNAs were identified as prognostic ferroptosis-related lncRNAs. Then, we constructed a ferroptosis-related lncRNA prognostic marker (Fe-LPM) according to the predictive capacity of eight ferroptosis-related lncRNAs. In this way, PC patients were categorized as high-risk and low-risk subgroups and we found many signal-transduction pathways converged on the low-risk subgroup according to GSEA analysis. Moreover, a competing risk nomogram model was established to predict the long-term survival of patients with PC. Last but not least, we constructed a ceRNA network, including 4 lncRNAs, 27 miRNAs and 57 mRNAs. The flowchart of the study was shown in Fig. 1.

Methods

Data processing

RNA-expression matrix and clinicopathological data of patients were extracted from the TCGA_PAAD (source: <https://portal.gdc.cancer.gov/>; $n = 182$) as a training set, and the data in ICGC_AU_PAAD (source: <https://daco.icgc.org/>; $n = 91$) were obtained from the ICGC website as a validation set. Normalized RNA-Seq counts were adopted, and patients with no prognostic information were excluded from our analysis. Additionally, based on previous literature, an expression matrix of 38 ferroptosis-related was retrieved from the TCGA and ICGC databases.

Generation and assessment of the Fe-LPM

Aimed at identifying the ferroptosis-related prognostic lncRNAs, Pearson correlation analysis was firstly implemented ($| \text{cor} | > 0.5$, $p < 0.01$), and we performed univariate Cox analysis among TCGA and ICGC datasets separately. The ferroptosis-related lncRNAs extracted from two datasets were converged to seize the 23 prognostic ferroptosis-related lncRNAs. Afterward, The R package “glmnet” was used to conduct the LASSO-Cox analysis, we established a ferroptosis-related lncRNA prognostic marker (Fe-LPM) for the

PC patient implicating 8 ferroptosis-related lncRNAs. The formula of the risk score was constructed as follows:

$$Risk\ score = \sum_{i=1}^n Coef_i * x_i$$

where $Coef_i$ represents the coefficients, x_i represents the normalized count of each ferroptosis-related lncRNAs. We performed the Principal component analysis (PCA) to check the identifiable capability of the Fe-LPM with the “gmodels” R package. Owing to analyzing the survival conditions of Fe-LPM, the optimal cut-off value was calculated using the R package “survminer”. The R package “timeROC” was applied to perform time-dependent receiver operating characteristic (ROC) curve to estimate the forecasting capacity of the Fe-LPM. We conducted the univariate and multivariate Cox analyses to identify the independence of the Fe-LPM in predicting OS of PC patients. Based on the result of multivariate Cox regression, we developed a nomogram, and the calibration plot validated the robustness of the nomogram.

Establishment of a ceRNA network

For understanding the co-expression network of lncRNAs, miRNAs and mRNAs, 4 of 23 ferroptosis-related lncRNAs were picked to predict 27 target miRNAs employing miRcode database. Then, we explored 57 target mRNAs of 27 miRNAs by the MiRDB, miRTarBase, StarBase and TargetScan databases. The ceRNA network was visualized with “Cytoscape” software (version 3.8.2).

Pathway enrichment analysis and GSEA analysis

Based on the TCGA_PAAD training set, 1213 differentially expressed mRNAs (DEmRNAs) between high-risk and low-risk groups were selected according to the criteria of $|\log_2(\text{Fold Change})| > 1$ and $p < 0.05$ by means of the “DESeq2” R package. The 1213 DEmRNAs and the 57 target mRNAs in the ceRNA network were individually imported into the “Metascape” website for pathway enrichment analysis. Gene Set Enrichment Analysis (GSEA) was also performed to interrogate the potential pathways enriched in the high-risk and low-risk subgroups, using GSEA software (version 4.1.0). Gene expression data from the TCGA_PAAD cohort were loaded into GSEA, the *c2.cp.kegg.v7.2.symbols.gmt* and *h.all.v7.2.symbols.gmt* were elected as the gene set database. The benchmark for which to be significantly enriched include nominal p -value < 0.05 , normalized enrichment score (NES) > 1 , and false discovery rate (FDR) q -value < 0.25 .

Statistical analysis

The student’s t -test was used to compare the distribution of risk scores. Kaplan-Meier (KM) analysis with a log-rank test was used to compare the OS between different subgroups. All statistical analyses were performed using the R programming language (Version 4.0.3). Unless specifically stated, a p -value of less than 0.05 was deemed to be statistically significant.

Results

Identification of ferroptosis-related lncRNAs

Through "GENCODE" database, we pinpointed 15071 and 11861 lncRNAs in the TCGA and ICGC datasets separately. Then, we obtained the expression matrix of 38 ferroptosis-related genes from each dataset. Pearson correlation analysis was first carried out to explore ferroptosis-related lncRNAs, thenceforth the univariate Cox regression was constructed to seek ferroptosis-related prognostic lncRNA ($p < 0.05$) in each dataset. Finally, we identified 23 ferroptosis-related lncRNAs which dramatically correlated with the survival among both datasets. The association between 23 ferroptosis-related lncRNAs and 38 ferroptosis-related genes was displayed in Fig. 2, the univariate Cox analysis of 23 ferroptosis-related lncRNAs was shown in Table 1.

Table 1
The 23 ferroptosis-related prognostic lncRNAs

lncRNA	TCGA				ICGC			
	HR	HR.95L	HR.95H	<i>p</i> -value	HR	HR.95L	HR.95H	<i>p</i> -value
OSMR-AS1	1.4915	1.0714	2.0763	0.0179	1.2615	1.0116	1.5732	0.0392
SLC16A1-AS1	1.4379	1.0252	2.0166	0.0354	1.2242	1.0028	1.4945	0.0469
SETBP1-DT	0.8096	0.6639	0.9872	0.0369	0.7414	0.6194	0.8874	0.0011
AC097639.1	0.8032	0.6709	0.9617	0.0170	0.7965	0.6595	0.9621	0.0182
TRAM2-AS1	0.7956	0.6417	0.9863	0.0370	0.7370	0.5433	0.9998	0.0498
FOXN3-AS1	0.7658	0.6139	0.9552	0.0179	0.7542	0.5733	0.9921	0.0437
ZNF793-AS1	0.7639	0.6051	0.9645	0.0236	0.7920	0.6888	0.9107	0.0011
LINC02777	0.7626	0.5860	0.9923	0.0437	0.8125	0.6668	0.9900	0.0395
LINC00526	0.7624	0.5873	0.9898	0.0417	0.7570	0.6133	0.9345	0.0096
SLC25A5-AS1	0.7556	0.5987	0.9535	0.0182	0.6088	0.4681	0.7917	0.0002
ZNF582-AS1	0.7524	0.5734	0.9871	0.0400	0.8326	0.7038	0.9850	0.0326
AC073896.2	0.7519	0.5795	0.9755	0.0318	0.5455	0.3846	0.7736	0.0007
AC009506.1	0.7479	0.5960	0.9386	0.0122	0.6740	0.4885	0.9298	0.0163
SGMS1-AS1	0.7370	0.5463	0.9942	0.0457	0.7119	0.5489	0.9233	0.0104
AC138356.1	0.7351	0.5439	0.9936	0.0453	0.7396	0.5989	0.9135	0.0051
AC008966.1	0.7348	0.5746	0.9397	0.0140	0.7491	0.5969	0.9402	0.0127
LINC00242	0.7280	0.5712	0.9279	0.0103	0.6744	0.5436	0.8366	0.0003
ST7-AS1	0.7146	0.5571	0.9167	0.0082	0.7633	0.6110	0.9534	0.0173
WDFY3-AS2	0.7067	0.5573	0.8961	0.0042	0.7835	0.6359	0.9655	0.0221
ZNF710-AS1	0.6972	0.5354	0.9079	0.0074	0.7130	0.5704	0.8912	0.0030
PXN-AS1	0.6910	0.5026	0.9501	0.0229	0.6138	0.4550	0.8279	0.0014
AC036176.1	0.6817	0.5213	0.8915	0.0051	0.6414	0.5054	0.8140	0.0003
AC016876.1	0.6378	0.4634	0.8778	0.0058	0.6581	0.4901	0.8838	0.0054
lncRNAs marked with bold font were risky lncRNAs and others were protective lncRNAs.								

Construction Of The Fe-lpm In Pc

The LASSO-Cox analysis was performed using the expression matrix of the 23 ferroptosis-related prognostic lncRNA mentioned above. The Fe-LPM encompassed 8 ferroptosis-related lncRNAs in the TCGA dataset was identified (Fig. 3A, B), the coefficient of the Fe-LPM was shown in Fig. 3C. A risk score was computed for all patients in TCGA dataset, and patients were then split for survival analysis into high-risk and low-risk subgroups on the threshold of median risk scores. The principal component analysis (PCA) indicated that we do obtain a high degree of discrimination between high-risk and low-risk subgroups in the TCGA cohort (Additional file 1: Figure S1A, B). KM survival curves considered that patients from the high-risk subgroup had considerably worse clinical outcomes than those from the low-risk subgroup (Fig. 3D). The distribution of the risk score was shown in Fig. 3E. The forecasting capability of the Fe-LPM for overall survival was appraised by ROC curves, and the area under the curve (AUC) reached 0.70 at 1 year, 0.71 at 2 years, 0.75 at 3 years (Fig. 3F).

Validation Of The Fe-lpm In Pc

To verify the stability of the prognostic markers constructed in the TCGA dataset, patients in the ICGC validation set were also assigned into high-risk and low-risk subgroups relative to the median risk scores calculated with the same formula as that from the training set. PCA in ICGC datasets was shown in Additional file 1: Figure S1C, D. Similar to the conclusion in the TCGA cohort, patients with high-risk scores had shorter OS than the low-risk ones in the ICGC dataset (Fig. 3G). The distribution of the risk score was shown in Fig. 3H. The AUC of the Fe-LPM in the ICGC cohort was 0.79 at 1 year, 0.71 at 2 years, 0.76 at 3 years (Fig. 3I).

Prognostic Analysis Of The Eight Fe-lpm

We implemented the univariate Cox analysis to estimate the prognostic role of the eight Fe-LPM. The result indicates that SLC16A1-AS1 is a risk factor with $HR > 1$, whereas SETBP1-DT, ZNF93-AS1, SLC25A5-AS1, AC073896.2, LINC00242, PXN-AS1 and AC036176.1 are protective factors (Fig. 4A). The heatmap exhibits that SLC16A1-AS1 expression increased with increasing risk score, while SETBP1-DT, ZNF93-AS1, SLC25A5-AS1, AC073896.2, LINC00242, PXN-AS1 and AC036176.1 expression decreased with increasing risk score (Fig. 4B). Also, the Kaplan-Meier curve certified that lower expression of SLC16A1-AS1 and higher expression of SETBP1-DT, ZNF93-AS1, SLC25A5-AS1, AC073896.2, LINC00242, PXN-AS1 and AC036176.1 corresponded with higher survival time in the TCGA cohort (Fig. 4C-J).

Clinical Relevance Of The Fe-lpm

In order to clarify the interdependency of risk scores and clinicopathological characteristics, we further scrutinize the clinicopathological information from the TCGA cohort. The result manifested that the risk score apparently corresponded with pathological T stage, TNM stage, grade and primary therapy

outcome of PC patients (Fig. 5A-D), while the tumor site, pathological N stage, age and tumor size proved to be nonsensical (Fig. 5E-H).

Pathway enrichment analysis and GSEA analysis in the TCGA dataset

For exploring the underlying pathway among the high-risk and low-risk subgroups, 1213 differentially expressed mRNAs (DEmRNAs) between high-risk and low-risk groups were selected according to the criteria of $|\log_2(\text{Fold change})| > 1$ and $p < 0.05$. These DEmRNAs were significantly enriched in chemical synaptic transmission, regulation of hormone levels, presynapse and regulation of ion transport (Additional file 1: Figure S2). The results of GSEA revealed that the genes in the low-risk subgroup were significantly enriched in fatty acid metabolism, peroxisome, oxidative phosphorylation, PI3K-AKT-mTOR signaling and lysosome (Fig. 6A-F).

The independence of the Fe-LPM in predicting OS in PC

To evaluate whether the Fe-LPM was an independent prognostic indicator for OS, univariate and multivariate Cox regression analyses were implemented. In the TCGA dataset, both univariate Cox analysis and multivariate Cox analysis showed that Fe-LPM was markedly correlated with survival status (Fig. 7A, B). To establish a predictive tool for quantitative analysis of OS in PC patients, we initiated a prognostic nomogram based on the pathological T stage, risk score, primary therapy outcome and age in the TCGA cohort (Fig. 7C). Calibration plots showed that the predictive concordance of the nomogram (Fig. 7D).

Construction Of The Cerna Regulatory Network

The ceRNA network comprising lncRNAs, miRNAs and mRNAs was constructed for the sake of systematically probing into the potential regulatory mechanism. According to the aforementioned 23 ferroptosis-related lncRNAs, 4 of 23 lncRNAs were filtered from the miRcode database, and 27 target miRNAs were distinguished. Then we use the MiRDB, miRTarBase, StarBase and TargetScan databases to extracted 57 target mRNAs on account of 27 miRNAs. At length, 4 lncRNAs-27miRNAs-57mRNAs ceRNA network was completed (Fig. 8A, Additional file 1: Figure S3). The pathway enrichment analysis of 57 target mRNAs elucidated that these mRNAs were enriched in lipid transport, anion transport and dephosphorylation (Fig. 8B-D).

Discussion

Ferroptosis, an extraordinary cell death pathway driven by iron-dependent lipid peroxidation, regulates the epigenetic program and metabolic changes in numerous cancers [16]. Multiple studies have already shown that specific lncRNAs can alleviate the malignancy of tumors by regulating the ferroptosis-related pathway. Interacted with G3BP1, the lncRNA P53RRA could restrain lung cancer progression via activating the p53 signaling pathway which correlated with ferroptosis [17]. LncRNA GABPB1-AS1

inhibits the translation of GABPB1, causing the accumulation of iron-dependent reactive oxygen species (ROS) and improving the overall survival of HCC patients [18]. Additionally, most studies have confirmed that lncRNAs might function as ceRNA of mRNAs, thereby manipulating ferroptosis-mediated tumor progression. In lung cancer, LINC00336 functions as an oncogene and regulates cystathionine- β -synthase (CBS) expression by competing for miR6852 [19]. The lncRNA MT1DP could sensitize NSCLC cells to erastin-induced ferroptosis by regulating the miR-365a-3p/NRF2 axis, mitigating the mortality of NSCLC [20]. After pondering these clues, we hypothesize that ferroptosis is tightly linked to lncRNAs, and we ought to concentrate on the potential interaction between lncRNAs and ferroptosis to uncover underlying prognostic biomarkers.

In the aggregate, 257 PC patients were involved to detect the prognostic value of ferroptosis-related lncRNAs. 23 ferroptosis-related prognostic lncRNAs were identified, and 8 of them were singled out as the Fe-LPM. LNC00242 was high-expressed in gastric cancer (GC) tissue regulated by LINC00242/miR-1-3p/G6PD axis [21], it may be a promising biomarker of GC [22]. Mediated by E2F1, SLC16A1-AS1 could progress bladder cancer to invasive stage [23], and sever as a new diagnostic indicator in hepatocellular carcinoma [24, 25]. It has been reported that SLC25A5-AS1 functions as a suppressor in the progression of gastric cancer to regulate cellular behaviors via miR-19a-3p/PTEN/PI3K/AKT signaling pathway [26]. PXN-AS1 stifles PC progression by serving as a sponge to suppress the expression of miR-3064 [27] and it also plays vital roles in hepatocarcinogenesis as a prognostic biomarker [28]. Since half of the eight lncRNAs were reported to be associated with tumor progression, the others need to be further explored by experiments in our future studies.

According to the median risk score calculated by the coefficient of eight Fe-LPM, the PC patients were stratified into the high-risk and low-risk subgroups, To further investigate the biological process and pathway between the two subgroups, GSEA revealed that gene-sets fatty acid metabolism, peroxisome, oxidative phosphorylation, PI3K-AKT-mTOR signaling and lysosome were enormously enriched in the low-risk subgroup. These signaling pathways have been confirmed to be indispensable for ferroptosis [29], implying that the low-risk subgroup is more sensitive to ferroptosis. Polyunsaturated fatty acid (PUFA) biosynthesis pathway has been reported to be a marker for predicting the ability of ferroptosis-mediated therapy in gastric cancer [30], peroxisome and oxidative phosphorylation also participate in the biological process of ferroptosis [31, 32]. Mutation of PI3K-AKT-mTOR signaling protects cancer cells from ferroptosis death through SREBP1/SCD1-mediated lipogenesis [33]. Besides, various studies have reported that ferroptosis can mitigate gemcitabine resistance and lead to a favorable prognosis in pancreatic cancer cells [34, 35], which is consistent with the result of our study: most of the Fe-LPM are protective factors based on the univariate Cox regression analysis.

Predicting clinical outcome endpoints are necessary for personalized and translational medicine [36]. Although numerous efforts have been exerted to research novel drugs that can suppress the PC, we can only expect to successfully translate genomic findings into clinical practice through the development of genome-sequencing technology [37]. Consequently, we made further efforts to explore the prognostic value of the Fe-LPM. The univariate and multivariate Cox analyses identified that Fe-LPM was an

independent indicator for OS, and a nomogram based on 4 clinicopathological features (pathological T stage, TNM stage, grade and primary therapy outcome of PC patients) was constructed and validated by calibration plot. Afterward, a ceRNA network including 4 lncRNAs-27miRNAs-57mRNAs was established. The above results confirmed that Fe-LPM may propose new prospects and intervention measures for the treatment of PC and provides a robust theoretical basis for future clinical judgments.

Conclusions

Given all the results offered above, we come to the conclusion that the eight Fe-LPM (including SLC16A1-AS1, SETBP1-DT, ZNF93-AS1, SLC25A5-AS1, AC073896.2, LINC00242, PXN-AS1 and AC036176) prove to be a prognostic biomarker of OS for PC patients. Function prediction further confirmed the importance of Fe-LPM in PC, offering us a comparatively systematic and comprehensive knowledge of the links among ferroptosis, lncRNAs and PC. However, it is observed that the shortage of clinical information in ICGC_AU_PAAD leads to a lack of profound validations in the ICGC cohort. On the other hand, our study should make further validations in vivo and vitro soon.

Abbreviations

AUC, area under the curve; CBS, cystathionine- β -synthase; ceRNA, competing endogenous RNA; DEmRNAs, differentially expressed mRNAs; Fe-LPM, ferroptosis-related lncRNA prognostic marker; FDR, false discovery rate; GSEA, gene set enrichment analysis; GC, gastric cancer; ICGC, International Cancer Genome Consortium; KM, Kaplan-Meier analysis; LASSO-Cox, least absolute shrinkage and selection operator Cox analysis; NES, normalized enrichment score; OS, overall survival; PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; PUFA, Polyunsaturated fatty acid; PCA, principal component analysis; RCD, regulated cell death; ROC, receiver operating characteristic; ROS, reactive oxygen species; TCGA, the Cancer Genome Atlas.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analysed during the current study are available in the TCGA_PAAD and ICGC_AU_PAAD repository, [<https://portal.gdc.cancer.gov/>, <https://daco.icgc.org/>]

Competing interests

The authors declare that they have no competing interests.

Funding

This research was funded by the National Natural Science Foundation of China (NO.81873589); National Natural Science Foundation For Young Scientists of China (NO.82000614); National Natural Science Foundation for Young Scientists of Hunan Province, China (2020JJ5876); Science and Technology Project of Changsha, Hunan, China (NO.kq2004146).

Authors' contributions

Conceptualization, Xiao Yu and Hongwei Zhu; Methodology, Dongjie Chen and Wenzhe Gao; Software, Dongjie Chen; Supervision, Wenzhe Gao, Longjun Zang and Hongwei Zhu; Validation, Wenzhe Gao, Xiao Yu and Hongwei Zhu; Writing – original draft, Dongjie Chen; Writing – review & editing, Wenzhe Gao, Longjun Zang, Xianlin Zhang and Zheng Li.

Acknowledgements

We would like to exert compelling appreciation for the TCGA and ICGC projects.

References

1. Vincent A, Herman J, Schulick R, Hruban RH, Goggins M. Pancreatic cancer. *Lancet*. 2011;378(9791):607–20.
2. Wolfgang CL, Herman JM, Laheru DA, Klein AP, Erdek MA, Fishman EK, et al. Recent progress in pancreatic cancer. *CA Cancer J Clin*. 2013;63(5):318–48.
3. Dreyer SB, Chang DK, Bailey P, Biankin AV. Pancreatic Cancer Genomes: Implications for Clinical Management and Therapeutic Development. *Clin Cancer Res*. 2017;23(7):1638–46.
4. Neoptolemos JP, Kleeff J, Michl P, Costello E, Greenhalf W, Palmer DH. Therapeutic developments in pancreatic cancer: current and future perspectives. *Nat Rev Gastroenterol Hepatol*. 2018;15(6):333–48.
5. Zheng J, Conrad M. The Metabolic Underpinnings of Ferroptosis. *Cell Metab*. 2020;32(6):920–37.
6. Tang D, Chen X, Kang R, Kroemer G. Ferroptosis: molecular mechanisms and health implications. *Cell Res*. 2021;31(2):107–25.
7. Stockwell BR, Jiang X, Gu W. Emerging Mechanisms and Disease Relevance of Ferroptosis. *Trends Cell Biol*. 2020;30(6):478–90.
8. Yee PP, Wei Y, Kim SY, Lu T, Chih SY, Lawson C, et al. Neutrophil-induced ferroptosis promotes tumor necrosis in glioblastoma progression. *Nat Commun*. 2020;11(1):5424.
9. Zhang H, Deng T, Liu R, Ning T, Yang H, Liu D, et al. CAF secreted miR-522 suppresses ferroptosis and promotes acquired chemo-resistance in gastric cancer. *Mol Cancer*. 2020;19(1):43.

10. Dai E, Han L, Liu J, Xie Y, Zeh HJ, Kang R, et al. Ferroptotic damage promotes pancreatic tumorigenesis through a TMEM173/STING-dependent DNA sensor pathway. *Nat Commun.* 2020;11(1):6339.
11. Sun Y, Ma L. New Insights into Long Non-Coding RNA MALAT1 in Cancer and Metastasis. *Cancers (Basel).* 2019;11(2).
12. Xiong G, Feng M, Yang G, Zheng S, Song X, Cao Z, et al. The underlying mechanisms of non-coding RNAs in the chemoresistance of pancreatic cancer. *Cancer Lett.* 2017;397:94–102.
13. Ren X, Chen C, Luo Y, Liu M, Li Y, Zheng S, et al. lncRNA-PLACT1 sustains activation of NF- κ B pathway through a positive feedback loop with I κ B α /E2F1 axis in pancreatic cancer. *Mol Cancer.* 2020;19(1):35.
14. Li N, Yang G, Luo L, Ling L, Wang X, Shi L, et al. lncRNA THAP9-AS1 Promotes Pancreatic Ductal Adenocarcinoma Growth and Leads to a Poor Clinical Outcome via Sponging miR-484 and Interacting with YAP. *Clin Cancer Res.* 2020;26(7):1736–48.
15. Xiong G, Liu C, Yang G, Feng M, Xu J, Zhao F, et al. Long noncoding RNA GSTM3TV2 upregulates LAT2 and OLR1 by competitively sponging let-7 to promote gemcitabine resistance in pancreatic cancer. *J Hematol Oncol.* 2019;12(1):97.
16. Wu Y, Zhang S, Gong X, Tam S, Xiao D, Liu S, et al. The epigenetic regulators and metabolic changes in ferroptosis-associated cancer progression. *Mol Cancer.* 2020;19(1):39.
17. Mao C, Wang X, Liu Y, Wang M, Yan B, Jiang Y, et al. A G3BP1-Interacting lncRNA Promotes Ferroptosis and Apoptosis in Cancer via Nuclear Sequestration of p53. *Cancer Res.* 2018;78(13):3484–96.
18. Qi W, Li Z, Xia L, Dai J, Zhang Q, Wu C, et al. lncRNA GABPB1-AS1 and GABPB1 regulate oxidative stress during erastin-induced ferroptosis in HepG2 hepatocellular carcinoma cells. *Sci Rep.* 2019;9(1):16185.
19. Wang M, Mao C, Ouyang L, Liu Y, Lai W, Liu N, et al. Long noncoding RNA LINC00336 inhibits ferroptosis in lung cancer by functioning as a competing endogenous RNA. *Cell Death Differ.* 2019;26(11):2329–43.
20. Gai C, Liu C, Wu X, Yu M, Zheng J, Zhang W, et al. MT1DP loaded by folate-modified liposomes sensitizes erastin-induced ferroptosis via regulating miR-365a-3p/NRF2 axis in non-small cell lung cancer cells. *Cell Death Dis.* 2020;11(9):751.
21. Deng P, Li K, Gu F, Zhang T, Zhao W, Sun M, et al. LINC00242/miR-1-3p/G6PD axis regulates Warburg effect and affects gastric cancer proliferation and apoptosis. *Mol Med.* 2021;27(1):9.
22. Zhong X, Yu X, Wen X, Chen L, Gu N. Activation of the LINC00242/miR-141/FOXC1 axis underpins the development of gastric cancer. *Cancer Cell Int.* 2020;20:272.
23. Logotheti S, Marquardt S, Gupta SK, Richter C, Edelhäuser BAH, Engelmann D, et al. lncRNA-SLC16A1-AS1 induces metabolic reprogramming during Bladder Cancer progression as target and co-activator of E2F1. *Theranostics.* 2020;10(21):9620–43.

24. Song M, Zhong A, Yang J, He J, Cheng S, Zeng J, et al. Large-scale analyses identify a cluster of novel long noncoding RNAs as potential competitive endogenous RNAs in progression of hepatocellular carcinoma. *Aging*. 2019;11(22):10422–53.
25. Pei S, Chen Z, Tan H, Fan L, Zhang B, Zhao C. SLC16A1-AS1 enhances radiosensitivity and represses cell proliferation and invasion by regulating the miR-301b-3p/CHD5 axis in hepatocellular carcinoma. *Environ Sci Pollut Res Int*. 2020;27(34):42778–90.
26. Li X, Yan X, Wang F, Yang Q, Luo X, Kong J, et al. Down-regulated lncRNA SLC25A5-AS1 facilitates cell growth and inhibits apoptosis via miR-19a-3p/PTEN/PI3K/AKT signalling pathway in gastric cancer. *J Cell Mol Med*. 2019;23(4):2920–32.
27. Yan J, Jia Y, Chen H, Chen W, Zhou X. Long non-coding RNA PXN-AS1 suppresses pancreatic cancer progression by acting as a competing endogenous RNA of miR-3064 to upregulate PIP4K2B expression. *J Exp Clin Cancer Res*. 2019;38(1):390.
28. Yuan JH, Liu XN, Wang TT, Pan W, Tao QF, Zhou WP, et al. The MBNL3 splicing factor promotes hepatocellular carcinoma by increasing PXN expression through the alternative splicing of lncRNA-PXN-AS1. *Nat Cell Biol*. 2017;19(7):820–32.
29. Stockwell BR, Friedmann Angeli JP, Bayir H, Bush AI, Conrad M, Dixon SJ, et al. Ferroptosis: A Regulated Cell Death Nexus Linking Metabolism, Redox Biology, and Disease. *Cell*. 2017;171(2):273–85.
30. Lee JY, Nam M, Son HY, Hyun K, Jang SY, Kim JW, et al. Polyunsaturated fatty acid biosynthesis pathway determines ferroptosis sensitivity in gastric cancer. *Proc Natl Acad Sci U S A*. 2020;117(51):32433–42.
31. Tang D, Kroemer G. Peroxisome: the new player in ferroptosis. *Signal Transduct Target Ther*. 2020;5(1):273.
32. Yusuf RZ, Saez B, Sharda A, van Gastel N, Yu VWC, Baryawno N, et al. Aldehyde dehydrogenase 3a2 protects AML cells from oxidative death and the synthetic lethality of ferroptosis inducers. *Blood*. 2020;136(11):1303–16.
33. Yi J, Zhu J, Wu J, Thompson CB, Jiang X. Oncogenic activation of PI3K-AKT-mTOR signaling suppresses ferroptosis via SREBP-mediated lipogenesis. *Proc Natl Acad Sci U S A*. 2020;117(49):31189–97.
34. Ye Z, Hu Q, Zhuo Q, Zhu Y, Fan G, Liu M, et al. Abrogation of ARF6 promotes RSL3-induced ferroptosis and mitigates gemcitabine resistance in pancreatic cancer cells. *Am J Cancer Res*. 2020;10(4):1182–93.
35. Ye Z, Zhuo Q, Hu Q, Xu X, Mengqi L, Zhang Z, et al. FBW7-NRA41-SCD1 axis synchronously regulates apoptosis and ferroptosis in pancreatic cancer cells. *Redox Biol*. 2021;38:101807.
36. Behrens D, Walther W, Fichtner I. Pancreatic cancer models for translational research. *Pharmacol Ther*. 2017;173:146–58.
37. Singh RR, Goldberg J, Varghese AM, Yu KH, Park W, O'Reilly EM. Genomic profiling in pancreatic ductal adenocarcinoma and a pathway towards therapy individualization: A scoping review. *Cancer*

Supplementary

Additional File 1 is not available with this version

Figures

Figure 1

Flow diagram of the study.

Figure 3

Construction and validation of the Fe-LPM in PC. The least absolute shrinkage and selection operator Cox (LASSO-Cox) analysis was implemented (A, B), and the coefficients were calculated by the 10-fold cross-validation based on minimum criteria (C). Kaplan-Meier analysis suggests the OS in the high-risk and low-risk groups in the TCGA cohort (D). Distribution of risk scores and survival status of PC patients in the TCGA cohort (E). Receiver operating characteristic (ROC) curve analysis for Fe-LPM in the TCGA cohort (F). In the ICGC validation cohort, the results of the Kaplan-Meier analysis were consistent with those in the TCGA cohort (G). Distribution of risk scores and survival status of PC patients in the ICGC cohort (H). Receiver operating characteristic (ROC) curve analysis for Fe-LPM in the ICGC validation cohort (I).

Figure 4

Prognostic analysis of eight Fe-LPS. Forest plot of the univariate Cox analysis in eight Fe-LPM (A). Heatmap showed the relationship between risk score and expression of the eight Fe-LPM (B). Kaplan-Meier analysis in different expression subgroups of the eight Fe-LPM (C-J).

Figure 5

Distribution of risk scores in different clinical characteristics. The risk score significantly correlated with pathological T stage, TNM stage, grade and primary therapy outcome of PC patients (A-D). The risk score was not related with the tumor site, pathological N stage, age and tumor size (E-H).

Figure 6

Gene Set Enrichment Analysis (GSEA) analysis in the TCGA dataset. GSEA illustrated that genes in the low-risk subgroup were significantly enriched in fatty acid metabolism (A, D), oxidative phosphorylation (B), PI3K-AKT-mTOR signaling (C), peroxisome (E) and lysosome (F).

Figure 7

The independence of the Fe-LPM in predicting OS in PC. Univariate and multivariate Cox analyses were performed based on risk scores and other clinical features in the TCGA dataset (A, B). A nomogram was constructed based on pathological T stage, risk score, primary therapy outcome and age in the TCGA cohort (C). A calibration plot confirmed the robustness of the Fe-LPM (D).

A

B

C

D

Figure 8

Construction of the ceRNA regulatory network. A ceRNA network including 4 lncRNAs-27 miRNAs-57 mRNAs was established (A). The heatmap (B), cluster ID network (C) and p-value network (D) of enriched pathways based on 57 target mRNAs.