A Novel Prognostic Risk Score Based on Ferroptosis-related LncRNAs Predicting Ovarian Cancer Patient Survival

Keyu Chen

Zhejiang Chinese Medical University

Xiaohong Li

Zhejiang Chinese Medical University

Caixia Qi (✉ ren000819@163.com)

Zhejiang Chinese Medical University

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Abstract

Background: Long non-coding RNAs (lncRNAs) are thought to be associated with several processes during cancer development and have been shown to be involved in the regulation of ferroptosis. Ovarian cancer is highly malignant tumour with a poor prognosis. The identification biomarkers with prognostic value in ovarian cancer may improve patient outcomes and can help to elucidate potential future therapeutic targets.

Results: We report differential expression of 187 ferroptosis-related lncRNAs in normal and ovarian cancer tissue. Using univariate and multivariable Cox regression analysis, we identified four lncRNAs that were strongly associated with prognosis. We constructed a prognostic risk score based on these four lncRNAs which was effectively able to distinguish between low- and high-risk OC patients based on survival time. Univariate and multivariable Cox regression analyses and time-related receiver operating characteristic curve analyses revealed that this risk score represented an independent prognostic factor in patients with ovarian cancer. For clinical implementation, we developed a nomogram based on the prognostic feature and patient age. Gene Ontology(GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that the four ferroptosis-related lncRNAs were related to tumour immunity.

Conclusions: we identify four novel ferroptosis-related lncRNAs as predictors of ovarian cancer prognosis and potential future therapeutic targets for ovarian cancer.

Background

Ovarian cancer (OC) is the fifth most common cause of cancer mortality in women [1]. In 2021, there were 21,410 new diagnoses of OC and 13,770 OC patients died from their disease in the US [2]. The prognosis of OC patients is heavily influenced by the stage at diagnosis [1]. Currently, the lack of effective screening tools and unspecific symptoms of early stage OC result in late diagnosis of a majority of patients, when metastases are already present [1,3]. The 5-year survival rate of advanced OC is 29%, and more than 80% of patients experience recurrence[3,4]. Despite significant developments in OC treatment options over the past two decades, overall survival (OS) rates have not improved drastically. Therefore, it is important to establish reliable prognostic features which may enable more accurate prognosis and could represent therapeutic targets in order to extend OS [5]. Certain lncRNAs have previously been shown to be associated with OC prognosis[6]. Due to the complex molecular mechanisms of tumours, polygenic models (incorporating several markers) tend to perform better at prognosis prediction than single genes.

lncRNAs are transcripts that are longer than 200 nucleotides which are not involved in protein translation. In recent years, lncRNAs have been reported to be involved in the proliferation and metastasis of cancer cells[7]. A related study reported that lncRNA MIR4697HG may be responsible for slowing down the proliferation of OC cells through ERK and AKT signaling pathways[8]. In addition, some specific

lncRNAs have been reported to predict cancer progression, which means that lncRNAs can be used as prognostic biomarkers for cancer[9].

Ferroptosis is a type of regulated cell death (RCD) distinct from apoptosis. It is closely linked to cellular iron levels and can be regulated by a variety of genes [10]. Cells undergoing ferroptosis exhibit normal nuclei and intact membranes, but have a reduced mitochondrial volume, increased mitochondrial membrane density, and loss of mitochondrial cristae [11,12]. Ferroptosis is characterized by the accumulation of lipid metabolites and cellular iron and excessive generation of reactive oxygen species. In addition, ferroptosis has been reported to contribute to low activity of glutathione peroxidase 4 (GPX4) [13]. Over last decade, the involvement of iron toxicity has been demonstrated in various tumours, and is furthermore related to treatment efficacy of tumours, including OC [14]. There is now increasing evidence that the iron metabolism may be strongly associated with cell growth and metastasis in OC. For instance, Basuli et al. observed a decrease in Ferroportin (FPN) and an increase in Transferrin receptor protein 1 (TfR1) levels in OC tissues. The authors hypothesized that OC cells exhibit both an increased iron content and iron dependence, suggesting that OC cells may be sensitive to ferroptosis-inducing drugs [15].

Based on the above discoveries, we hypothesized that lncRNAs and ferroptosis may play a critical role in tumour development and treatment. As the involvement of lncRNAs and ferroptosis in OC has not yet been assessed in depth, further studies exploring the role of lncRNAs and ferroptosis in OC development are warranted. Current therapeutic options for OC include surgical resection and platinum-based chemotherapy [16]. To explore whether the expression of ferroptosis-related genes differs between normal and ovarian cancer tissues, we conducted an in-depth study using relevant data from the the Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) databases. The predictive prognostic performance of these genes was further explored and prognostic features based on four ferroptosis-related genes were developed. In conclusion, we successfully explore the expression of ferroptosis-related genes and constructed prognostic features with potential application to clinical treatment in this study.

Methods

Data

Gene expression data and individual clinical information of OC patients were derived from TCGA. Due to an absence of data on normal ovarian sample in the TCGA cohort, we have supplemented data from normal samples (n=88) derived from GTEx. It is worth noting that the GTEx and TCGA databases are based on the same technology and platform. A list of ferroptosis-related genes (n=259) (Supplementary Table 3) was obtained from the FerrDb [14].

Screening and of ferroptosis-related lncRNAs

RNA-seq data accessed from TCGA and GTEx were combined. Based on FerrDb, we identified ferroptosis-related mRNAs. We used pearson correlation to further identify ferroptosis-related lncRNAs that met the following criteria: correlation coefficient $|R^2| > 0.6$, $p < 0.001$.

Identification of DEGs

DEGs associated with iron toxicity were identified using the limma package, based on the following criteria: $FDR < 0.05$, $|\log_2 FC| > 1$. A heatmap reflecting expression levels of DEGs in tumour and normal samples was created using the R heatmap package.

Construction and examination of prognostic features

We randomly assigned 184 of the 364 OC patients with complete survival information from the TCGA cohort (“entire dataset”) to the “primary dataset”. We did not observe significant differences in clinical characteristics (age and grading) between the primary dataset and the entire dataset. The prognostic value of ferroptosis-related lncRNAs in the primary dataset was assessed via univariate Cox regression and identified ferroptosis-related lncRNAs with a high correlation with the survival status ($p < 0.1$) as candidate genes. We then used multivariable Cox regression analysis to further explore the prognostic potential of candidate genes for prognostic features ($p < 0.05$). The prognostic score was calculated as follows:

$$\text{Risk Score} = (a_1 * gene_1exp) + (a_2 * gene_2exp) + \dots (a_n * gene_nexp)$$

We further subdivided patients in the primary dataset into a low-risk and high-risk subgroup by median prognostic features. We assessed the predictive performance of prognostic features on OS via receiver operating characteristic curves and Kaplan-Meier survival curves. The risk score for every patient in the entire dataset was calculated using the same formula as in the primary dataset to validate prognostic features. We then divided the “entire dataset” of patients into low- and high-risk subgroups based on the median risk score of the “primary dataset” and further validated the predictive performance using receiver operating characteristic curves and Kaplan-Meier curves.

Construction of Nomogram based on prognostic features

We performed univariate and multivariable COX regression analyses on the “entire dataset” and “primary dataset”, respectively, to determine whether prognostic characteristics were independent prognostic factors. Nomograms based on data from the “entire dataset” was then created in order to predict the survival rates of OC patients after 1, 2, and 3 years, with the calibration curves plotted to validate the predictive effect.

Function enrichment analyses

We identified mRNAs co-expressed with OS-related lncRNAs ($|R^2| > 0.6$, $p < 0.001$) and subjected them to KEGG and GO analyses to assess molecular functions, cellular components, and major signalling pathways involved in OS-related lncRNAs. $p < 0.05$ was considered statistically significant.

Statistical analysis

We used Student’s *t*-test to identify DEGs, and Pearson correlation test to assess the co-expression of lncRNAs and mRNAs. The PERL programming language was used to process clinical and genetic data (version 5.30.2, <http://www.perl.org>). We used R software (version 4.1.0) for statistical analyses and all *p*-values were two-tailed.

Results

Identification of ferroptosis-related genes

Combining RNA-seq data from TCGA and GTEx, we identified 19,555 mRNAs and 13,829 lncRNAs. We assessed 240 ferroptosis-related mRNAs and found 315 ferroptosis-related lncRNAs via correlation analysis ($|R^2|>0.6$, $p<0.001$).

Identification of differentially Expressed lncRNAs

Comparing the expression of 315 ferroptosis-related lncRNAs in 379 OC tissues from TCGA and 88 normal tissue from GTEx, we identified 187 differentially expressed genes (DEGs) ($p<0.01$), of which 48 were downregulated in OC while 139 were upregulated. Figure 1A shows a heatmap of expression levels of the abovementioned lncRNAs. A volcano plot in Figure 1B shows the differential expression of ferroptosis-related lncRNAs in normal and tumor sample.

Identification of candidate lncRNAs associated with overall survival

We next assessed the association of ferroptosis-associated lncRNAs with overall survival (OS). Fifteen patients were excluded from the TCGA dataset due to a lack of complete clinical data. Among the 364 remaining OC patients (“entire dataset”), we randomly assigned 184 patients to the “primary dataset”(Patient IDs in the primary dataset can be found in Supplementary Table 1), which did not exhibit significant differences in clinical data compared with the entire dataset (Table 1). We evaluated the prognostic performance of 187 ferroptosis-related DEGs by Kaplan-Meier analysis and univariate Cox regression analysis, resulting in the identification of 7 candidate lncRNAs closely associated with OS ($p<0.1$) (Table 2). Figure 2A shows a forest plot of these 7 genes, three of exhibited a hazard ratio (HR) >1 and are therefore likely associated with a worse prognosis, while the remaining four lncRNAs had a HR <1 . Kaplan-Meier curves of the 7 lncRNAs showed the same trend as the above results (Figure 2B-H).

Table 1. Comparison of clinical data for the primary and entire dataset

Character	primary dataset n=170	entire dataset n=339	pvalue
Age[year]			0.701
≥65	35.87%	35.44%	
<65	64.13%	64.56%	
Grade			0.833
G1	0.54%	0.27%	
G2	10.87%	11.54%	
G3	87.50%	87.64%	
G4	1.09%	0.55%	

Table 2. Univariate Cox results for lncRNAs based on the primary dataset

id	HR (95%CI)	pvalue
AC138904.1	0.722(0.515-1.011)	0.058
DNM3OS	1.105(1.005-1.214)	0.039
USP30-AS1	0.842(0.718-0.987)	0.034
FOXP4-AS1	0.780(0.614-0.991)	0.042
AC020916.2	1.349(1.010-1.801)	0.042
LINC01150	1.273(0.958-1.693)	0.096
AC068870.2	0.874(0.749-1.020)	0.088

Prognosis feature construction and examination

We used a stepwise multivariable Cox regression analysis to identify the optimal combination of OS-related lncRNAs. The Akaike information criterion (AIC) was employed to prevent overfitting that it can not over-fitted. Finally, four OS-related lncRNAs (AC138904.1, DNM3OS, USP30-AS1, and FOXP4-AS1) were selected from the multivariable Cox proportional hazards model (Table 3). A prognostic risk score based on the ferroptosis-related lncRNAs was created based on the following formula:

$$\text{Risk Score} = -(0.326 \times \text{AC138904.1exp}) + (0.100 \times \text{DNM30Sexp}) - (0.172 \times \text{USP30 - AS1exp}) - (0.248 \times \text{FOXP4 - AS1exp})$$

We next calculated this risk score for each patient in the primary dataset and divided patients into high-(n=92) and low-risk groups (n=92) based on median risk scores in the primary dataset. Risk scores, OS,

and expression profiles of the four lncRNAs were visualised in Figure 3A-C. Kaplan-Meier analysis demonstrated a significantly worse prognosis in the high-risk group than in the low-risk group ($p < 0.001$, Figure 3D). Next, we utilized time-dependent receiver operating characteristic curves to assess the performance of the prognostic features for prediction of OS (Figure 3E-G). This revealed we were able to obtain AUC (area under the curve) values of 0.738, 0.737, and 0.637 at 1, 2, and 3 years, respectively, indicating a high accuracy of the prediction model. From this, we concluded that the prognostic features we had selected performed well for risk and overall survival prediction in the primary dataset.

We further validated the predictive performance of the prognostic features using the entire dataset ($n = 364$). The median risk score of the primary dataset was used as the threshold to divide the entire dataset into a high- ($n = 191$) and a low-risk group ($n = 173$). The risk score, OS status, and four OS-related lncRNAs in the entire dataset exhibited a similar trend as results of the primary dataset (Figure 4A-C). We also found significant differences in survival between the high- and low-risk groups (Figure 4D). To evaluate the ability to predict survival at 1, 2, and 3 years, we again calculated AUCs from time-dependent receiver operating characteristic curves (Figure 4E-G). AUCs were 0.702, 0.714, and 0.632, respectively. While AUC values were decreased slightly compared with the primary dataset, they still indicated an excellent predictive performance of prognostic risk score.

Table 3. Multivariable Cox analysis of lncRNAs in the primary dataset.

id	coef	HR (95%CI)	pvalue
AC138904.1	-0.326	0.722(0.515-1.011)	0.058
DNM3OS	0.100	1.105(1.005-1.214)	0.039
USP30-AS1	-0.172	0.842(0.718-0.987)	0.034
FOXP4-AS1	-0.248	0.780(0.614-0.991)	0.042

Independent prognostic performance of prognostic features

Using univariate Cox regression analysis, we found that the prognostic risk score acted as an independent prognostic factor in both datasets we evaluated ($HR = 1.6013$ and $p = 1.79 \times 10^{-5}$ in the primary dataset, and $HR = 1.5758$ $p = 5.69 \times 10^{-7}$ in the entire dataset) (Table 4, Figure 5A). Multivariable COX regression analysis revealed that the risk score was still an independent prognostic feature even after correction for other confounders ($HR = 1.5680$, $p = 3.69 \times 10^{-5}$ in the primary dataset; $HR = 1.5672$, $p = 5.05 \times 10^{-7}$ in the entire dataset) (Table 4, Figure 5B). To evaluate predictive accuracy of the risk score, the receiver operating characteristic curves were plotted and further AUC values were calculated for the entire dataset (Figure 5C). This revealed that assessing prognosis at three years, the ferroptosis-related lncRNA risk score performed better than age or grade (AUCs of prognostic risk score, age, and grade were 0.714, 0.631, 0.555, respectively).

Table 4. Univariate and multivariable Cox proportional hazards regression analysis of the prognostic risk score and clinical risk factors in the entire dataset.

Characteristic	Univariate analysis		Multivariate analysis	
	HR (95%CI)	pvalue	HR (95%CI)	pvalue
Age (≥65 vs. <65)	1.019(1.006-1.033)	0.005	1.020(1.007-1.034)	0.003
grade(GI-GIV)	1.387(0.922-2.084)	0.116	1.267(0.840-1.911)	0.259
riskScore	1.576(1.319-1.883)	<0.001	1.567(1.315-1.867)	<0.001

Production of nomograms based on prognostic features

Traditional clinical risk factors remain important predictors of OS in OC patients. To better predict the OS of OC patients, prognostic features and a number of traditional clinical factors have previously been combined to develop models for OS prediction. Examining independent prognostic performance in the entire dataset, we discovered that in addition to ferroptosis-related lncRNAs, age was also a prognostic marker, which may have been overlooked due to the smaller sample size in the primary dataset. We therefore created a nomogram based on these two risk factors (ferroptosis-related lncRNA prognostic risk score and age) and utilized it to predict 1, 2 , and 3 year survival rates (Figure 6A). In the clinic, this simple graphical tool could be utilized to easily calculate the survival probabilities of each OC patient. We used the C-index and calibration curves to evaluate the predictive effectiveness of the nomograms (Figure 6B-D) which revealed that these nomograms were good predictors of survival time for OC patients.

Enrichment analysis

In order to infer the potential functions of ferroptosis-related lncRNAs in the development of OC, we identified 417 lncRNA-mRNA pairs ($|R2|>0.6$, <0.001) (Supplementary Table 2) and subjected the co-expressed mRNAs to GO and KEGG pathway analysis. GO results showed that the co-expressed mRNAs were enriched for genes involved in immune response and immune regulation, such as the activation of T cells (Figure 7A). KEGG results revealed that the 417 co-expressed mRNAs were predominantly involved in Human T-lymphotropic virus 1 infection, Epstein-Barr virus infection, antigen handling, and presentation (Figure 7B).

Discussion

Survival times for OC patients vary widely, spanning from less than 5 months to more than 10 years [17]. Nonetheless, due to a lack of choice in treatment approaches, all patients are treated relatively similarly. Prognostic features may help to accurately identify patients with different survival times and to individualize treatment. Over the past decades, clinical characteristics such as age and a range of serum markers have been used to determine tumour progression and predict the prognosis of OC patients. However, these clinical characteristics factors are often not sufficiently accurate prognostic predictors to help improve treatment options. In this study, we developed and validated a four lncRNA-based

prognostic risk score using data from the TCGA cohort for which both clinical and gene expression data are available.

With the intensive study of various common cancers, there is growing evidence that ferroptosis contributes to cancer development [18,19,20]. Ferroptosis acts as a double-edged sword [21]: it can cause immunosuppression by triggering a cellular inflammatory response, which in turn accelerates tumour growth [22]; on the other hand, pharmacological induction of ferroptosis can inhibit tumour growth [23]. Ferroptosis has therefore shown great promise in cancer treatment, particularly in cancers that are resistant to conventional therapies [24]. Ferroptosis-associated drugs such as artesunate, FIN56, FINO2, erastin, and sorafenib have been successively discovered to exert antitumor effects. For instance, artesunate, a herbal medicine originally used for malaria control, has been found to inhibit the growth of OC cells via induction of reactive oxygen species-related (ROS) ferroptosis [25,26]. With increasing research, more ferroptosis-associated genes are being identified. Due to a lack of systematic reports, previous studies have tended to identify ferroptosis-related genes only based on existing literature, which may have resulted in some ferroptosis-related genes being overlooked [27]. The FerrDb database includes 259 regulators derived from ferroptosis articles in the PubMed database [28]. In this study, we used the FerrDb database to identify a comprehensive list of ferroptosis-related genes.

Our study identified four ferroptosis-related lncRNAs (FOXP4-AS1, USP30-AS1, DNM3OS, AC138904.1) with a prognostic role in OC. Among these four lncRNAs, three lncRNAs (FOXP4-AS1, USP30-AS1, DNM3OS) have been previously studied either in OC or other cancers. Among these, FOXP4-AS1 and USP30-AS1 were associated with a good prognosis for OC, while DNM3OS was associated with a poor prognosis for OC. For instance, FOXP4-AS1 has been proposed an emerging cancer-related biomarker. While a study by Huang et al. identified inconsistent effects of FOXP4-AS1 in different types of cancers [29], Yang et al. found that FOXP4-AS1 promoted osteosarcoma development by binding to LSD1 and EZH2 to downregulate LATS1 [30], and similar effects have been reported in prostate, cervical, and nasopharyngeal cancers [31,32,33]. Interestingly, the role of FOXP4-AS1 in OC may be different from other cancers. Liao et al. suggested that FOXP4-AS1 improves OC prognosis by participation in the CTLA4 pathway [34]. The authors found that FOXP4-AS1 was highly expressed in the low-risk group of OC patients. Based on these results and our own study, we infer that FOXP4-AS1 may be associated with a favourable OC prognosis. USP30-AS1 is transcribed from the antisense strand of the autophagy-related USP30 gene located on chromosome 12 [35]. In our study, elevated levels of USP30-AS1 was associated with an improved prognosis. This is again consistent with reports from previous studies which found abnormal expression of USP30-AS1 in ovarian, bladder, and cervical cancers as well as melanoma, where it acted as a good prognostic feature [36,37,38,39]. Wang et al. showed that the lncRNA DNM3OS may promote cell proliferation, invasion, and metastasis of hepatocellular carcinoma [40]. Similarly, DNM3OS has been shown to positively regulate SMAD6 in retinoblastoma via competitive interaction with miR-134-5p, where high SMAD6 expression promotes tumour cell proliferation, migration, and epithelial-mesenchymal transition [41]. Another previous study reported that DNM3OS knockdown resulted in altered EMT-related genes/pathways, mesenchymal to epithelial transformation, and reduced cell migration and invasion, which suggested that high DNM3OS expression may lead to a reduced survival

rate in OC patients [42]. He et al. found that DNM3OS promoted OC progression via regulation of the miR-193a-3p/MAP3K3 axis [43], which again suggested that DNM3OS may represent a poor prognostic factor in OC, and DNM3OS has also been reported as a molecular marker for cancer progression in a variety of different cancer types [44,45,46]. In conclusion, the abovementioned three lncRNAs are thought to be involved in the development of OC or other cancers, and may represent therapeutic targets in OC. The other ferroptosis-related lncRNA in our prognostic risk score, AC138904.1, has been rarely reported, and the present study may provide a new perspective on its functional pathways and involvement in ovarian carcinogenesis and progression.

To further explore how the lncRNAs may be involved in OC development, we performed a functional enrichment analysis and found that the risk score-associated genes were highly associated with the immune response. GO analysis identified a significant enrichment in pathways linked to the activation and regulation of T cells, which suggested that T-cell activation may be closely linked to OC development. Yeung et al. previously showed that ISG15 can inhibit OC progression via activation of CD8⁺ T cells [47]. CD8⁺ T cells have been reported to inhibit cysteine uptake by tumor cells and may promote ferroptosis in cancer cells by releasing IFN γ , which may represent a novel anti-tumour mechanism [48,49]. Akyol et al. found that HSP10 produced by ovarian tumour cells suppressed the expression of CD3-zeta, a key component of T cell activation, in T cells [50], and OC patients with lower T cell CD3-zeta expression have been found to exhibit shorter survival times [51]. In addition, IL4I1 has been found to impair T-cell activation, block T cell proliferation and differentiation, and thus promote OC proliferation, migration, and invasion [52]. From this, we hypothesized that T cell activation may prolong OC patient survival, while T cell suppression may be closely associated with low survival in OC patients. In addition, the negative regulation of immune system processes was significantly enriched in ferroptosis-associated lncRNAs. The immune system is known to play a significant role in cancer progression, and a previous study reported progressive impairment of the immune response in patients with advanced OC [53,54]. The most significant cellular component enrichment in GO analysis was the external side of the plasma membrane, including proteins which are attached, immobilised or embedded in the plasma membrane. LAG-3 is a cell surface molecule with diverse biological effects on T cell function. A study by Matsuzaki et al. found that LAG-3 indirectly inhibited T cell proliferation, which the authors proposed to be associated with a reduction in T cell receptor-induced calcium flux [55]. In conclusion, we may speculate that proteins attached to the external side of the plasma membrane are intimately involved in the development of OC.

KEGG results revealed that Human T-lymphotropic virus 1 infection, Epstein-Barr virus infection, and antigen handling and presentation pathways were significantly enriched. Human T-lymphotropic virus 1 (HTLV-1) is a retrovirus that can induce adult T cell leukaemia [56]. So far, there have been no studies reporting an association between HTLV-1 and OC. However, HTLV-1 infection can promote T cell proliferation by tampering with several core signalling pathways of T cell function [57], and the relationship between T cell function and OC has been demonstrated in several studies [49,50,52]. From this, we speculate that HTLV-1 may regulate OC via T cell function and offers a new direction for the treatment of OC. Epstein-Barr virus (EBV) is a double-stranded DNA virus [58]. In two different studies, the EBV positivity rate has been reported to be lower in the normal group than in OC patient groups [59,60]. It

has moreover been reported that the miR-BART7 of EBV was more highly expressed in the OC patients than in cancer-free controls, and miR-BART7 may be related to poor OC prognosis [61]. Littman et al. showed that elevated EBV antibody titers may lead to increased risk of OC [62]. Although the potential role of EBV in OC has not been extensively researched, its role in other cancers has been clearly demonstrated [63,64]. In addition to HTLV-1 and EBV, pathways involved in processing and presentation of antigens were significantly enriched in KEGG analysis. A previous study showed that CCL22, expressed by macrophages in the tumour microenvironment of OC patients, helped to attract regulatory T cells to the tumour environment [65]. Interestingly, regulatory T cells have been shown to be related to a higher risk of death in OC patients [66]. Macrophage-expressed B7-H4 has also been found to suppress tumor-associated antigen-specific T cell immunity, and B7-H4 expression levels were negatively associated with patient survival [67,68]. Plasmacytoid dendritic cells (pDCs) are innate immune cells that appear in peripheral lymphoid organs and the circulation. A review reported that OC-related pDCs induce the activation of CD8⁺ Treg cells and promote tumour angiogenesis [68,69]. Therefore, we speculated that macrophages, plasmacytoid dendritic cells, and other antigen-presenting cells, may indirectly influence OC prognosis. In short, the four prognostic ferroptosis-related lncRNAs identified in this study are thought to be involved in OC development and prognosis.

We developed a prognostic profile of four lncRNAs associated with iron toxicity, and found it could effectively identify patients with high-risk OC. As OC has been studied closely, many prognostic markers have been identified. However, some studies screened prognostic markers with criteria that ignored the clinical significance by limiting their focus to the statistical performance [70]. Age has been reported as a survival and prognostic factor for OC patients in a recent study [71]. In this study, we analysed the prognostic value of clinical risk factors and prognostic features. Finally, we constructed a nomogram based on the prognostic risk score and age for easy clinical implementation [72].

The current study has several limitations. Firstly, some clinical data (e.g., stage) were not collected by TCGA, so we were unable to conduct a full survival analysis for these factors. Second, the prognostic features we established need to be validated in other independent cohorts for further confirmation and to ensure their robustness. Third, further experiments are needed to explore how ferroptosis-related lncRNAs are involved in OC development.

Conclusion

We successfully constructed a prognostic risk score based on four ferroptosis-related lncRNAs that can be used as a new biomarker for the prediction OC patient survival. Additionally, this novel signature may enable further insights into the association between ferroptosis and tumorigenesis for clinical ferroptosis-related targeted therapies. For easy clinical implementation, we developed a prognostic feature-based nomogram which can aid in the individualized management of OC patients.

Abbreviations

Long non-coding RNAs	lncRNAs
area under the curve	AUC
Gene Ontology	GO
Kyoto Encyclopedia of Genes and Genomes	KEGG
overall survival	OS
regulated cell death	RCD
glutathione peroxidase 4	GPX4
Ferroportin	FPN
Transferrin receptor protein 1	TfR1
Cancer Genome Atlas	TCGA
Genotype-Tissue Expression	GTEX
differentially expressed genes	DEGs
Ovarian cancer	OC
hazard ratio	HR
Akaike information criterion	AIC
reactive oxygen species	ROS
Human T-lymphotropic virus 1	HTLV-1
Epstein-Barr virus	EBV
Plasmacytoid dendritic cells	pDCs

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 and GTEx datasets that provide free online tools and resources.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: Chen, first author: conception of the research content idea, design of the research proposal, finding data and writing the paper. Li: data processing, data analysis, visual presentation of research results. Qi, corresponding author: theoretical guidance of the research proposal, review and revision of the first draft, responsible for communication with the journal for submission of the manuscript.

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Figures

Figure 1

Identification of DEGs between ovarian cancer samples and normal ovarian tissue. (A) Heatmap of 315 ferroptosis-related lncRNAs (green and red indicate lower and higher lncRNA expression, respectively) (B) Volcano plot of 315 ferroptosis-related lncRNAs in ovarian cancer samples and normal ovarian tissue.

Figure 2

Seven candidate ferroptosis-related lncRNA associated with overall survival. (A) Forest plots of 7 OS-related candidate genes identified by univariate Cox regression. (B-H) Single gene Kaplan-Meier curves assessing survival in association with expression of the seven lncRNAs AC138904.1, DNM3OS, USP30-AS1, AC020916.2, LINC01150, AC068870.2, AC068870.2.

Figure 3

The predictive power of a novel, four lncRNA prognostic risk score in the primary dataset. (A) Risk score grouping based on prognostic features. (B) OS status scatter plot. (C) Heatmap of four OS-related lncRNAs. (D) Low- and high-risk group Kaplan-Meier curves for OS. (E-G) receiver operating characteristic curves were used to validate the predictive accuracy of prognostic features for 1, 2, and 3 year OS in the primary dataset.

Figure 4

The predictive power of a four lncRNA prognostic risk score in the entire dataset. (A) Risk score grouping based on prognostic features. (B) OS status scatter plot. (C) Heatmap of four OS-related lncRNAs. (D) Low- and high-risk group Kaplan-Meier curves for OS. (E-G) receiver operating characteristic curves were used to validate the predictive accuracy of prognostic features for 1, 2, and 3 year OS in the entire dataset.

Figure 5

Estimated prognostic accuracy of the ferroptosis-related lncRNAs prognostic risk score and other clinicopathological variables in OC patients. (A) Univariate Cox regression analysis showed that age, risk score, and OS significantly associated. (B) Multivariable Cox regression analysis showed that age and the risk score act as independent prognostic indicators for OC patients. (C) receiver operating characteristic curve analysis of the prognostic accuracy of the prognostic risk score, age and grade.

Figure 6

Construction and validation of prognostic nomograms. (A) Prognostic nomogram based on a four-ferroptosis-related lncRNA prognostic risk score and age. (B-D) Calibration curves for the prognosis-based nomograms for high- and low-risk OC patients highlight the small deviation between predicted and actual survival rates.

Supplementary Files

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