Ferroptosis-Related Long Non-Coding RNA Signature Predicts the Prognosis of Bladder Cancer

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Abstract

Background: Ferroptosis is an iron-dependent programmed cell death modality that may have a tumor suppressor function. Therefore, regulating ferroptosis in tumor cells could serve as a novel therapeutic approach. This article focuses on ferroptosis-associated long non-coding RNAs (lncRNAs) and their potential application as a prognostic model for bladder cancer (BCa).

Methods: We retrieved bladder cancer-related transcriptome information and clinical information from the TCGA database and ferroptosis-related gene sets from the FerrDb database. Minimum absolute shrinkage and selection operator regression and Cox regression models were used to identify and develop predictive models and validate the models' accuracy. Finally, we explore the inter-regulatory relationships between ferroptosis-related genes and immune cell infiltration, immune checkpoints, and m⁶A methylation genes.

Results: Kaplan-Meier analyses revealed 11 differentially expressed lncRNAs associated with poor BCa prognosis. This signature (AUC = 0.720) could potentially be utilized to predict BCa prognosis. Our risk assessment model outperformed traditional clinicopathological features in predicting BCa prognosis. Additionally, GSEA revealed immune and tumor-related pathways in individuals in the low-risk group. TCGA showed that the p53 signaling pathway, ferroptosis, Kaposi sarcoma-associated herpesvirus infection, IL-17 signaling pathway, MicroRNAs in cancer, TNF signaling pathway, PI3K-Akt signaling pathway and HIF-1 signaling pathway were significantly different from those in the high-risk group. Immune checkpoints, such as PDCD-1 (PD-1), CTLA4, and LAG3, were also differentially expressed between the two risk groups. m⁶A methylation-related genes were likewise significantly differentially expressed between the two risk groups.

Conclusion: A new ferroptosis-associated lncRNAs signature on the prognosis of BCa patients will provide new ideas for the treatment and management of BCa patients.

Introduction

Bladder cancer (BCa) is one of the most common malignancies of the urogenital system and one of the ten predominant malignancies worldwide[1]. According to whether it invades the muscle layer of the bladder, bladder cancer is divided into muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC)[2]. Although surgical treatment and postoperative Bacillus Calmette-Guerin (BCG) perfusion and other immunotherapy are applied to the clinical treatment of BCa[3], still about 20% of BCa cases invade the bladder muscle. Despite the available treatments, MIBC recurrence, progression, and mortality are high[4]. The five-year overall survival (OS) rate for all stages of urothelial cancer patients is approximately 66–68%[5]. The effective clinical management of BCa is limited by the best preclinical models and the lack of accurate biomarkers for early cancer diagnosis. Therefore, it is crucial to explore other forms of cell death to overcome tumor cells' resistance and discover new and effective prognostic biomarkers of early BCa.

Ferroptosis is a newly discovered form of programmed cell death, different from apoptosis and autophagy[6]. Ferroptosis is iron-dependent as it is triggered by the accumulation of intracellular iron, lipid peroxides, and reactive oxygen species (ROS). The primary mechanism of ferroptosis is the induction of cell death by lipid peroxidation in the presence of divalent iron or ester oxygenase, which catalyzes the high expression of unsaturated fatty acids in the cell membrane[7]. Ferroptosis is involved in various critical biological processes, including cancer, ischemia-reperfusion injury, and neurodegenerative diseases[8]. Furthermore, more and more recent studies have confirmed that the regulation of ferroptosis could be used as a new therapeutic tool[9]. Therefore, a novel field is emerging, and new studies are investigating the link between ferroptosis and cancer.

Long non-coding RNA (lncRNA) refers to a class of non-coding RNA longer than 200 nucleotides in length. lncRNA can regulate different physiological and biochemical cellular processes by mediating chromosomal modifications, transcriptional activation, and interference[10]. In addition to gene regulation, lncRNAs are involved in various bioregulatory processes, including those related to tumorigenesis, progression, and metastasis[11]. However, the association between ferroptosis, lncRNAs, and cancer is only now beginning to be explored. For example, one report from Wang et al. showed that the lncRNA LINC00336 regulates tumor progression by inhibiting ferroptosis mechanisms in lung cancer through interaction with ELAVL1[12]. lncRNA LINK-A, an oncogene, plays a vital role in endogenous tumor suppression and presentation of cancer cell antigens[13]. In addition, the lncRNAMT1DP showed increased sensitivity of non-small cell lung cancer to ferroptosis by regulating the miR-365a-3p/NRF2 signaling pathway[14]. Therefore, lncRNAs can act as an independent prognostic factor for tumors, providing new directions for individualized tumor treatment.

Nonetheless, there are no studies on the association of ferroptosis-related lncRNAs with BCa overall survival to date. This study constitutes the first prognostic model of differentially expressed ferroptosis-related lncRNAs with prognostic multiple lncRNAs based on The Cancer.

Methods

Data collection:

BCa transcriptome expression data were retrieved from the *TCGA* data portal (<https://cancergenome.nih.gov/>). The data included 414 tumor samples and 19 healthy samples. The clinical characteristics of the BCa patients obtained included age, stage, TNM stage, survival time, and survival status. Patients with missing information were excluded from our analysis. Afterward, samples with OS $\leq$ 30 days were excluded because of the factors of non-neoplastic death [15] (Table 1). The corresponding ferroptosis-related genes were downloaded from the FerrDb database[16]. FerrDb is a comprehensive, manually curated, and up-to-date database for ferroptosis markers and regulators in health and disease. In this study, we identified 247 genes related to triggering effects (Table 2). The relationship between the ferroptosis-related lncRNAs and BCa was assessed using Pearson correlation. The association was considered significant if the correlation coefficient $|R2| > 0.3$ at $P < 0.001$. Statistical

significance of differential expression of ferroptosis-related lncRNAs was set at a fold-change (*FC*) value of > 1.0 and *FDR*-corrected value of $P < 0.01$.

Enrichment Analysis of miRNA ferroptosis-related DEGs:

Functional enrichment analysis of differentially expressed genes (DEGs) was performed using *Metascape* (<http://metascape.org>) and the Database for Annotation, Visualization and Integrated Discovery (*DAVID*) [17]. In addition, the functional analysis of biological processes (BP), molecular functions (MF), and cellular components (CC) regulated by the differentially expressed ferroptosis-related lncRNAs were analyzed based on Kyoto Encyclopedia of Genes and Genomes (KEGG) data using R software and *Metascape database*. The P-value was set to $P < 0.05$ as the critical value.

Development of the ferroptosis-related lncRNAs prognostic signature

We used *Lasso-penalized Cox regression analysis* and *Univariate Cox regression analysis* using the "glmnet" package in R to construct the ferroptosis-related lncRNAs signature. The risk score was calculated as below [18] and each BCa patient's risk score was evaluated. With the median value as a bound, the RNAs were divided into low-risk ($<$ median value) and high-risk ($\geq$ median value) groups.

$(\text{Coefficient lncRNA}_1 \times \text{expression of lncRNA}_1) + (\text{Coefficient lncRNA}_2 \times \text{expression of lncRNA}_2) + \dots + (\text{Coefficient lncRNA}_n \times \text{expression lncRNA}_n)$

The predictive nomogram

We performed Gene set enrichment analyses (GSEA) to define the lncRNAs signatures in the KEGG pathways, which were then searched in the TCGA-BLCA database. Statistical significance was set at $P < 0.05$ and false discovery rate (FDR) at $q < 0.25$. A nomogram was constructed by integrating the prognostic signatures for predicting 1, 3, and 5-year OS of BCa patients.

Immunity analysis and gene expression

The CIBERSORT [19], ESTIMATE [20], MCPcounter [21], single-sample gene set enrichment analysis (ssGSEA) [22] and TIMER [23] algorithms were compared to assess cellular components or cell immune responses between high-risk and low-risk groups based on ferroptosis-related lncRNAs signature. The differences in the immune response under different algorithms were uncovered using a Heatmap. In addition, ssGSEA was used to quantify the tumor-infiltrating immune cell subgroups between the two groups and assess their immune function. The potential immune checkpoint was also retrieved from previous literature.

Cell culture

Bladder cancer cell lines T24 and EJ and normal bladder epithelial cells line SV-HUC were obtained from the Shanghai Branch, Chinese Academy of Sciences. The cells were cultured in 1640 medium with 10% fetal calf serum and passaged by 0.25% trypsinization with EDTA (Invitrogen, Grand Island, NY). Tumor cells in logarithmic phase are selected for experiment.

Validation of the diff-miRNAs

The identified diff-LncRNAs were further validated by real-time-quantitative PCR (RT-qPCR) analysis using the following human cell lines: Bladder cancer cells (T24 and EJ) and normal bladder epithelial cells line SV-HUC. The cell lines were obtained from the Shanghai Institute of Cell Science, Chinese Academy of Sciences. The RT-qPCR was done according to the following procedures. Briefly, TRIzol (Invitrogen; Thermo Fisher Scientific, Inc., Carlsbad, CA, USA) was adopted to extract total RNA from cells. The LncRNAs primers and real-time fluorescent quantitative PCR testing kit were purchased from FuleGen Co., Ltd. (Guangzhou, China). GAPDH was used as an endogenous control. The relative quantification of LncRNA levels is determined by the $\Delta\Delta C_t$ method. The synthesis of our first cDNA was synthesized by using EntiLink™ 1st Strand cDNA Synthesis Kit (ELK Biotechnology, EQ003), and real-time fluorescent quantitative PCR was completed on the StepOne™ Real-Time PCR instrument from Life technologies, and each sample was made 3 double holes, using EnTurbo™ SYBR Green PCR SuperMix kit (ELK Biotechnology, EQ001): the specific primer sequence list is shown in Table 6.

Statistical analysis

Data were analyzed using Bioconductor packages in R software (version 4.0.2). Normal and non-normal distributed variables were analyzed using the unpaired student's t-test and the Wilcoxon test, respectively. Benjamini-Hochberg method was used to identify the differentially expressed lncRNAs, based on FDR. The ssGSEA-normalized BCa DEGs were compared with a human genome using "GSVA" (R-package). The sensitivity and specificity of the derived prognostic signatures for BCa in comparison to other clinicopathological was assessed using the receiver operating characteristic (ROC) curve and decision curve analysis (DCA)[24]. The relationship between ferroptosis-related lncRNAs and clinicopathological manifestations was evaluated using logistic regression analyses and a heatmap graph. Finally, the survival analysis of BCa patients based on the ferroptosis-related lncRNAs signature was assessed using the Kaplan–Meier survival analysis. For each analysis, statistical significance was set at $P < 0.05$.

Results

Extraction and functional enrichment analysis of differential genes associated with ferroptosis.

First, we started analyzing the BCa transcriptome expression data obtained from *TCGA*. By differential enrichment analysis by DAVID database, we found 47 DEGs associated with ferroptosis. Of these genes, 22 were downregulated, and 25 were upregulated (Table 3). BP functional enrichment analysis revealed that these genes are involved in the following pathways: intrinsic apoptotic signaling pathway, multicellular organismal homeostasis, and response to toxic substances. Among the MF specific terms, we found “regulate iron ion binding”, “oxidoreductase activity”, “acting on single donors with incorporation of molecular oxygen”, and “protein kinase”. Among the CC terms, we mainly found “chromatin”, “receptor complex”, and “endoplasmic reticulum lumen”. KEGG-based analysis showed that overexpression of genes mainly involved the p53 signaling pathway, ferroptosis, Kaposi sarcoma-associated herpesvirus infection, IL-17 signaling pathway, MicroRNAs in cancer, the TNF signaling pathway, PI3K-Akt signaling pathway, and HIF-1 signaling pathway (Figure 1A-D).

Prognostic features and survival analysis based on ferroptosis-associated lncRNAs

We identified 270 lncRNAs associated with ferroptosis (Table 4). In addition, univariate Cox analysis identified 140 important lncRNAs associated with ferroptosis, which were included in the multivariate Cox analysis. Overall, 11 differentially expressed lncRNAs (Table 5) were found to be independent prognostic indicators for BCa.

Therefore, we proceeded to calculate risk scores and construct a BCa prognostic model using lncRNAs. Kaplan-Meier analysis showed that patients with high-risk lncRNAs expression had poorer survival than low fractional risk lncRNAs ($P < 0.001$, Figure 2A). This risk model (AUC = 0.720) showed superior performance and predictive power than traditional clinicopathological features (Figure 2B). Using patients' risk survival status maps, we found that patients' risk scores were inversely correlated with the survival of BCa patients. Interestingly, the heat map also demonstrated that most of the novel lncRNAs identified in this study were negatively correlated with our risk model (Figure 2C). The AUC predictive values of these new lncRNA models for 1-year, 3-year, and 5-year survival were 0.720, 0.697, and 0.706, respectively (Figure 2D). Univariate and multifactorial Cox analyses showed that lncRNAs model (HR: 1.05, 95CI: 1.02-1.07) and tumor stage (HR: 1.66, 95CI: 1.37-2.02) were independent prognostic factors for OS in BCa patients (Figure 3A-B). lncRNA-mRNA relationships are shown in Figure 3D. A heatmap of the association between prognostic models and clinicopathological manifestations of lncRNAs associated with ferroptosis was also analyzed (Figure 4A). A hybrid column line plot combining clinicopathological characteristics and the novel ferroptosis-related lncRNAs prognostic signature (Figure 4B) was stable and accurate, thus may be applied in clinical management of BCa patients.

Gene set enrichment analysis

The gene set enrichment analysis (GSEA) revealed various immune and tumor-related pathways that are prognostic signature regulators of most novel lncRNAs associated with ferroptosis, such as Adhesion junction, ECM receptor interaction, Chemokine signaling pathway, B cell receptor signaling pathway, TGF- β signaling pathway, MAPK receptor signaling pathway, Notch signaling pathway, and Bladder cancer. (Figure 5).

Expression of immune-related genes

Next, we created a heat map of the immune response based on CIBERSORT, ESTIMATE, MCPcounter, single sample gene set enrichment analysis (ssGSEA), and TIMER algorithm (Figure 6A). ssGSEA based on the TCGA-BLCA data, correlation analysis between immune cell subsets and related functions showed that T cell functions include checkpoint (suppression), lysis, HLA, inflammatory regulation, co-stimulation, co-inhibition, and type II INF response. Significant differences between low-risk and high-risk patients (Figure 6B). As checkpoint inhibitor-based immunotherapy strategies are emerging as one of the best cancer treatment tools for some drug-resistant tumors, we further explored the differences in immune checkpoint expression between the two groups and found significant differences in PD-1, CTLA4, LAG3, and BTLA expression (Figure 6C). In addition, the comparison of m⁶A-related mRNA expression and the expression of METTL3, KIAA1429, RBM15, ZC3H13, YTHDC1, YTHDF1, YTHDF2, HNRNPC, and FTO in the high-risk and low-risk groups were significant (Figure 6D).

Validation of the identified Diff-miRNAs

Figure 7 shows the results of the qRT-PCR. It is shown that the expression of lncRNAs (AL031775.1, AC018653.3, AC011468.1, AL583785.1, AC021321.1, AP003352.1, *ETV7* – *AS1*, U47924.1, AC010326.3) is significantly decreased in the SV-HUC cell line as compared with that in the T24 cell line, while LINC02762 expression did not show significant differences in the two cell lines. Similarly, the expression of lncRNAs (AL031775.1, AC018653.3, AC011468.1, AL583785.1, AC021321.1, *ETV7* – *AS1*, U47924.1, AC010326.3) was significantly upregulated in EJ cell lines, differing from SV-HUC cells. However, the expression of AP003352.1 and LINC02762 did not show significant differences in the two cell lines. The result shows us that eight lncRNAs, AL031775.1, AC018653.3, AC011468.1, AL583785.1, AC021321.1, *ETV7* – *AS1*, U47924.1, AC010326.3, could be used as ferroptosis-related biomarkers to predict the prognosis of BCa patients. provide new targets for their treatment and research. The specific primer sequences are listed in Table 6

Discussion

Ferroptosis or iron-dependent cell death can regulate tumor proliferation, invasion, and progression[25]. Therefore, ferroptosis induction is emerging as a promising anti-cancer therapeutic strategy by triggering tumor cell death, especially in those patients with drug-resistant tumors[26]. In this study, through analysis, we identified 47 DEGs associated with ferroptosis. By GO analysis, we obtained that these DEGs

are involved in the BP intrinsic apoptotic signaling pathway, multicellular organismal homeostasis, and response to toxic substances. MF terms were mainly related to “regulate iron ion binding”, “oxidoreductase activity”, “acting on single donors with incorporation”, and CC terms to chromatin, receptor complex, and endoplasmic reticulum lumen. KEGG signaling pathway prediction analysis further elaborated that these overexpressed genes are mainly involved in the p53 signaling pathway, Ferroptosis, Kaposi sarcoma-associated herpesvirus infection, IL -17 signaling pathway, MicroRNAs in cancer, TNF signaling pathway, PI3K-Akt signaling pathway, and HIF-1. A recent study showed that p53 could enhance SLC7A11 (solute carrier family 7 member 11), SAT1 (spermidine/spermine N1-acetyltransferase 1), and GLS2 (glutaminase 2) expression by repressing ferroptosis. On the other hand, p53 inhibits ferroptosis by directly inhibiting DPP4 (dipeptidyl peptidase 4) activity or by inducing CDKN1A/p21 (cell cycle protein-dependent kinase inhibitor 1A) expression (8, 16). Another independent study also confirmed that ferroptosis could be regulated by p53 signaling and the tumor-associated mutant p53 (mutp53). The primary manifestation is that the regulation of ferroptosis by p53 contributes to the tumor-suppressive function of p53 and, in addition, the accumulation of mutp53 protein in cancer cells creates cancer cells sensitive to ferroptosis[27]. Pretreatment with FG-4592, an inhibitor of prolyl hydroxylase of HIF, reduces renal injury via AKT/GSK-3 β -mediated activation of Nrf2 of advanced stages of ferroptosis [28]. In addition, Takahashi et al. reported that cancer spheroids proliferate using mammalian targets of rapamycin (mTOR) and utilize the lipid peroxidase GPX4 for defense against ferroptosis[29].

In this study, we also retrieved the TCGA database and, based on the TCGA-BLCA dataset, identified lncRNAs associated with iron-dependent cell death. LncRNA expression patterns show specificity for specific tumors and tissue types, highlighting lncRNAs as potential therapeutic targets for cancer. With advances in biotechnology such as genome editing, high-throughput sequencing, and nanotechnology, lncRNAs could potentially serve as molecular targets for cancer therapy. Thus, depending on their tissue-specific expression properties, lncRNAs are considered an emerging and viable candidate for precision medicine. To date, several studies have shown that lncRNAs can act as anti-cancer targets by regulating ferroptosis. Targeting lncRNA-associated key ferroptosis molecules may create new opportunities for a novel and promising means of treating drug-resistant tumors[30]. A recent independent studies suggest that iron-dependent cell death-associated lncRNAs can be a prognostic factor in patients with colorectal cancer and HNSCC patients[31]. In our present study, however, we constructed a model characterized by 11 lncRNAs associated with iron-dependent cell death to predict the prognosis of BCa patients. First, we obtained 270 lncRNAs associated with ferroptosis, and lasso and Cox regression models were used to classify these lncRNAs based on their expression into two groups, high-risk and low-risk, and to explore their potential role in BCa. The classification into high-risk groups was based on risk scores, with statistically significant OS between low-risk groups. AUC also suggested the predictive power of the lncRNA signature, which compared favorably with the traditional cancer stage or cancer grade model. Cox analysis further showed that risk score was an independent prognostic factor for BCa. Finally, we identified 11 lncRNAs signatures, including AL031775.1, AC024060.2, AC018653.3, AC011468.1, AL583785.1, AC021321.1, AP003352.1, *ETV7* — *AS1*, U47924.1, AC010326.3, and LINC02762. Subsequently, using GSEA, we revealed potential signaling pathways for the 11 ferroptosis-associated

lncRNAs. For example, Adhesion junction, ECM receptor interaction, Chemokine signaling pathway, B cell receptor signaling pathway, TGF- β signaling pathway, MAPK receptor signaling pathway, Notch signaling pathway, and Bladder cancer. It is well documented that ferroptosis promotes neutrophil adhesion to coronary vascular endothelial cells via the TLR4/Trif/I type IFN signaling pathway, thereby coordinating neutrophil recruitment to damaged myocardium and promoting programmed cell death[32]. Several recent studies have confirmed the interaction between ferroptosis and immune checkpoint inhibitors and immune cell infiltration [33]. Similarly, lncRNAs and miRNAs have critical roles in ferroptosis. It was shown that LINC00618 promoted VCR-induced ferroptosis and apoptosis, while LINC00618 accelerated ferroptosis in an apoptosis-dependent manner. LINC00618 attenuated lymphatic-specific decapping enzyme (LSH) expression and LSH enhanced SLC7A11 promoter region after recruitment to SLC7A11 transcription, further inhibiting ferroptosis[34]. lncRNA OIP5-AS1 promotes PCa progression and ferroptosis resistance through miR-128-3p/SLC7A11 signaling[35]. However, more interestingly, we also identified a relationship between ferroptosis-associated lncRNAs and m⁶A methylation genes.

In recent years, we have witnessed that ferroptosis-associated lncRNAs play a crucial role in tumor cells and the treatment of tumor drug resistance[36]. Long non-coding RNAs have emerged as novel potential markers for many diseases due to their stability and presence in the circulation[37]. However, the mechanism of action of ferroptosis-associated lncRNAs in BCa patients has not been elucidated. Therefore, this study aimed to use high-throughput sequencing data to identify 11 lncRNA signatures associated with ferroptosis in the hope that their use as ferroptosis-related biomarkers to predict the prognosis of BCa patients could inform the therapeutic modalities of this disease. However, there have been no studies on the role of ferroptosis-related lncRNAs in BCa prognosis to date. These findings may provide a new target for the future treatment and management of cancer.

Despite the encouraging data, many key questions such as the interconnection of ferroptosis with other types of cell death and host immunogenicity remain unresolved. This study explored novel ferroptosis biomarkers in the context of BCa prognosis. These biomarkers could be used to predict and potentially treat bladder cancer. This signature profile deserves further investigation using a different cohort since our findings were not validated using clinical samples. Therefore, an in-deep validation with more clinical data from BCa patients is needed before translating our results from the lab to the clinic.

Conclusion

In summary, we developed a BCa score model based on eleven ferroptosis-related genes (AL031775.1, AC024060.2, AC018653.3, AC011468.1, AL583785.1, AC021321.1, AP003352.1, *ETV7* — *AS1*, U47924.1, AC010326.3, LINC02762). This novel model lays the foundation for developing new research strategies to explore the mechanisms of ferroptosis and present individualized predictions for BCa patients' prognosis.

Declarations

Data Availability

All data generated or analyzed during this study are included in this article.

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Not applicable.

Author contributions

Jian Hou, Zhenquan Lu , Xiaobao Cheng and Runan Dong wrote the main manuscript text, GenYi Qu and Yong Xu performed experiments, Yi Jiang and Guoqing Wu collected data, All the authors reviewed the manuscript and discussed the results and edited the manuscript.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflicts of interest

The authors declare that they have no competing interests.

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Tables

Table 1

The clinical characteristics of patients in the TCGA dataset.

Variable	Number of samples
Age at diagnosis	
$\leq 65 / > 65$	160/237
gender	
Male/Female	294/103
Grade	
Low Grade/High Grade/NA	18/376/3
stage	
I/II/III/IV/NA	2/124/136/133/2
T	
T0/T1/T2/T3/T4/NA	1/3/114/191/57/31
M	
M0/M1/NA	187/10/200
N	
N0/N1/N2/N3/NA	228/45/76/8/40

Table 6

The specific primer sequence list.

Gene name	sequence
AL031775.1	Forward GGTGTCTGTATATTGTCCTATCCA Reverse CAGTCCATCATCAAGATTGTAAGG
AC018653.3	Forward CTACCTGTCCTGCCTCCTTC Reverse GCCCATGCTTTCCAGATGTATT
AC011468.1	Forward AAGCAGTATTTGGAAGCACTT Reverse AACTCCTGACTTCTAGGTTGAGA
AL583785.1	Forward CTGGGAAAGGCAAGGATGTG Reverse ACAAACTGGGTGGCTTACAAC
AC021321.1	Forward GCATCTGTCACTGTTCTGTTCTAT Reverse TAGCCTTCCTAAATCTGGTCACT
AP003352.1	Forward TGCCTCAGCCTCTCAAGTAG Reverse CGTGGCTCACACCTGTAATC
<i>ETV7 – AS1</i>	Forward CAACGGTGCTAGTGGTAGTAGT Reverse GCTTCTCCTTCTCGGTGACA
U47924.1	Forward AATGGGTGATGTGGGAGAAATG Reverse TCCTGGGTCTGTCTTTGTGT
AC010326.3	Forward GCTTCCGAGATCAGACGAGAT Reverse TCAAAGAGAGATGCCACACATTG
LINC02762	Forward CATTGAGGAGTCAAGCAGCAAA Reverse TGAGGCAGGAGAATCACTTGA
GAPDH	Forward Reverse

Figures

Figure 1

GO and KEGG analyses for ferroptosis-related differentially expressed genes. (B) GO and (A) KEGG. GO and KEGG analyses for ferroptosis-related differentially expressed genes by metascape(C-D).

Figure 2

Ferroptosis-related lncRNAs signature based on TCGA. (A) Kaplan-Meier curves result, (B). The AUC values of the risk factors, (C). Risk survival status plot, (D) The AUC of the for the prediction of 1, 3, 5-year survival rate of BCa and (E) The DCA of the risk factors.

Figure 3

Univariate and multivariate COX analysis for the expression of ferroptosis-related lncRNAs. (A). univariate, (B). multivariate, (C). Univariate COX analysis for the expression of ferroptosis-related lncRNAs (D). The relationship between the novel lncRNA and mRNA expression.

Figure 4

Heatmap for ferroptosis-related lncRNAs prognostic signature and clinicopathological manifestations(A). A nomogram for both clinic-pathological factors and prognostic ferroptosis-related lncRNAs(B).

Figure 5

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A **B**

Figure 6

(A). Heatmap for immune responses based on CIBERSORT, ESTIMATE, MCPcounter, ssGSEA, and TIMER algorithms among high and low risk group.(B) ssGSEA for the association between immune cell subpopulations and related functions (C). Expression of immune checkpoints among high and low BCa risk groups. (D)The expression of m6A-related genes between high and low BCa risk group.

Figure 7

The results of qRT-PCR shows the differential expression of iron death-associated LncRNAs associated with bladder cancer prognosis between bladder cancer cells(T24 and EJ cell lines) and normal bladder epithelial cells line SV-HUC.(* Means $p < 0.05$, ** means $p < 0.001$, *** means $p < 0.0001$).

Supplementary Files

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