Integrated Analysis of lncRNA-associated ceRNA Network Identifies Prognostic lncRNAs in Bladder Cancer Based on Whole Transcriptome Sequencing and TCGA Database

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Abstract

Background

Bladder cancer (BC) is a serious urinary tract malignancy with high incidences and deaths. Here, we aim to explore the OS-related lncRNAs and mRNAs, and constructed a reliable predicting model for accurately predict BC prognosis.

Methods

3 matched BC tumor samples and adjacent normal samples were processed by whole transcriptome sequencing. Differentially expressed lncRNAs and mRNAs (DE-lncRNAs and DE-mRNAs) were identified with the thresholds $|\log_2(FC)| > 2$ and p value < 0.05 using DEGseq2 R package. Meantime, 408 BC samples and 19 normal samples were downloaded from TCGA database. And DE-lncRNAs and DE-mRNAs were screened with thresholds $|\log_2(FC)| > 0.5$ and p value < 0.05 . The DE-lncRNAs and DE-mRNAs were overlapped between RNA-sequencing data and TCGA data. Then, overall survival (OS)-related DE-lncRNAs and DE-mRNAs were screened and a prognostic gene signature and risk model were constructed using Univariate Cox and stepwise multiple regression analysis. Risk score was calculated based on prognostic gene signature. The independent risk factors were identified with incorporation into clinical risk factors using Univariate Cox and stepwise multiple regression analysis. A predicting model was constructed based on independent factors, and calibrated using Time-dependent ROC curves. Finally, the differentially expressed genes (DEGs) between high-risk and low-risk groups were identified with thresholds $|\log_2(FC)| > 0.5$ and p value < 0.05 . And the biofunction was determined by Gene Set Enrichment Analysis (GSEA).

Results

A total 2210 DE-lncRNAs and 2334 DE-mRNAs were identified based on whole transcriptome sequencing. And a total 3724 DE-lncRNAs and 2689 DE-mRNAs were identified based on TCGA database. Then, 137 DE-lncRNAs and 278 DE-mRNAs were screened by overlapping RNA sequencing data and TCGA data. A total 13 gene signature were identified to be closely related with OS in BC. Moreover, these 13-OS-related gene signature were identified as independent risk factors with clinical risk factors. Then, a nomogram was constructed and confirmed as the calibration and accuracy predicating model for OS in BC. Finally, a total 739 DEGs were identified between high-risk and low-risk groups, most of DEGs enriched in immune-related pathways. And an OS-related ceRNA network was selected based on 13-OS-related signature.

Conclusion

Our finding provided novel OS-related prognostic signature and reliable predicting model for BC patients, which might facilitate individualized treatment and prognostic evaluation.

Introduction

Bladder cancer (BC) is a malignant urinary system neoplasm, which causes 549,000 new cases and 200,000 deaths in 2018, and among in men than women about 4 times [1, 2]. Exposure to chemical, drinking water pollution, and smoking contribute to BC initiation and developing [3], it has been estimated that approximate 50% of BC cases attribute to smoking in the United States [4, 5]. In China, BC emerges prevalent with the rapid industrialization, which increasing exposure to occupational chemical [6]. However, the treatment for BC remains unfavorable nowadays. Due to the high heterogeneous malignancy and limited therapeutic strategy, including surgery, immunotherapy, and chemotherapy, the 5-years survival rates of BC reveal flat [7-9]. Therefore, it is urgent to explore the efficient diagnostic and therapeutic biomarkers for BC.

BC reveals the clinically and biologically heterogeneous features, thus, molecular characteristics of cancer reflect the cancer initiation, progression, and clinical prognosis, as well as contribute to explore the biomarkers for diagnosis and therapy [10, 11]. Based on advanced the genetic sequencing technologies and computational analysis cause the explosive increasing large cohorts of tumors as well as genomic researches [12, 13]. Abundant genomic subtypes, molecular classifications, and patient stratification haven been constructed in BC [14, 15]. Such as, a novel mutant gene HECW1 has been identified according to

whole-exome sequencing in muscle-invasive transitional cell carcinoma of BC [16]. Moreover, risk prognostic signature of conventional urothelial carcinoma and high grade T1 urothelial BC are identified based on whole transcriptome sequencing analysis [17]. Therefore, transcriptomics and next-generation sequencing (NGS) are powerful for deciphering genome structure and function, exploring genetic network and cell-to-cell interaction [18].

MRNA processing is essential for gene expression to regulate tumorigenesis and development, but also non-coding RNAs (ncRNAs), including circular RNAs (circRNAs), long non-coding RNAs (lncRNAs), and microRNAs (miRNAs) contribute to tumor initiation, progression, and clinical prognosis [19]. lncRNAs is a group of ncRNAs in length more than 200 nucleotides that associate to various functions. lncRNAs exert biological functions display tissue/ell-specific and distribution of subcellular [20]. The biological contributions of lncRNAs include regulating transcription in cis and trans manners, regulating mRNA expression, and organizing nuclear domains [21]. In general, lncRNAs compete with miRNAs to up-regulate mRNA expression by acting as competitive endogenous RNAs (ceRNAs) [22]. Increasing evidences have indicated that lncRNAs play the vital roles in BC progression. Such as lncRNA SOX2OT enhances BC developing and progressing via promoting SOX2 expression [23]. And lncRNA DANCER acts as an oncogene by increasing MSI2 expression via competing miR-149 in BC [24]. Of interest, abundant lncRNAs have been identified as the prognostic biomarkers based on the bioinformatics analysis, for example, autophagy-related LINC02178, AC108449.2, Z83843.1, FAM13A-AS1 and USP30-AS1 function as the prognostic signature for bladder urothelial carcinoma patients [25]. Moreover, UCA1 has been demonstrated as a diagnostic biomarker, and HOTAIR and GAS5 act the biomarkers for disease-free survival, recurrence-free survival, and disease-specific survival in BC [26]. AC005014.2, AC010503.4, AL450384.2, LINC00930 and SH3BP5-AS1 have been found upregulation in BC and associate to immune cell infiltration [27]. However, OS-related signature and ceRNA network in predicting the survival and prognosis of BC remain unclear.

Here, we integrated analysis of whole transcriptomic RNA sequencing and the RNA expression files from the Cancer Genome Atlas (TCGA) database to identify the 13-OS-related signature, and construct and validate the risk model and prognostic predicting model for BC. Finally, the prognostic ceRNA network, LINC01322-hsa-miR-125b-2-3p-IGF2 and LINC01322-hsa-miR-6507-5p-MLLT11 were identified as key prognostic ceRNA in BC.

Methods

Patients and clinical specimen collection

Three paired tumor tissues and matched normal tissues were obtained from BC patients who underwent surgical resection before chemotherapy or radiotherapy. All patients were known the purpose of this study and signed the inform consents. All experiments performances were approved by the Medical Ethics Committee of the Second Affiliated Hospital of Kunming Medical University. The pathology of BC was confirmed by at least three pathologists before surgery. All specimen were collected and stored in liquid nitrogen.

RNA extraction and quality control

Total RNA was extracted from tumor tissues and matched normal tissues using TRIzol reagent (Takara, Dalian, China) following the manufacturer's instruction. Then, RNA purity, concentration, and integrity were detected using a NanoPhotometer® spectrophotometer (IMPLEN, CA, USA), Qubit® RNA Assay Kit in Qubit® 2.0 Fluorometer (Life Technologies, CA, USA), and RNA Nano 6000 assay Kit of a Bioanalyzer 2100 system (Agilent, Technologies, CA, USA), respectively.

Library construction

After that, the library preparation was performed successive using Epicentre Rbio-zero™ rRNA Removal Kit (Epicentre, USA) and NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina® (NEB, USA) according to the manufacturer's protocol. Briefly, amount of 3 µg RNA per sample was used to the preparation. Firstly, the rRNA was removed using Epicentre Rbio-zero™ rRNA Removal Kit (Epicentre, USA) and then cleaned up using ethanol precipitation. Secondary, the sequencing libraries were

generated using NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina® (NEB, USA) according to the manufacturer's protocol. The procedures of sequencing libraries construction shown as following, the first strand cDNA was synthesized using random hexamer primer and M-MuL V Reverse Transcriptase (RNase H⁻), the second cDNA then synthesized using DNA Polymerase I and RNase H⁻. Subsequently, the 3' ends of DNA fragments were adenylation via exonuclease and polymerase activities, and NEBNext adaptor with hairpin loop structure were ligated to prepare for hybridization. The cDNA fragments of preferentially 150-200 bp in length were screened with AMPure XP system (Beckman, Coulter, Beverly, USA). After that, the second cDNA fragments which containing uracil (U) were degraded with USER Enzyme (NEB, USA). Then, PCR was performed with Phusion High-Fidelity DNA polymerase, Universal PCR primers and Index (X) Primer. At last, products were purified with AMPure XP system (Beckman, Coulter, Beverly, USA) and library quality was evaluated using Agilent Bioanalyzer 2100 system.

RNA sequencing

Finally, the cDNA libraries of index-coded samples were clustered using cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS (Illumina) following the manufacturer's protocol. Then, the libraries were sequenced on an Illumina HiSeq 4000 platform and 150 bp paired-end reads were generated.

Data analysis

After RNA-sequencing, raw data of fastq format, which containing adapter, poly-N, and low-quality reads were converted into high-quality clear data. Then, high-quality clear data were mapped to the reference genome using HISAT2 v2.2.1. The reads numbers mapped to each gene were counted using HTSeq v0.13.5. After that, Fragments Per Kilobase of transcript sequence per Millions (FPKM) of each gene was calculated based on the length of the gene and reads count mapped to this gene. Then, the DE-mRNAs and DE-lncRNAs between tumor samples and normal samples were identified using DEGseq2 R package with the thresholds $|\log_2(\text{Fold change (FC)})| > 2$ and $p \text{ value} < 0.05$.

Data acquisition and processing

The expression profiles and clinical information were downloaded from the TCGA-BLCA project of the Genomic Data Commons Data Portal of TCGA (<https://portal.gdc.cancer.gov/>), which contained 433 BC cases, excluding the non-primary tumors and samples lacking the clinical information, finally, 408 tumor samples and 19 normal samples were used for this study. The RNA expression profiles were normalized using DEGseq2 R package.

Screening of DE-lncRNAs and DE-mRNAs

DE-mRNAs and DE-lncRNAs were screened using DEGseq2 R package according to the thresholds $|\log_2(\text{FC})| > 0.5$ and $p \text{ value} < 0.05$, respectively. Then, differences between tumor and normal were visualized using volcano plots and heatmaps, which performed using ggplot2 R package and pheatmap R package, respectively.

Overlapping the RNA-seq data and TCGA data

To identify the significant DE-mRNAs and DE-lncRNAs in bladder cancer, the DE-mRNAs and DE-lncRNAs were overlapped and identified both in RNA-seq data and TCGA data.

Construction of the lncRNA-miRNA-mRNA network

The binding relationship between lncRNAs and miRNAs were speculated using miRanda database, and the binding relationship between miRNAs and mRNAs were speculated using Targetscan database. The positive association between lncRNAs and mRNAs was analyzed. Then, the ceRNA network was constructed based on the regulatory speculation, and visualized by Cytoscape software.

Identification of the OS-related DE-lncRNAs and DE-mRNAs in BC patients

To identify the OS-related DE-lncRNAs and DE-mRNAs, all BC patients were distributed into training set and validation set at a ratio of 7:3. The correlation between DE-lncRNAs/DE-mRNAs and OS were assessed by Kaplan-Meier analysis and Univariate Cox regression analysis. DE-lncRNAs and DE-mRNAs with $p\text{ value}<0.05$ and with the consistence between expression and prognostic effects were considered as the candidates OS-related DE-lncRNAs and DE-mRNAs in training set.

Construction and evaluation of the risk model in BC

After univariate Cox regression analysis, total 35 OS-related candidates (10 DE-lncRNAs and 25 DE-mRNAs) were incorporated into a stepwise multivariate regression model to predicate the prognostic values of 35 OS-related candidates, and 13-OS-related signature was confirmed as the risk signature. Then, the risk score was calculated based on predict. Coxph fuction of survival R package, and shown as follows: $\text{risk score} = e^{\sum (\text{each gene's expression levels} \times \text{corresponding coefficient})} / e^{\sum (\text{each gene's mean expression levels} \times \text{corresponding coefficient})}$. Then, each BC patients in entire sets were assigned a risk score according to the formula. And the patients were divided into high-risk and low-risk groups according to the median risk score. Subsequently, the survival differences between high-risk and low-risk groups were determined by Kaplan-Meier methods and log-rank test. In addition, association between clinical characteristics (such as T/N/M stages, pathological stages, and other clinical risk factors) and independent prognostic signature were determined by a stratified analysis. Moreover, the efficiency of the prognostic model was evaluated by Kaplan-Meier survival curves and time-dependent ROC curves. Furthermore, the association between risk score and clinicopathological characteristics was examined to confirm the reliable and accuracy of risk model.

Construction of the risk signature-based prognostic nomograms

To identify independent predictors of OS in BC, the clinical factors were incorporated into univariate and stepwise multivariate regression analysis. And a nomogram was constructed to predicate median survival time and 3- and 5-years survival rates of BC patients using rms R package. the accuracy of nomograms was calculated by concordance index (C-index) and calibration curves.

Screening of the DEGs between high-risk and low-risk groups in BC patients

To explore the potential function and mechanism between high-risk and low-risk score groups, the DE-mRNA between high-risk and low-risk score groups were screened out according to the cutoff values $|\log_2(\text{FC})|>0.5$ and $p\text{ value}<0.05$.

GSEA analysis (high/low risk score group)

For further to identify the regulatory mechanism between high-risk and low-risk score groups, GSEA was performed to explore the biofunction of the risk score-related genes. And the GO term, KEGG pathway, and Reactome term were enriched by GSEA. The cutoff was $\text{FDR}<0.05$ and $|\text{NES}|>1$.

Results

Identification of the DE-lncRNAs and DE-mRNAs and construction of a ceRNA network in BC

The workflow diagram was constructed to illustrate the designs of this study (Fig. 1). Next-generation sequencing was performed to investigate the differences between tumor tissues and adjacent normal tissues. A total 2210 DE-lncRNAs and 2334 DE-mRNAs between tumor tissues and normal tissues were screened out according to the criteria $|\log_2(\text{FC})|>2$ and $p\text{ value}<0.05$ (Fig. 2A-B, Table S1-2). DE-lncRNAs included 1255 upregulated DE-lncRNAs and 955 downregulated DE-lncRNAs. DE-mRNAs consisted of 689 upregulated DE-mRNAs and 1645 downregulated DE-mRNAs. Moreover, hierarchical clustering displayed that significant difference between tumor samples and normal samples (Fig. 2C-D). Furthermore, the DE-lncRNAs and DE-mRNAs between 408 bladder cancer samples and 19 normal samples were identified. Results indicated that 3,724 DE-lncRNAs (2675 upregulated and 1067 downregulated DE-lncRNAs) and 2,689 DE-mRNAs (1261 upregulated DE-mRNAs and 1428 downregulated DE-mRNAs) were identified with threshold $|\log_2(\text{FC})|>0.5$ and $p\text{ value}<0.05$ (Table S3-4). Then, 137 overlapped DE-lncRNAs and 278 overlapped DE-mRNAs were obtained from RNA-seq data and TCGA data (Fig. 2E-F, Table S5-

6), which contained 96 upregulated DE-lncRNAs and 41 downregulated DE-lncRNAs, and 47 upregulated DE-mRNAs and 231 downregulated DE-mRNAs. Based on the speculated miRNAs, which directly targeted lncRNAs and mRNAs, the ceRNA network was constructed and visualized in Fig. 2G. The ceRNA network constituted with 17 lncRNAs, 32 mRNAs, and 29 miRNAs, and formed 312 edges and 78 nodes (Table S7).

Screening of 13-OS-related signature from BC based TCGA database

In order to determine whether these DE-lncRNAs and DE-mRNAs related to the OS in BC patients, these RNAs were filtered with KM methods and log-rank test. The results suggested that there are 18 DE-lncRNAs and 44 DE-mRNAs were assessed as OS-related candidates (Fig. 3, Table 1). Then, univariate Cox analysis was performed to determine whether 18 DE-lncRNAs and 44 DE-mRNAs are the risk factors according to threshold $P < 0.05$ (Table 2). Therefore, a total 10 DE-lncRNAs and 25 DE-mRNAs were identified as the risk factors (Fig. 4A, Table 3). Furthermore, the 35 OS-related candidates were incorporated into a stepwise multiple regression model, 13-OS-related candidates, which including 6 DE-lncRNAs and 7 DE-mRNAs were identified (Fig. 4B, Table 4). The stepwise multiple regression model was confirmed as the finally predicated model, and the 13-OS-related candidates which selected from the stepwise multiple regression model were integrated into predicative signature to calculate the risk score. Based on the median of risk score, entire BC patients were divided into high-risk and low-risk groups, the OS statues and expression files of each patient indicated that high-risk group with low survival rate than low-risk group (Fig. 4C-D). Moreover, the discrimination ability and predictive sensitivity of the predicated model were determined by Kaplan-Meier and time-dependent ROC curves. Kaplan-Meier curve revealed that high-risk group showed shorter survival time compared with low-risk group (Fig. 4E). Kaplan-Meier curve displayed the AUC value of 1-years, 2-years, 3-years, 4-years, and 5-years OS predication of the prognostic predicted model were 0.695, 0.784, 0.771, 0.760, and 0.754 in training set, and showed the AUC value of 1-years, 2-years, 3-years, 4-years, and 5-years OS predication of the prognostic predicted model were 0.619, 0.641, 0.657, 0.699, and 0.715 (Fig. 4F-G). Then, we investigated the expression of thirteen OS-related signature in training and validation sets. As shown in Fig. 4H, 13-OS-related signature were separated into two risk subgroups, IGF2, MLT11, KIAA1462, GPIHBP1, PTGIS, LHFP, and EGFR_AS1 upregulated in high-risk group whereas RASL12, RP4_761J14.8, RP3_412A9.17, RP11_92F20.1, RP11_338L22.2, and XXbac_BPG299F13.17 downregulated in low-risk group, in both sets. These data suggested that discrimination ability and predictive sensitivity of the prognostic predictive model based on 13-OS-related signature.

Association between risk score and clinical characteristics

We further investigated the correlation between risk score and clinical characteristics using Wilcox test or Kruskal-Wallis test. We found that the increasing risk scores positively associated with distant metastasis (M), advanced pathological stages (III/IV), malignant primary tumor (T) (Fig. 5A). In addition, high-risk group showed shorter survival time in patients and not correlated with age and gender stratification (Fig. 5B-C). Besides, the high-risk patients exhibited shorter survival time with or without distant metastasis and lymph node metastasis (Fig. 5D-E). The high-risk patients also showed shorter survival time in III/IV stages (Fig. 5F). Moreover, high-risk patients exhibited poor survival with or without prior malignancy, treatment, and different treatment types (Fig. 5H-J).

Identification and validation of OS-related signature as independent prognostic factors for BC patients

To identify of the independent risk factors for BC patients, Univariate Cox and stepwise multivariate regression analyses were performed with clinicopathological factors, such as age, gender, T/N/M stages, pathological stages, prior malignancy, treatment, BMI in entire sets. The results indicated that risk score ($P < 0.001$) was independent prognostic indicator in BC (Fig. 6A-B, Table 5).

Construction and validation of a nomogram for prognostic prediction in BC

We integrated traditional risk factors, such as age, gender, T/N/M stages, pathological stages, prior malignancy, treatment, BMI, and risk score to construct the prognostic nomograms predicting median survival time, 3-year, and 5-year OS of BC patients using a stepwise multiple regression model (Fig. 7A-B). The concordance index (C-index) and calibration plots were used to

evaluate the discrimination and calibration abilities of the predictive nomogram. The C-index of predictive nomogram was 0.7312959, and the C-index after calibrating this nomogram was 0.7122346 (Fig. 7C). Then, 3-year and 5-year OS probabilities generated by the nomogram, and results indicated the predicated 3-year and 5-year OS probabilities closed to acute 3-year and 5-year OS probabilities (Fig. 7D-E). Our finding indicated that the effectiveness and sensitivity of the predictive nomogram.

Function analysis of 13 OS-related signature

GO annotation and KEGG pathway enrichment were performed to analysis the biofunction of 13-OS-related signature. The GO terms including biological processes, cellular components, and molecular processes, in this study, the molecular processes primarily enriched in insulin-like growth factor receptor binding, protein transmembrane transporter activity, protein transporter activity, insulin receptor binding, macromolecule transmembrane transporter activity, protein serine/threonine kinase activator activity, lipoprotein particle binding, peptide transmembrane transporter activity, and intramolecular oxidoreductase activity, IGF2, GPIHBP1, and PTGIS were involved this processes (Fig. S1A). However, there is no significant KEGG pathway enrichment analysis (Fig. S1B). These data suggested that OS-related signature might affect the several biological processes to regulate BC progression.

Identification of the DEGs between high-risk score group and low-risk score group in BC

We also examined whether significant differences and potential regulatory mechanism between high-risk score group and low-risk score group according to the TCGA dataset. As shown in Fig. 8 and Table S8, total 739 DEGs were identified between high-risk score and low-risk score groups, which including 481 upregulated and 258 downregulated DEGs with threshold $|\log_2(FC)| > 0.5$ and p value < 0.05 . The hierarchical clustering revealed that significant differences between high-risk score and low-risk score groups (Fig. 8B). There are abundant numbers of DEGs between high-risk score and low-risk score groups. In addition, we also found IGF2, PTGIS, LHFPL6, RASL12, MLLT11, JCAD, GPIHBP1, and EGFR-AS1 upregulated in high-risk group compared to low-risk group, but RP4_761J14.8, RP3_412A9.17, RP11_92F20.1, RP11_33BL22.2, and XXbac_BPG299F13.17 downregulated in high-risk group compared to low-risk group (Table S9).

Function analysis of the risk score-related DEGs in BC

The underlying mechanism of risk score-based DEGs was explored by GSEA. Results revealed that high-risk group mostly associated to biological processes, such as mitotic cell cycle, mRNA metabolic process, DNA metabolic process, etc. (Fig. 9A, 9F, Table 6, Table S10). The related cell components included mitochondrial part, chromosome, chromosome part, nuclear body and so on (Fig. 9B, 9G, Table 6, Table S10). And the molecular functions included double-stranded DNA binding, chromatin binding, ubiquitin-like protein transferase activity and so on (Fig. 9C, 9H, Table 6, Table S10). Besides, KEGG pathway enrichment analysis indicated risk score-based DEGs associated to Herpes simplex virus 1 infection, RNA transport, Spliceosome, mRNA surveillance pathway, etc. (Fig. 9D, 9I, Table 7, Table S11). And the Reactome pathways enrichment revealed risk score-based DEGs mostly enriched in Metabolism of RNA, Cell Cycle, Transcriptional Regulation by TP53, etc. (Fig. 9E, 9J, Table 8, Table S12). Of interest, as shown in Table 9, risk score-based DEGs involved in multiple immune-related pathways. Above results indicated that high risk score patients associated to multiple biological function alterations, including immune dysregulation.

Prognostic ceRNA network analysis

Based on previous studies, we identified 13 prognostic signature and constructed the ceRNA networks. Therefore, the prognostic ceRNA network, LINC01322-hsa-miR-125b-2-3p-IGF2 and LINC01322-hsa-miR-6507-5p-MLLT11 were selected from the ceRNA network.

Discussion

BC remains a tough malignant tumor with the high incidence and mortality, which challenges accurate diagnosis and efficient treatment. More than 90% patients have been diagnosed as transitional cell carcinoma (TCC), which divides into muscle

invasive and non-muscle invasive, the muscle invasive BC patients exhibit poorer prognosis and survival rate than others [28]. In the past decades, endoscopic evaluation, biopsies, and intravesical treatment have been used to diagnose and extent periods of survival time, moreover, the high risk patients are treated with chemotherapy, radical cystectomy, and immunotherapy [29, 30]. However, the prognosis and survival rate for BC patients remain unfavorable. Therefore, based on the whole transcriptomic sequencing and bioinformatic analysis, we found 13-OS-related signature associated to BC, which including 7 mRNAs and 6 lncRNAs. Besides, we also constructed a prognostic model and a predicating model for BC patients, and selected a significant OS-related ceRNA network.

Emerging evidences indicate that lncRNAs exert vital roles in BC initiation and progression, and act as powerful biomarkers for diagnosis and treatment. It has been reported that lncRNA CASC11 plays as an oncogene to accelerate BC cell growth by sponging miR-150 [31]. lncRNA CCAT1 remains promoting BC proliferation, migration, and invasion via inhibiting several miRNAs [32]. Moreover, lncRNA RMRP may be a potentially therapeutic target for BC patients [33]. In addition, abundant numbers of lncRNAs have been identified as the prognostic predictors in BC. Such as, upregulation of lncRNA LINC00641 associates to poor prognosis and promote progression via regulating h miR-197-3p/KLF10/PTEN/PI3K/AKT cascade in BC [34]. lncRNA GCLNC1 increasing relates to poor survival rates in patients with BC via enhancing MYC expression [35]. Here, we identified that EGFR-AS1 acted as a high-risk factor for BC patients, whereas RP4_761J14.8, RP3_412A9.17, RP11_92F20.1, RP11_338L22.2, and XXbac_BPG299F13.17 were identified as the low-risk factors for BC patients. EGFR-AS1 has been reported that acts as an oncogene in multiple cancers, such as prostate cancer, esophageal squamous cell carcinoma, glioma, renal cancer, non-small lung cancer, and BC [36-41]. Our finding confirmed that EGFR-AS1 promotes BC progression. Nevertheless, 5 novel lncRNAs, P4_761J14.8, RP3_412A9.17, RP11_92F20.1, RP11_338L22.2, and XXbac_BPG299F13.17 have been found that associated to favorable prognosis of BC patients.

Additionally, IGF2, MLLT11, RASL12, KIAA1462, GPIHBP1, PTGIS, and LHFP were identified as the risk factors for BC. RASL12 and KIAA1462 associated to optimistic prognosis whereas IGF2, MLLT11, GPIHBP1, PTGIS, and LHFP related to poor prognosis of BC patients. RASL12 is one number of RAS-related guanosine triphosphatases (GTPases), which function as the tumor suppresser [42]. However, the tumor suppressor function of RASL12 rarely has been reported. KIAA1462, also named as Junctional cadherin 5 associated (JCAD5), is a coronary artery disease associated gene [43]. It has been reported acting as an oncogene in liver cancer, but its role and regulatory mechanism in BC remain unknown. Our finding predicated that KIAA1462 might act as a tumor suppressor in BC. IGF2 is a mitogenic peptide hormone and expressed in liver and many other tissues, it also associates to metabolic processes [44]. Previous studies have indicated that IGF2 expression promotes BC aggressive behaviors and serves as a prognostic biomarker for BC [45, 46]. In the present study, IGF2 also has been demonstrated as a prognostic biomarker for BC. MLLT11, known as ALL1-fused gene from the chromosome 1q (AF1q), has been found as an oncogene in multiple cancers and is related to poor outcome of cancers [47-51]. Despite MLLT11 has been found relating to BC development, the prognostic predicting value remains unclear. Here, our results revealed MLLT11 served as a prognostic biomarker for BC. GPIHBP1, also named as glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein, which is a GPI-anchored protein of capillary endothelial cells and necessary for lipoprotein lipase (LPL)-mediated triglyceride-rich lipoproteins (TRLs) processing [52, 53]. It has been found upregulation in gliomas and breast cancer [53, 54]. There are firstly found in BC. PTGIS, a prostacyclin synthase, have the function of dilating blood vessels and inhibiting platelet aggregation [55]. High expression of PTGIS associates liver metastasis and poor prognosis in colon cancer patients [56]. It also has bene found PTGIS associates to immune cell infiltration in lung cancer, ovarian cancer, and gastric cancer [57]. However, the regulatory mechanism of PTGIS in BC remains unclear. LHFP, is a member of LHFP family, upregulation of LHFP associates with mesenchymal differentiation in gliosarcoma [58, 59]. It almost has no reported in other cancers. Although LHFP has been identified as a prognostic biomarker in BC, the role and mechanism of it also need furtherly investigate.

Furthermore, we also demonstrated that T/N/M stages, pathologic stage, and 13-OS-related signature were identified as the independent risk factors in BC. And we also constructed a reliable predicting model for BC patients. Traditionally, T/N/M stages and pathologic stage are the criterion for establishing the treatment and assessing the prognosis for cancer patients [60]. Moreover, it also exhibits various responses for similar treatment in different stages of patients [61]. Therefore, in our study, the clinical factors combine with molecular profiling are need for constructing the reliable predicting model. Hence, the prognostic

model and predicating model in this study are reliable in BC. The BC samples from TCGA database were divided into high-risk and low-risk groups, the DEGs and regulatory mechanism between high-risk and low-risk groups were explored. We found most of DEGs enriched in mRNA processing, DNA replication, and metabolic processing. Of interest, numerous immune-related pathways have been enriched in high-risk and low-risk groups. Finally, based on the OS-related signature, an OS-related ceRNA network in BC has been selected.

Conclusion

Taken together, we identified 13-OS-related signature, which acted as prognostic biomarkers for BC. Based on 13-OS-related signature we constructed a reliable predicting model to predict the survival time in BC. In addition, we also identified DEGs and pathways in high-risk and low-risk groups based on 13-OS-related signature, and selected an OS-related ceRNA network in BC. Our finding supplied 13 prognostic factors and predicting model in BC. Therefore, we further to explore the function and potential mechanism of these genes in the subsequent studies.

Abbreviations

BC: Bladder cancer; lncRNA: Long non-coding RNA; ceRNA: Competing endogenous RNA; miRNA: MicroRNA; DEGs: Differentially expressed genes; DE-lncRNAs: Differentially expressed lncRNAs; DE-mRNAs: Differentially expressed mRNAs; TCGA: The cancer genome atlas; OS: Overall survival; GSEA: Gene set enrichment analysis; FPKM: Fragments Per Kilobase of transcript sequence per Millions; FC: Fold change

Declarations

Acknowledgements

Not applicable

Authors' contributions

Conceptualization ideas: Shi-wei Xiao, Jian-song Wang.

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All authors read and approved this final manuscript.

Availability of data and materials

The datasets analyzed during the current study are available in the TCGA database (<https://portal.gdc.cancer.gov/>).

Ethics approval and consent to participate

All patients were known the purpose of this study and signed the inform consents. All experiments performances were approved by the Medical Ethics Committee of the Second Affiliated Hospital of Kunming Medical University.

Consent for publication

All authors agree to publish.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 62 significant survival-related DE-mRNAs and DE-lncRNAs in BC.

gene or Clinical features	P value
C14orf132	0.0028
C8orf88	0.0161
CALD1	0.0096
CCDC80	0.0165
CD248	0.001
CHRD1	0.0311
CLIP3	0.0449
COL14A1	0.01
COL21A1	0.0406
COL6A2	0.0203
CTSG	0.0162
CYR61	0.0114
EFEMP1	0.0098
EGR2	0.0385
FLNC	0.0301
GEM	0.0435
GPIHBP1	0.0281
IGDCC4	0.0056
IGF2	4.00E-04
KCNK3	0.0339
KIAA1462	0.0458
KLHL23	0.0354
LHFP	0.0039
MFAP4	0.0032
MLLT11	0.0246
NFE2L3	0.0359
P2RY1	0.0411
PID1	5.00E-04
PTGFR	0.0141
PTGIS	0.023
PTRF	0.0459
RASL12	0.0037
SMYD2	0.017
SOBP	0.0248

SPON1	0.006
SPRN	0.0206
TGFB1I1	5.00E-04
TMEM100	0.028
TNFAIP8L3	0.0343
TPM2	0.0278
TPPP3	0.0177
UNC5C	0.0168
ZNF117	0.0199
ZNF521	0.0032
CTB_186G2.1	0.0088
CTB_31020.4	0.0287
EGFR_AS1	0.036
IL10RB_AS1	0.0142
LINC00702	0.005
LINC01322	0.0143
RP11_338L22.2	0.0171
RP11_355N15.3	0.0319
RP11_362K14.5	0.0442
RP11_417E7.2	0.0191
RP11_7F17.8	0.0098
RP11_92F20.1	0.0059
RP3_412A9.17	0.0068
RP3_508I15.18	0.0371
RP4_635E18.7	0.0467
RP4_761J14.8	0.0021
SPAG5_AS1	0.0349
XXbac_BPG299F13.17	0.0126

Table 2 Univariate Cox analysis of the of 25 DE-mRNAs and 10 DE-lncRNAs in BC.

Gene symbol	Coefficient	HR (95% CI for HR)	Wald. test	z	P. value
SMYD2	0.33	1.4 (1.1-1.8)	6.6	2.6	0.01
SPRN	-0.31	0.73 (0.54-0.99)	4	-2	0.045
IGF2	0.088	1.1 (1-1.1)	13	3.5	0.00039
MLLT11	0.14	1.2 (1-1.3)	4.6	2.2	0.031
CALD1	0.14	1.1 (1-1.3)	5.3	2.3	0.021
FLNC	0.093	1.1 (1-1.2)	4.6	2.1	0.032
RASL12	0.14	1.2 (1-1.3)	4.9	2.2	0.027
PID1	0.17	1.2 (1-1.4)	4.2	2	0.04
CLIP3	0.13	1.1 (1-1.3)	4.4	2.1	0.037
TGFB11I	0.2	1.2 (1.1-1.4)	7.3	2.7	0.0068
COL14A1	0.18	1.2 (1.1-1.3)	8.5	2.9	0.0036
MFAP4	0.11	1.1 (1-1.2)	6.1	2.5	0.014
KIAA1462	0.16	1.2 (1-1.3)	4.9	2.2	0.027
GPIHBP1	0.18	1.2 (1-1.4)	4.2	2	0.041
PTGIS	0.1	1.1 (1-1.2)	6	2.5	0.014
CCDC80	0.13	1.1 (1-1.3)	6.7	2.6	0.0096
LHFP	0.25	1.3 (1.1-1.5)	9.7	3.1	0.0018
SPON1	0.11	1.1 (1-1.2)	5.1	2.3	0.024
UNC5C	0.29	1.3 (1.1-1.7)	6.9	2.6	0.0088
IGDCC4	0.24	1.3 (1-1.6)	5.2	2.3	0.023
CD248	0.16	1.2 (1-1.3)	6.2	2.5	0.013
ZNF521	0.21	1.2 (1.1-1.4)	6.8	2.6	0.0093
COL6A2	0.1	1.1 (1-1.2)	4.6	2.1	0.032
TPPP3	0.14	1.2 (1-1.3)	5.8	2.4	0.016
EFEMP1	0.099	1.1 (1-1.2)	6.2	2.5	0.013
RP4_635E18.7	-1	0.36 (0.15-0.89)	4.9	-2.2	0.026
RP4_761J14.8	-1.1	0.33 (0.12-0.89)	4.8	-2.2	0.029
RP11_417E7.2	0.16	1.2 (1-1.3)	5.9	2.4	0.015
RP3_412A9.17	-13	2.6e-06 (2e-10-0.034)	7.1	-2.7	0.0078
SPAG5_AS1	-0.5	0.6 (0.39-0.94)	5	-2.2	0.026
EGFR_AS1	0.39	1.5 (1.1-1.9)	7.7	2.8	0.0056
RP11_92F20.1	-1.5	0.21 (0.066-0.69)	6.6	-2.6	0.01
RP11_338L22.2	-3.2	0.042 (0.0046-0.39)	7.8	-2.8	0.0054
XXbac_BPG299F13.17	-0.52	0.6 (0.41-0.86)	7.8	-2.8	0.0051

RP11_7F17.8	-0.88	0.41 (0.22-0.8)	6.9	-2.6	0.0085
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Table 3 35 significant genes both by Univariate Cox analysis and KM analysis in BC.

Gene symbol	Coefficient	HR (95% CI for HR)	Wald. test	z	Cox Pvalue	KM Pvalue
SMYD2	0.33	1.4 (1.1-1.8)	6.6	2.6	0.01	0.016977168
SPRN	-0.31	0.73 (0.54-0.99)	4	-2	0.045	0.020553935
IGF2	0.088	1.1 (1-1.1)	13	3.5	0.00039	0.000399633
MLLT11	0.14	1.2 (1-1.3)	4.6	2.2	0.031	0.024637757
CALD1	0.14	1.1 (1-1.3)	5.3	2.3	0.021	0.009588017
FLNC	0.093	1.1 (1-1.2)	4.6	2.1	0.032	0.03006371
RASL12	0.14	1.2 (1-1.3)	4.9	2.2	0.027	0.003715353
PID1	0.17	1.2 (1-1.4)	4.2	2	0.04	0.000487617
CLIP3	0.13	1.1 (1-1.3)	4.4	2.1	0.037	0.044899614
TGFB11	0.2	1.2 (1.1-1.4)	7.3	2.7	0.0068	0.000481368
COL14A1	0.18	1.2 (1.1-1.3)	8.5	2.9	0.0036	0.00997827
MFAP4	0.11	1.1 (1-1.2)	6.1	2.5	0.014	0.003225683
KIAA1462	0.16	1.2 (1-1.3)	4.9	2.2	0.027	0.045800571
GPIHBP1	0.18	1.2 (1-1.4)	4.2	2	0.041	0.028058123
PTGIS	0.1	1.1 (1-1.2)	6	2.5	0.014	0.022982575
CCDC80	0.13	1.1 (1-1.3)	6.7	2.6	0.0096	0.016467292
LHFP	0.25	1.3 (1.1-1.5)	9.7	3.1	0.0018	0.003923056
SPON1	0.11	1.1 (1-1.2)	5.1	2.3	0.024	0.005957644
UNC5C	0.29	1.3 (1.1-1.7)	6.9	2.6	0.0088	0.016820479
IGDCC4	0.24	1.3 (1-1.6)	5.2	2.3	0.023	0.005605966
CD248	0.16	1.2 (1-1.3)	6.2	2.5	0.013	0.000988525
ZNF521	0.21	1.2 (1.1-1.4)	6.8	2.6	0.0093	0.003152521
COL6A2	0.1	1.1 (1-1.2)	4.6	2.1	0.032	0.020323121
TPPP3	0.14	1.2 (1-1.3)	5.8	2.4	0.016	0.017684884
EFEMP1	0.099	1.1 (1-1.2)	6.2	2.5	0.013	0.009837627
RP4_635E18.7	-1	0.36 (0.15-0.89)	4.9	-2.2	0.026	0.046653654
RP4_761J14.8	-1.1	0.33 (0.12-0.89)	4.8	-2.2	0.029	0.002109411
RP11_417E7.2	0.16	1.2 (1-1.3)	5.9	2.4	0.015	0.019106815
RP3_412A9.17	-13	2.6e-06 (2e-10-0.034)	7.1	-2.7	0.0078	0.006753141
SPAG5_AS1	-0.5	0.6 (0.39-0.94)	5	-2.2	0.026	0.034855029
EGFR_AS1	0.39	1.5 (1.1-1.9)	7.7	2.8	0.0056	0.036033844
RP11_92F20.1	-1.5	0.21 (0.066-0.69)	6.6	-2.6	0.01	0.005874754
RP11_338L22.2	-3.2	0.042 (0.0046-0.39)	7.8	-2.8	0.0054	0.017103922
XXbac_BPG299F13.17	-0.52	0.6 (0.41-0.86)	7.8	-2.8	0.0051	0.012648268

RP11_7F17.8	-0.88	0.41 (0.22-0.8)	6.9	-2.6	0.0085	0.009768362
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Table 4 Stepwise multivariable regression analysis of 13-OS-related signature in BC.

Gene symbol	Coefficient	HR (95% CI for HR)	z	Pvalue
IGF2	0.072	1.1 (1-1.1)	2.7	0.008
MLLT11	0.28	1.3 (1.1-1.5)	3.6	0.00034
RASL12	-0.25	0.78 (0.59-1)	-1.8	0.075
KIAA1462	-0.2	0.82 (0.65-1)	-1.7	0.097
GPIHBP1	0.21	1.2 (0.96-1.6)	1.6	0.1
PTGIS	0.13	1.1 (0.99-1.3)	1.8	0.064
LHFP	0.31	1.4 (1-1.8)	2.1	0.037
RP4_761J14.8	-1.5	0.22 (0.069-0.67)	-2.6	0.0083
RP3_412A9.17	-8.2	0.00027 (1.1e-08-6.3)	-1.6	0.11
EGFR_AS1	0.73	2.1 (1.6-2.8)	5	7.10E-07
RP11_92F20.1	-0.89	0.41 (0.12-1.4)	-1.4	0.16
RP11_338L22.2	-2.8	0.06 (0.0051-0.71)	-2.2	0.026
XXbac_BPG299F13.17	-0.62	0.54 (0.36-0.79)	-3.1	0.0017

Table 5 Univariate and stepwise multivariate regression analysis of 13-OS-related signature and clinical factors in BC.

	Univariate					Multivariate			
Characteristic	Coefficient	HR (95% CI for HR)	Wald. test	z	P. value	Coefficient	HR (95% CI for HR)	z	P. value
Age	0.41	1.5 (0.68-3.4)	1	1	0.31	NA	NA	NA	NA
BMI	-0.0097	0.99 (0.93-1.1)	0.1	-0.31	0.75	NA	NA	NA	NA
Gender	0.38	1.5 (0.78-2.8)	1.4	1.2	0.24	NA	NA	NA	NA
M	1.2	3.3 (1.3-8.3)	6.1	2.5	0.014	0.72	2.1 (0.64-6.6)	1.2	0.23
N	0.34	1.4 (1-1.9)	4.5	2.1	0.035	0.024	1 (0.56-1.9)	0.08	0.94
Pathologic Stage	0.43	1.5 (1.1-2.2)	5	2.2	0.025	0.25	1.3 (0.61-2.7)	0.66	0.51
Prior malignancy	-0.042	0.96 (0.53-1.7)	0.02	-0.14	0.89	NA	NA	NA	NA
Risk Score	0.29	1.3 (1.2-1.5)	17	4.2	2.90E-05	0.29	1.3 (1.2-1.5)	4	6.20E-05
T	0.42	1.5 (1-2.3)	4	2	0.047	0.15	1.2 (0.66-2.1)	0.53	0.6
Treatment or therapy	-0.52	0.59 (0.31-1.1)	2.4	-1.6	0.12	NA	NA	NA	NA

Table 6 Top 10 GO items between high-risk group and low-risk groups.

Category	ID	Description	Set Size	Enrichment Score	NES	Pvalue	P. adjust
GO_BP	GO:0000278	mitotic cell cycle	992	-0.464724267	-1.3614549	0.001001001	0.061778905
GO_BP	GO:0016071	mRNA metabolic process	848	-0.542660019	-1.587106501	0.001001001	0.061778905
GO_BP	GO:0006259	DNA metabolic process	952	-0.545942243	-1.598313641	0.001002004	0.061778905
GO_BP	GO:0010608	posttranscriptional regulation of gene expression	988	-0.437354846	-1.280957399	0.001002004	0.061778905
GO_BP	GO:0032446	protein modification by small protein conjugation	863	-0.488265611	-1.427585831	0.001003009	0.061778905
GO_BP	GO:1903047	mitotic cell cycle process	849	-0.47915529	-1.400713629	0.001003009	0.061778905
GO_BP	GO:0006974	cellular response to DNA damage stimulus	808	-0.526701319	-1.539454663	0.001005025	0.061778905
GO_BP	GO:0007017	microtubule-based process	735	-0.465135709	-1.357317291	0.001005025	0.061778905
GO_BP	GO:0010564	regulation of cell cycle process	761	-0.453381982	-1.323138862	0.001006036	0.061778905
GO_BP	GO:0016567	protein ubiquitination	781	-0.484549462	-1.415369216	0.001006036	0.061778905
GO_CC	GO:0044429	mitochondrial part	989	-0.502561227	-1.469216881	0.001	0.024793568
GO_CC	GO:0005694	chromosome	960	-0.591508504	-1.727406672	0.001003009	0.024793568
GO_CC	GO:0044427	chromosomal part	839	-0.581404731	-1.693270033	0.001003009	0.024793568
GO_CC	GO:0016604	nuclear body	756	-0.532684555	-1.549009555	0.001007049	0.024793568
GO_CC	GO:1990234	transferase complex	760	-0.54196285	-1.575933532	0.001008065	0.024793568
GO_CC	GO:0005740	mitochondrial envelope	700	-0.474912399	-1.377157665	0.001011122	0.024793568
GO_CC	GO:0031966	mitochondrial membrane	659	-0.47798788	-1.383462124	0.001011122	0.024793568
GO_CC	GO:0005815	microtubule organizing center	717	-0.496117777	-1.438036473	0.001012146	0.024793568
GO_CC	GO:0000228	nuclear chromosome	536	-0.560614936	-1.609688185	0.00102145	0.024793568
GO_CC	GO:0019866	organelle inner membrane	499	-0.529177603	-1.514318611	0.001027749	0.024793568
GO_MF	GO:0003690	double-stranded DNA binding	951	-0.438323917	-1.285738833	0.001002004	0.078819444
GO_MF	GO:0003682	chromatin binding	506	-0.485755154	-1.398894504	0.00101833	0.078819444
GO_MF	GO:0019787	ubiquitin-like protein transferase activity	394	-0.50980879	-1.458055509	0.001033058	0.078819444

GO_MF	GO:0004842	ubiquitin-protein transferase activity	369	-0.519072957	-1.480260991	0.00104712	0.078819444
GO_MF	GO:0140098	catalytic activity, acting on RNA	344	-0.64201376	-1.825584896	0.001053741	0.078819444
GO_MF	GO:0016747	transferase activity, transferring acyl groups other than amino-acyl groups	222	-0.544216869	-1.510691791	0.001092896	0.078819444
GO_MF	GO:0016741	transferase activity, transferring one-carbon groups	215	-0.563259955	-1.556126762	0.00110011	0.078819444
GO_MF	GO:0008168	methyltransferase activity	204	-0.57625549	-1.582657961	0.001103753	0.078819444
GO_MF	GO:0140097	catalytic activity, acting on DNA	200	-0.667001877	-1.831792815	0.001103753	0.078819444
GO_MF	GO:0004518	nuclease activity	191	-0.581259246	-1.591485284	0.001104972	0.078819444

Table 7 Top 10 KEGG pathway enrichment between high-risk and low-risk groups.

ID	Description	Set Size	Enrichment Score	NES	P value	P. adjust
hsa05168	Herpes simplex virus 1 infection	488	-0.6446174	-1.842130	0.0010183	0.0351334
hsa03013	RNA transport	181	-0.6186630	-1.666905	0.0011148	0.0351334
hsa03040	Spliceosome	150	-0.6288256	-1.678070	0.0011312	0.0351334
hsa03015	mRNA surveillance pathway	98	-0.6341924	-1.619593	0.0011806	0.0351334
hsa03008	Ribosome biogenesis in eukaryotes	100	-0.6383869	-1.634230	0.0011834	0.0351334
hsa03460	Fanconi anemia pathway	51	-0.7493155	-1.754771	0.0012626	0.0351334
hsa03440	Homologous recombination	41	-0.7138885	-1.630394	0.0012739	0.0351334
hsa03030	DNA replication	36	-0.7239130	-1.620366	0.0013072	0.0351334
hsa03410	Base excision repair	32	-0.7507609	-1.642526	0.0013280	0.0351334
hsa00640	Propanoate metabolism	33	-0.6984884	-1.535068	0.0026455	0.0351334

Table 8 Top 10 Reactome pathway enrichment between high-risk and low-risk groups.

ID	Description	Set Size	Enrichment Score	NES	P value	P. adjust
R-HSA-8953854	Metabolism of RNA	654	-0.5878868	-1.709269	0.0010081	0.0207553
R-HSA-1640170	Cell Cycle	580	-0.6190954	-1.795344	0.0010101	0.0207553
R-HSA-69278	Cell Cycle, Mitotic	476	-0.6098167	-1.754112	0.0010225	0.0207553
R-HSA-3700989	Transcriptional Regulation by TP53	358	-0.5412421	-1.535369	0.0010471	0.0207553
R-HSA-68886	M Phase	333	-0.6182648	-1.751180	0.0010482	0.0207553
R-HSA-73894	DNA Repair	281	-0.6328865	-1.774354	0.0010650	0.0207553
R-HSA-69620	Cell Cycle Checkpoints	259	-0.6739822	-1.879255	0.0010811	0.0207553
R-HSA-5688426	Deubiquitination	262	-0.5316360	-1.482592	0.0010823	0.0207553
R-HSA-72203	Processing of Capped Intron-Containing Pre-mRNA	240	-0.6935057	-1.927120	0.0010929	0.0207553
R-HSA-3247509	Chromatin modifying enzymes	206	-0.6358033	-1.757258	0.0010929	0.0207553

Table 9 Top 30 immune-related GO items between high-risk and low-risk groups.

Category	ID	Description	Set Size	Enrichment Score	NES	Pvalue	P. adjust
GO_BP	GO:0061844	antimicrobial humoral immune response mediated by antimicrobial peptide	64	0.656427085	1.880885659	0.004545455	0.061778905
GO_BP	GO:0002920	regulation of humoral immune response	72	0.692362073	1.998668651	0.004784689	0.061778905
GO_BP	GO:0002698	negative regulation of immune effector process	119	0.479991273	1.50663113	0.006410256	0.061778905
GO_BP	GO:0061760	antifungal innate immune response	6	0.944371109	1.671379401	0.006644518	0.061778905
GO_BP	GO:0002825	regulation of T-helper 1 type immune response	27	0.694207786	1.694245404	0.007194245	0.061778905
GO_BP	GO:0050777	negative regulation of immune response	149	0.429811218	1.37517549	0.0078125	0.061778905
GO_BP	GO:0002455	humoral immune response mediated by circulating immunoglobulin	46	0.597003903	1.63760526	0.008130081	0.061778905
GO_BP	GO:0002460	adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	249	0.41445012	1.404280462	0.012195122	0.075469812
GO_BP	GO:0006959	humoral immune response	242	0.596316105	2.011824756	0.012195122	0.075469812
GO_BP	GO:0002566	somatic diversification of immune receptors via somatic mutation	14	-0.746642029	-1.416986547	0.023611111	0.105846068
GO_BP	GO:0002367	cytokine production involved in immune response	100	0.460689547	1.394236368	0.023809524	0.105846068
GO_BP	GO:0002697	regulation of immune effector process	382	0.434712763	1.524381063	0.024390244	0.107845588
GO_BP	GO:0002313	mature B cell differentiation involved in immune response	19	0.752170817	1.698308816	0.025362319	0.110685264
GO_BP	GO:0002828	regulation of type 2 immune	30	0.612415889	1.523211012	0.028776978	0.11939051

		response					
GO_BP	GO:0002250	adaptive immune response	399	0.374636813	1.314964677	0.03125	0.125055212
GO_BP	GO:0016064	immunoglobulin mediated immune response	111	0.426648404	1.325134841	0.031446541	0.12561978
GO_BP	GO:0042092	type 2 immune response	35	0.570714804	1.476162204	0.033210332	0.130132589
GO_BP	GO:0002683	negative regulation of immune system process	433	0.473489834	1.663316675	0.034482759	0.132547218
GO_BP	GO:0002699	positive regulation of immune effector process	206	0.389608877	1.276417783	0.034482759	0.132547218
GO_BP	GO:0042088	T-helper 1 type immune response	42	0.548051796	1.473951212	0.034749035	0.133288
GO_BP	GO:0002700	regulation of production of molecular mediator of immune response	133	0.411048764	1.295197795	0.035714286	0.135783226
Category	GO:0002283	neutrophil activation involved in immune response	479	0.545581181	1.977918089	0.037037037	0.137241536
GO_BP	GO:0002275	myeloid cell activation involved in immune response	533	0.539350874	1.967939907	0.038461538	0.139552078
GO_BP	GO:0002757	immune response-activating signal transduction	515	0.317517933	1.149727314	0.041666667	0.145248172
GO_BP	GO:0002718	regulation of cytokine production involved in immune response	82	0.465493608	1.367849793	0.042553191	0.148053731
GO_BP	GO:0002253	activation of immune response	595	0.3727582	1.359115478	0.045454545	0.153893192
GO_BP	GO:0002764	immune response-regulating signaling pathway	548	0.32806093	1.187034741	0.045454545	0.153893192
GO_BP	GO:0002440	production of molecular mediator of immune response	193	0.368868951	1.219083215	0.047169811	0.15751317
GO_BP	GO:0002720	positive regulation of cytokine production involved in immune response	52	0.471727597	1.311127536	0.049180328	0.161732766

Figures

Figure 1

Flowchart illustrated the study design and analysis analytical processing.

Figure 2

Identification of DE-lncRNA and DE-mRNA and construction of ceRNA network in BC A, B The volcano plot of DE-lncRNAs and DE-mRNAs between three paired bladder cancer samples and adjacent normal samples. C, D The heatmap of DE-lncRNAs and DE-mRNAs between three paired bladder cancer samples and adjacent normal samples. E, F The Venn of DE-lncRNAs and DE-mRNAs by overlapping between RNA-seq data and TCGA data. G The lncRNA-miRNA-mRNA ceRNA network based on overlapped DE-lncRNAs and DE-mRNAs

Figure 3

Kaplan-Meier curves exhibited 62 significant survival-related DE-mRNAs and DE-lncRNAs in BC.

Figure 4

Screening of 13-OS-related signature from BC based TCGA database. A The forest plots of 35 variates associated to OS in training set. B The forest plots of 13-OS-related signature associated to OS in training set. C The PCA plot shown the difference between high-risk and low-risk groups in training set and validation set. D Up: The risk score distribution, and Bottom: The survival statues between high-risk and low-risk groups in training set and validation set. E Kaplan-Meier curves for high-risk and low-risk bladder cancer based on 13-OS-related signature in training set and validation set. F Time-dependent ROC curves illustrated the predictive of the 1, 2, 3, 4, 5-years based on 13-OS-related signature in training set and validation set. G Time-dependent ROC curves illustrated the predictive of the 5-years based on 13-OS-related signature in training set and validation set. H The heatmap of 13-OS-related signature expression and association to clinical features in training set and validation set.

Figure 5

Association between risk score and clinical characteristics in BC. A The box plots shown association between risk score and M stages, pathologic stages, and N stages in entire datasets. B-J Kaplan-Meier curves exhibited association between risk score and age stratification with 60 years, gender stratification, M/N/T stages stratification, pathologic stages, prior malignancy statues, and treatment or therapy types in entire sets.

Figure 6

Identification and validation of the 13-OS-related signature in BC patients. A The forest plot of 13-OS-related signature and clinical risk factors in the entire datasets. B The forest plot of 13-OS-related signature and T/M/N stages and pathological stage in the entire datasets.

Figure 7

Construction and validation of a nomogram for prognostic prediction in BC. A, B The nomogram for predicating median survival time, 3- and 5-years survival time. C The C-index and calibration plots shown discrimination and calibration ability of nomogram for 1, 2, 3, 4, 5-years survival time. D, E The C-index and calibration plots shown discrimination and calibration ability of nomogram for 3- and 5-years survival time.

Figure 8

Identification of the DEGs between high-risk score group and low-risk score group in BC. A The volcano plot of DEGs between high-risk and low-risk groups in bladder cancer. B The heatmap of DEGs between high-risk and low-risk groups in bladder cancer.

Figure 9

Function analysis of the risk score-related DEGs in BC. A-E The peak diagrams of GO annotation, KEGG and Reactome pathways enrichment by GSEA, respectively. F-J The ridge line graphs of GO annotation, KEGG and Reactome pathways enrichment by GSEA, respectively.

Supplementary Files

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