Comprehensive analysis of the functions and prognostic significance of RNA-binding proteins in bladder urothelial carcinoma

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Primary research

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Abstract

Background

Alterations in RNA-binding proteins (RBPs) are reported in various cancer types; however, the role of RBPs in bladder urothelial cancer (BLCA) remains unknown. This study aimed to systematically examine the function and prognostic significance of RBPs in bladder cancer using bioinformatics analyses.

Methods

RNA sequencing and clinical data for BLCA were downloaded from The Cancer Genome Atlas (TCGA) database, and differentially expressed RBPs (DERBPs) between normal and cancer tissues were identified. Protein-protein interaction (PPI) network of DERBPs was established, and enrichment analysis and visualizations were performed. A total of 404 patients with BLCA from TCGA database were randomly divided into training and testing groups. A prognostic model was constructed using the data from training group, and validated in the testing group. Receiver operating characteristic (ROC) curve and survival analysis were performed to explore the prognostic value of the model. A nomogram was established to predict survival in bladder cancer patients. Finally, the verification of prognosis-related hub RBP survival analysis were performed.

Results

A total of 388 DERBPs were identified, including 219 upregulated and 169 downregulated RBPs. All RBPs were screened for prognostic model establishment and 9 RBPs (TRIM71, YTHDC1, DARS2, XPOT, ZNF106, FTO, IPO7, EFTUD2, and CTU1) were regarded as prognosis-related hub RBPs in BLCA. Further analysis revealed worse overall survival (OS) in the high-risk cohort compared to the model-based low-risk cohort. The area under the ROC curve was 0.752 in the training group and 0.701 in the testing group, which confirms the good prediction ability. A nomogram was established according to nine prognosis-related RBPs, which showed well predicting ability for BLCA. BLCA patients with high DARS2, XPOT, ZNF106, FTO, and IPO7 expression (on the contrary, low YTHDC1 and CTU1 expression) were correlated to poor overall survival.

Conclusions

The prognosis-related hub RBPs may be involved in oncogenesis, development, and metastasis of BLCA. Our results will be of great significance in revealing the pathogenesis of BLCA, and developing new therapeutic targets and prognostic molecular markers.

Background

Bladder urothelial cancer (BLCA) is the tenth most common malignant tumor in the world. More than five hundred thousand new cases of bladder cancer and two hundred thousand deaths are estimated to have occurred in 2018, and it is more common in men than in women [1]. Based on pathological diagnosis, bladder cancer can be categorized into non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). Most of BLCA cases originate from epithelial cells, of which approximately 90% are urothelial tumors, whereas squamous and glandular tumors are the less common histologic subtypes; bladder cancer originates very rarely from mesenchymal cells [2]. General treatment includes operation, intravesical treatment, radical treatment, immunotherapy and radiotherapy, and various other therapies chosen according to cancer-risk assessment [3]. High-risk patients with NMIBC have 60–70% chance of recurrence and 10–45% chance of progression to muscle invasive or metastatic disease within 5 years [4]. Unfortunately, the recurrence rate of BLCA is quite high. Treatment needs to be repeated frequently, which in turn inevitably leads to resistance [5].

Important players in RNA-mediated, post-transcriptional regulation are RNA-binding proteins (RBPs) [6]. These proteins, among other diverse biological functions, facilitate the regulation by miRNAs and sRNAs [7, 8]. Till date, more than 1500 RBP genes have been identified in the human genome through genome-wide analysis [9]. Over the past decade, many studies have revealed abnormal expression of RBPs in tumors, suggesting them to be involved in carcinogenesis. IGF2BP1 causes increase in proliferation and tumorigenesis, and the leukemia cell line with low expression of IGF2BP1 has less ability to form colonies and initiate tumors [10]. MSI1 is reported to be a potential therapeutic target for glioblastoma, since luteolin has been shown to inhibit the RNA-binding characteristics of MSI1 and destroy the cancer phenotype in glioblastoma [11]. The hnRNP K has both oncogenic and tumor suppressor properties. However, it mostly behaves as a tumor suppressor in acute leukemia [12]. RBPs constitute a key factor of the post-transcriptional process and play an important role in the regulation of RNA in gastrointestinal [13] and colorectal cancers [14]. Despite the emergence of RBPs as key regulators of every cancer hallmark, very little is known about their potential mechanisms and downstream carcinogenic targets, particularly with regard to bladder cancer. Therefore, all relevant BLCA data were downloaded from TCGA and a comprehensive analysis was conducted to investigate the potential molecular function and clinical significance of RBPs in BLCA. In this study, we selected a number of DERBPs related to BLCA, which have provided new insights into the pathogenesis of the disease. Some of them may be potential biomarkers for the diagnosis and prognosis of BLCA.

Materials And Methods

Data preprocessing and identification of differentially expressed RBPs

The RNA-sequencing dataset and corresponding clinical data were downloaded from TCGA (https://portal.gdc.cancer.gov/); it included 19 normal bladder tissue samples and 411 BLCA samples. The raw data of BLCA were preprocessed using the limma package [15] in R software. We used the Wilcoxon test in R software to select DERBPs between normal bladder and BLCA tissues, considering

$|\log_2 FC \text{ (fold change)}| \geq 0.5$ and FDR (false discovery rate) < 0.05. Finally, we applied R and pheatmap R package to draw volcano map and heatmap of the DERBPs.

PPI network establishment and module selection

To construct a protein-protein interaction network with a confidence score above 0.4 as the cut-off criterion, protein interactions were first analyzed using the Search Tool for the Retrieval of Interacting Genes (STRING, version 11.0; http://string-db.org) [16]. The Cytoscape 3.7.1 software, which is a platform with an open bioinformatic source [17], was utilized to establish and visualize the PPI network. The key modules were identified by Molecular Complex Detection (MCODE) plug-in [18]. For all data, $P \leq 0.05$ suggested significant difference.

GO and KEGG enrichment analysis

Gene ontology (GO) is considered to be one of the main bioinformatics methods to analyze biological processes [19]; the Kyoto Encyclopedia of Genes and Genomes (KEGG) is a database that allows users to study biological systems and advanced functions from widespread molecular datasets [20]. The biological functions of DERBPs were systematically explored by GO and KEGG enrichment analysis. GO and KEGG enrichment analysis and visualizations were performed using R software, which included ggplot2 R package, org.Hs.eg.db package, enrichplot package, and clusterProfiler package [21]. Adjusted p-value or p-value < 0.05 was considered statistically significant.

Prognosis-related RBP identification

Univariate Cox regression analysis or Kaplan-Meier test was performed for DERBPs using survival R package. Values with p < 0.01 were considered to correspond to prognosis-related candidate hub RBPs in univariate Cox regression test. Kaplan-Meier test was used to evaluate the prognostic value of DERBPs, and p-value < 0.05 was considered to indicate candidate hub RBPs related to prognosis. Thereafter, multivariate Cox regression test was applied to prognosis-related candidate hub RBPs in order to further identify the prognosis-related hub RBPs.

Prognostic model establishment and evaluation

All patients with BLCA, from TCGA, were randomly divided into training group and testing group. A multivariate Cox proportional hazards regression model was established, based on prognosis-related RBPs in the training group, which calculated the risk score to evaluate patient prognosis using the survival, caret, glmnet [22], survminer, and survivalROC packages in R. Using the model, we calculated the risk score of each patient with BLCA based on the following formula: Risk score = $\beta 1 \times Exp1 + \beta 2 \times Exp2 + \beta I \times Expi$, where β is regression coefficient and Exp is expression level.

On the basis of median risk score from the formula, the training group was divided into low-risk cohort and high-risk cohort; thereafter, the testing group was divided into low-risk cohort and high-risk cohort, depending on the median score of training group and risk score from the formula. Patients in the testing

group served as validation cohort to verify the predictive ability of the model. Difference in overall survival rate between high- and low-risk cohorts was compared by log-rank test using survival and survminer R packages in the training and testing group, respectively. The ROC curve was constructed using survivalROC R package to evaluate the predictive ability of model in both training and testing groups, and the pheatmap R package was used to draw risk plot and heatmap. Finally, based on the nine hub RBPs, a nomogram was constructed to predict the possibility of OS using the rms R package.

Mutation analysis and prognostic value of clinical parameters

Mutation analysis of nine hub RBPs was executed using the cBioPortal platform (http://www.cbioportal.org) [23]. We applied the survival R package for Cox regression analysis to assess the prognostic significance of different clinical parameters in the training and testing groups of patients with BLCA, respectively.

Hub RBP expression levels and prognostic analysis

Prognostic analysis of hub RBPs was conducted to generate Kaplan-Meier curve and calculate p-value using the UCSC Xena Functional Genomics Explorer (https://xenabrowser.net/) [24]. The online database of Human Protein Atlas (http://www.proteinatlas.org/) was utilized to explore the expression of hub RBPs at translational level [25].

Results

Differentially expressed RBP (DERBP) identification

The research design is shown in Fig. 1a. In this study, we performed a comprehensive analysis of crucial functions and prognostic significance of RBPs in BLCA. Data regarding BLCA were acquired from TCGA, including 411 bladder cancer samples and 19 normal bladder samples. Relevant packages in R were utilized to process the data and select the DERBPs. A total of 388 (out of 1542) RBPs [9] fulfilled the screening criteria of the study, consisting of 219 upregulated and 169 downregulated RBPs. The heatmap and volcano map of DERBPs are displayed in Fig. 1b, c.

PPI network establishment and module selection

To demonstrate the functions of DERBPs in BLCA, we established the PPI network using STRING database and Cytoscape software, which consisted of 4145 edges and 376 nodes (Fig. 2a). The PPI network was analyzed using MCODE to determine top three important modules, which consisted of 3302 edges and 104 nodes (Fig. 2b).

GO and KEGG enrichment analysis

We performed GO and KEGG functional enrichment analysis of DERBPs using R software and correlation packages. The downregulated DERBPs were remarkably enriched in the GO analysis related to RNA

splicing, and regulation of mRNA metabolic process, cytoplasmic ribonucleoprotein granule, ribonucleoprotein granule, translation factor activity, RNA binding, and mRNA 3'-UTR binding (Fig. 3a); the upregulated DERBPs were remarkably enriched in ncRNA processing, tRNA metabolic process, cytoplasmic ribonucleoprotein granule, ribonucleoprotein granule, and catalytic activity, acting on RNA, and catalytic activity, acting on a tRNA (Fig. 3c). We found the downregulated DERBPs to be mainly enriched in KEGG analysis related to mRNA surveillance pathway, RNA transport, and RNA degradation (Fig. 3b) while the upregulated DERBPs were remarkably enriched in RNA transport, spliceosome, and mRNA surveillance pathway (Fig. 3d). Further, we performed GO and KEGG functional enrichment analysis of key modules, results of which are shown in Table 1.

Prognosis-related RBP screening

A total of 388 DERBPs were identified. In order to study the prognostic value of these RBPs, univariate Cox regression analysis was performed, and 19 candidate hub RBPs related to prognosis were obtained (Fig. 3e). Multivariate Cox regression analysis was performed on the 19 RBPs, of which 9 hub RBPs were identified as independent predictors of BLCA (Fig. 3f, Table 2).

Prognosis-related model construction and analysis

A total of 404 patients with BLCA were randomly divided into training group (202 patients) and testing group (202 patients). The 9 prognosis-related hub RBPs were utilized to establish a predictive model based on training-group data. We calculated the risk score of every patient based on the following formula:

Risk score = (0.2707 × ExpTRIM71) + (-0.1148 × ExpYTHDC1) + (0.0417 × DARS2) + (0.0272 × ExpXPOT) + (0.1341 × ExpZNF106) + (0.2806 × ExpFTO) + (-0.023 × ExpIPO7) + (0.0521 × ExpEFTUD2) + (-0.0812 × ExpCTU1)

Next, we aimed to evaluate the predictive ability. Results in the training group indicated patients in high-risk cohort to have worse OS than those in the low-risk cohort (Fig. 4b). ROC analysis demonstrated prognostic value of the nine hub RBPs. Area under the ROC curve (AUC) of the model was 0.752 in the training group (Fig. 4c), suggesting it to have better diagnostic capability. In the training group, Fig. 4a showed the expression heatmap, patient survival status, and risk scores for the low- and high-risk cohorts based on nine RBPs. In order to evaluate whether the risk score model had the same prognostic significance in the testing group, the same formula was used in the latter; high-risk cohort patients were found to have worse OS than those in the low-risk cohort, and area under the ROC curve was 0.701 (Fig. 5a–c). It thus suggested better sensitivity and specificity of the model for predicting prognosis.

A nomogram based on nine RBPs

In order to develop a quantitative approach for predicting prognosis in bladder cancer, nine RBPs were integrated to construct a nomogram (Fig. 6a). Based on multivariate Cox regression analysis, the point scale in nomogram was used to assign values to individual variables. By drawing a vertical line between

the prognosis axis and total-point axis, we could calculate the estimated survival rate of 1 year, 3 years, and 5 years, which could eventually help doctors to make clinical decisions for patients with BLCA.

Mutation analysis and prognostic value of clinical parameters

Mutation analysis of the hub genes TRIM71, YTHDC1, DARS2, XPOT, ZNF106, FTO, IPO7, EFTUD2, and CTU1 was performed using the cBioPortal platform. Results indicated that in 226 samples from 404 patients with BLCA, the 9 hub RBPs had changed (56%) (Fig. 6b, c). The high mRNA levels of DARS2 was the maximum alteration among the 9 hub RBPs.

Cox regression analysis was used to evaluate the effect of different clinical characteristics on the prognosis of patients with BLCA. Univariate Cox regression analysis results suggested age, stage, and risk score to be related to OS of patients with BLCA, in both training and testing groups (Fig. 6d, f). Multivariate Cox regression analysis results indicated age, stage, and risk score to be independent prognostic factors associated with OS in the training and testing groups (Fig. 6e, g).

Survival analysis and expression level of hub RBPs

To demonstrate prognostic significance of the nine hub RBPs in BLCA, the overall survival of every hub RBP was analyzed to draw Kaplan-Meier curve; p-value was calculated using UCSC online tool. Results showed that patients with BLCA, having high DARS2, FTO, IPO7, XPOT, and ZNF106 expression (Fig. 7b, d, e, g, i) and low CTU1 and YTHDC1 expression (Fig. 7a, h) were correlated with worse OS. We used the immunohistochemical results of Human Protein Atlas database to explore the expression of hub RBPs in BLCA, and found that DARS2, EFTUD2, FTO, TRIM71, and ZNF106 levels (Fig. 8b-d, f, h) in bladder cancer tissues were significantly higher than in normal bladder tissues. However, the antibody staining levels of CTU1, IPO7, and YTHDC1 (Fig. 8a, e, g) in bladder cancer tissues were relatively reduced.

Discussion

Although early diagnosis and multimodal treatment of bladder cancer have achieved promising results recently, metastatic diseases are usually incurable, and the 5-year survival rate remains only 15% [26]. Metastasis and recurrence are the main causes of death in patients with bladder cancer, especially MIBC [27]. Therefore, it would be highly significant to understand the molecular mechanism of bladder cancer further, and develop effective early-screening and diagnostic approaches to enhance treatment effect and quality of life in patients. RNA-binding proteins play an important role in the regulation of various RNA processes, including splicing, transport, translation, and degradation of coding and non-coding RNAs [28]. RBPs and RNAs assemble into a dynamic complex, called ribonucleoprotein (RNP), which regulates almost every stage of RNA lifecycle [29]. An important regulatory mechanism of lncRNAs is RNA-binding protein-mediated post-transcriptional regulation [30]. This post-transcriptional regulation is a vital approach of coding and non-coding RNAs, and is mainly promoted by RNA-binding proteins, since they dynamically coordinate the maturation, transport, and stability of all RNA types [9]. Identification of pathogenic gene variation in cancer has always been the subject of in-depth study, and colorectal cancer

[31], prostate cancer [32], glioblastoma [33], ovarian cancer [34], and melanoma [35] have been reported to be related to RNA-binding proteins. However, the mechanisms of RBPs in BLCA are currently only little understood.

In this study, RNA sequencing data of BLCA were integrated to identify the DERBPs between bladder cancer tissues and normal bladder tissues. We established a PPI network of these RBPs. GO and KEGG enrichment analysis of the DERBPs indicated the downregulated RBPs to be remarkably enriched in RNA splicing, and regulation of mRNA metabolic process, cytoplasmic ribonucleoprotein granule, mRNA surveillance pathway, RNA transport, and RNA degradation. The upregulated RBPs were remarkably enriched in ncRNA processing, tRNA metabolic process, cytoplasmic ribonucleoprotein granule, RNA transport, spliceosome, and mRNA surveillance pathway. Burdelski et al. had previously reported the role of RNA processing in various cancer types [36]. In some cases, tumors utilize the mRNA surveillance pathway to downregulate gene expression by destroying key tumor-suppressor mRNAs, whereas in other cases, tumors adapt to their microenvironment by regulating the activity of mRNA surveillance pathway [37]. Therefore, RNA-binding proteins can regulate tumor-cell proliferation through a variety of biological processes, such as regulation of mRNA surveillance pathways and RNA processing.

In our study, univariate Cox regression analysis was used to screen candidate hub RBPs related to prognosis, and multivariate Cox regression analysis was used to identify hub RBPs related to prognosis; finally, we identified the following nine hub RBPs: TRIM71, YTHDC1, DARS2, XPOT, ZNF106, FTO, IPO7, EFTUD2, and CTU1. Using multivariate Cox regression analysis, according to the data of training group, the risk score model was constructed with the 9 RBPs to predict the prognosis of patients with BLCA. In the training group, ROC curve of the nine-RBP risk score model had medium ability to predict OS (AUC = 0.752), and high-risk patients with BLCA showed remarkably worse overall survival time. In the testing group, as a validation cohort, ROC curve of the nine-RBP risk score model also had medium ability to predict OS (AUC = 0.701), and high-risk patients with BLCA showed remarkably worse overall survival time. The nomogram was established to enable professionals to predict 1-, 3-, and 5-year OS for patients with BLCA. Based on the predicted results by risk score model, high-risk score patients had worse prognosis, suggesting the treatment plan and individualized treatment to possibly require adjustment. We further demonstrated that patients with BLCA, having high DARS2, XPOT, ZNF106, FTO, and IPO7 expression, and low YTHDC1 and CTU1 expression, were correlated with worse overall survival. Moreover, TRIM71, DARS2, ZNF106, FTO, and EFTUD2 expression was significantly higher in bladder cancer tissues than in normal bladder tissues. However, the staining levels of CTU1, IPO7, and YTHDC1 in bladder cancer tissues were relatively lower.

The hub RBPs have been reported in many studies. ELP3 and CTU1/2, partner enzymes in uridine 34(U34) mcm^5s^2-tRNA modification, are upregulated and promote metastasis in human breast cancers [38]. CTU1 copy number amplifications were identified in 25% of myxopapillary ependymomas [39]. Qin et al. had demonstrated DARS2 as a hepatocarcinoma gene that could promote the progression of hepatocarcinoma cell cycle and inhibit the apoptosis of hepatocarcinoma cells [40]. EFTUD2 gene expression was upregulated in hepatocarcinoma, and had prognostic significance in patients with

hepatocarcinoma [41]. Liu et al. found the expression of FTO in esophageal squamous cell carcinoma (ESCC) to be higher than in adjacent normal tissues, and the corresponding survival curve showed the high expression of FTO to tend toward poor prognosis. In terms of function, FTO silencing inhibited the growth and migration of ESCC cells in CCK8 and transwell assays, whereas FTO overexpression showed the opposite results [42]. Inhibition of IPO7 by siRNA is known to lead to reduced proliferation of prostate cancer cells [43]. Torres-Fernández et al. had indicated TRIM71 to be correlated with advanced stages and poor prognosis in hepatocellular carcinoma. TRIM71 could inhibit the mRNA expression of cell cycle inhibitor and tumor suppressor CDKN1A/p21, and promote the proliferation of tumor cells [44]. XPOT belongs to the Ran-GTPase exportin family that mediates export of tRNA from nucleus to the cytoplasm, and high expression of XPOT in hepatocellular carcinoma is associated with worse prognosis [45]. Celona et al. had reported ZFP106 knockout mice to have severe degeneration of motoneurons while transgenic recovery of ZFP106 specifically inhibited the degeneration [46].

In summary, the study proposed new insights regarding the functions of RBPs in BLCA oncogenesis and development. In addition, the model indicated better predictive ability in terms of survival, which may be helpful in the exploitation of novel BLCA prognostic biomarkers. However, this research had some restriction factor. Firstly, our findings are only based on RNA sequencing data without other omics data. Secondly, the risk score model was established based on the TCGA BLCA data, and prospective study should be conducted to prove it. Thirdly, The TCGA data lacked some clinical characteristics that may have reduced the statistical validity and reliability of multivariate Cox regression analysis. Finally, since we had adopted a bioinformatics approach, further biological experiments would be required to verify the claims.

Conclusions

In conclusion, we comprehensively investigated the function and prognostic significance of DERBPs in BLCA through extensive bioinformatics analysis. The hub RBPs may be involved in oncogenesis, development, and metastasis of BLCA. A risk score model, or RBP-related prognostic model, was established, and might be used as an independent prognostic factor for BLCA. Our results will be of great significance in revealing the pathogenesis of BLCA, and developing new therapeutic targets and prognostic molecular markers.

Abbreviations

RBPs: RNA-binding proteins; BLCA:Bladder urothelial cancer; TCGA:The Cancer Genome Atlas; DERBPs:Differentially expressed RBPs; PPI:Protein-protein interaction; ROC:Receiver operating characteristic; OS:overall survival; NMIBC:Non-muscle invasive bladder cancer; MIBC:Muscle invasive bladder cancer; MCODE:Molecular Complex Detection; GO:Gene ontology; KEGG:Kyoto Encyclopedia of Genes and Genomes; AUC:Area under the curve; BP:Biological processes; CC:Cell component; MF:Molecular function; FDR:false discovery rate; HR:Hazard ratio; FC:Fold change.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

All authors have agreed for this publication.

Availability of data and materials

The data was available in TCGA database. The data used to support the results of this study are available from the corresponding author upon request.

Competing interests

The authors declare that there are no conflict of interests.

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Authors' contributions

All authors helped to design and conduct this study. C. G. and T. S. performed the data collection, bioinformatics analyses and manuscript preparation. D. W., Z. W., and M. L. carried out the data collection and statistical analyses. X. J. and G. B. supervised the whole project and performed manuscript preparation and revision. All authors read and approved the final manuscript.

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Tables

Table 1 GO and KEGG enrichment analysis of top three modules

Category		ID	Description	Gene Ratio	FDR
Module 1	BP	GO:0000377	RNA splicing, via transesterification reactions with bulged adenosine as nucleophile	33/33	2.00E-55
	BP	GO:0000398	mRNA splicing, via spliceosome	33/33	2.00E-55
	BP	GO:0000375	RNA splicing, via transesterification reactions	33/33	2.00E-55
	CC	GO:0005681	spliceosomal complex	22/33	1.52E-36
	CC	GO:0005684	U2-type spliceosomal complex	18/33	7.50E-34
	CC	GO:0071005	U2-type precatalytic spliceosome	15/33	4.74E-31
	MF	GO:0017069	snRNA binding	5/33	7.27E-07
	MF	GO:0036002	pre-mRNA binding	5/33	1.63E-06
	MF	GO:0001055	RNA polymerase II activity	3/33	7.19E-06
	KEGG	hsa03040	Spliceosome	17/27	3.23E-23
	KEGG	hsa03015	mRNA surveillance pathway	8/27	5.80E-10
	KEGG	hsa03020	RNA polymerase	4/27	3.14E-06
Module 2	BP	GO:0042254	ribosome biogenesis	19/27	6.25E-27
	BP	GO:0016072	rRNA metabolic process	16/27	3.81E-22
	BP	GO:0006364	rRNA processing	15/27	2.01E-21
	CC	GO:0030684	preribosome	8/30	3.65E-12
	CC	GO:0030687	preribosome, large subunit precursor	5/30	1.90E-09
	CC	GO:0032040	small-subunit processome	3/30	0.000172
	MF	GO:0140098	catalytic activity, acting on RNA	15/31	3.38E-16
	MF	GO:0003724	RNA helicase activity	9/31	6.12E-14
	MF	GO:0004386	helicase activity	9/31	3.63E-11
	KEGG	hsa03008	Ribosome biogenesis in eukaryotes	2/4	0.001193
	KEGG	hsa03020	RNA polymerase	1/4	0.008072
Module 3	BP	GO:0006414	translational elongation	16/39	1.41E-22
	BP	GO:0006415	translational termination	14/39	1.86E-20
	BP	GO:0070125	mitochondrial translational elongation	12/39	1.39E-17

	CC	GO:0005840	ribosome	17/39	2.41E-20
	CC	GO:0044391	ribosomal subunit	15/39	1.45E-19
	CC	GO:0000313	organellar ribosome	10/39	1.01E-14
	MF	GO:0003735	structural constituent of ribosome	13/38	7.39E-15
	MF	GO:0008135	translation factor activity, RNA binding	8/38	2.04E-10
	MF	GO:0003746	translation elongation factor activity	4/38	1.32E-06
	KEGG	hsa03010	Ribosome	11/24	2.01E-12
	KEGG	hsa03013	RNA transport	5/24	0.000674
	KEGG	hsa03015	mRNA surveillance pathway	3/24	0.006711

GO Gene Ontology, *KEGG* Kyoto Encyclopedia of Genes and Genomes, *BP* biological processes, *CC* cell component, *MF* molecular function, *FDR* false discovery rate

Table 2 Nine prognosis-related RBPs selected by multivariate Cox regression analysis

RBP name	Full name	coefficient	HR	p-value
TRIM71	tripartite motif containing 71	0.2707	1.3108	0.0243
YTHDC1	YTH domain containing 1	-0.1148	0.8916	0.0065
DARS2	aspartyl-tRNA synthetase 2, mitochondrial	0.0417	1.0426	0.0257
XPOT	exportin for tRNA	0.0272	1.0276	0.0876
ZNF106	zinc finger protein 106	0.1341	1.1435	0.0105
FTO	FTO alpha-ketoglutarate dependent dioxygenase	0.2806	1.3240	0.0043
IPO7	importin 7	-0.0231	0.9771	0.01419
EFTUD2	elongation factor Tu GTP binding domain containing 2	0.0521	1.0535	0.1063
CTU1	cytosolic thiouridylase subunit 1	0.0812	0.9220	0.1837

RBP RNA binding protein, *HR* hazard ratio

Figures

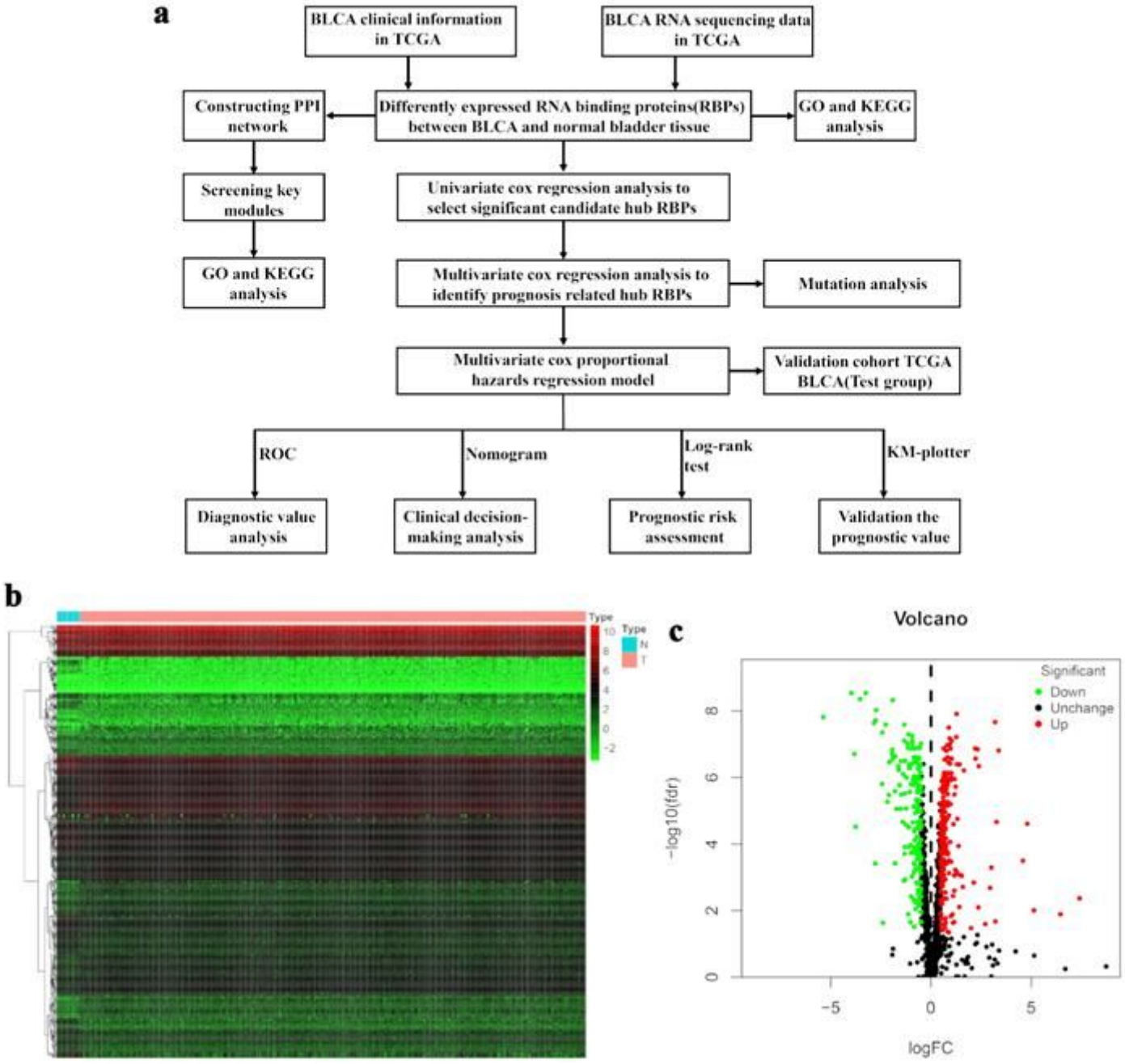

Figure 1

Flow diagram of the study, and the DERBPs in bladder urothelial carcinoma. a Flowchart for the analysis of RBPs in bladder urothelial carcinoma. b The differentially expressed RBPs in each sample were displayed in heatmap. c Volcano diagram showed the remarkably differentially expressed RBPs between BLCA tissues and normal tissues

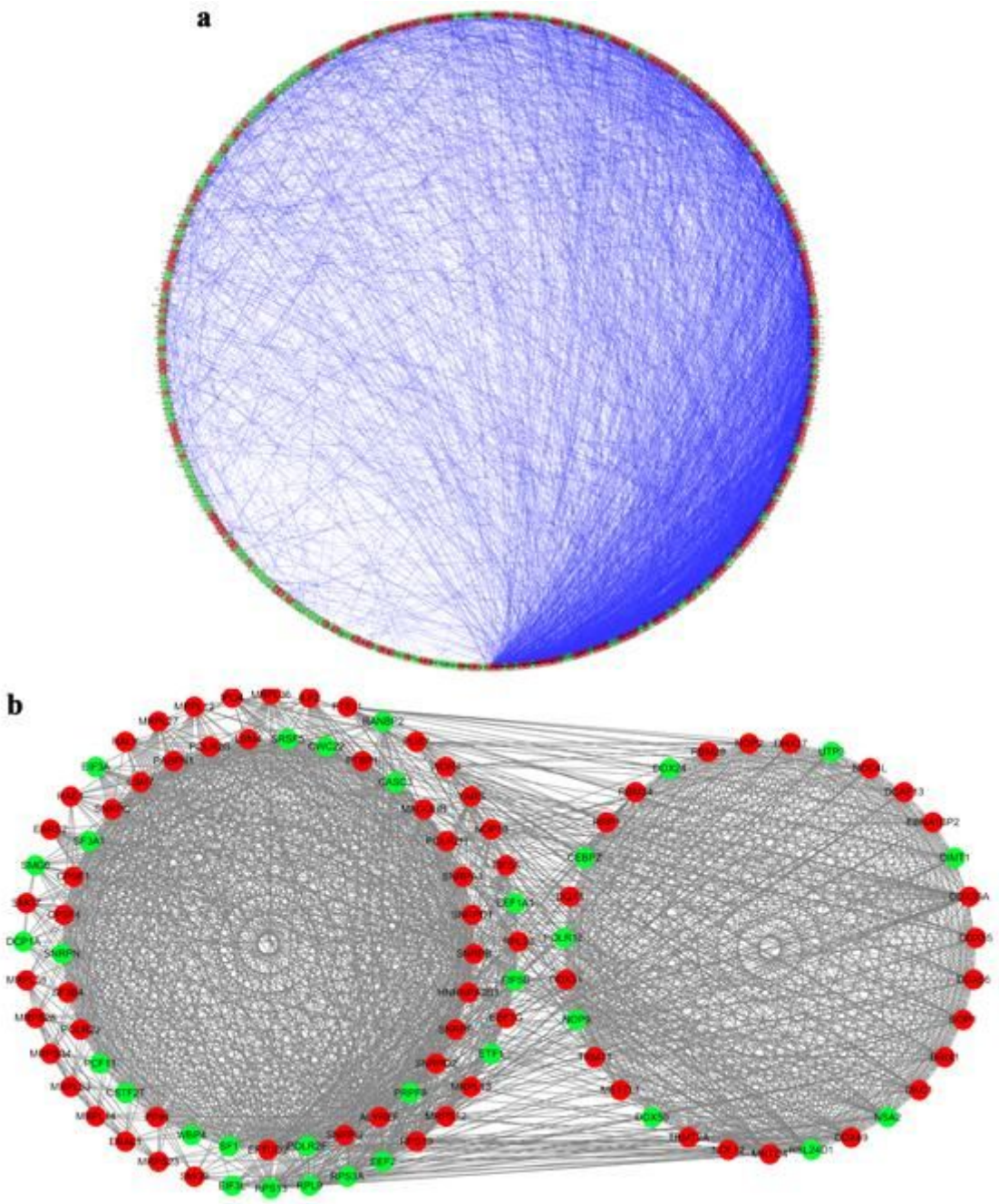

Figure 2

PPI network and module analysis of the DERBPs. a PPI network of the 376 DERBPs, and 376 nodes and 4145 edges were contained. b Top 3 critical modules from PPI network. Upregulated RBPs were red nodes, and downregulated RBPs were green nodes

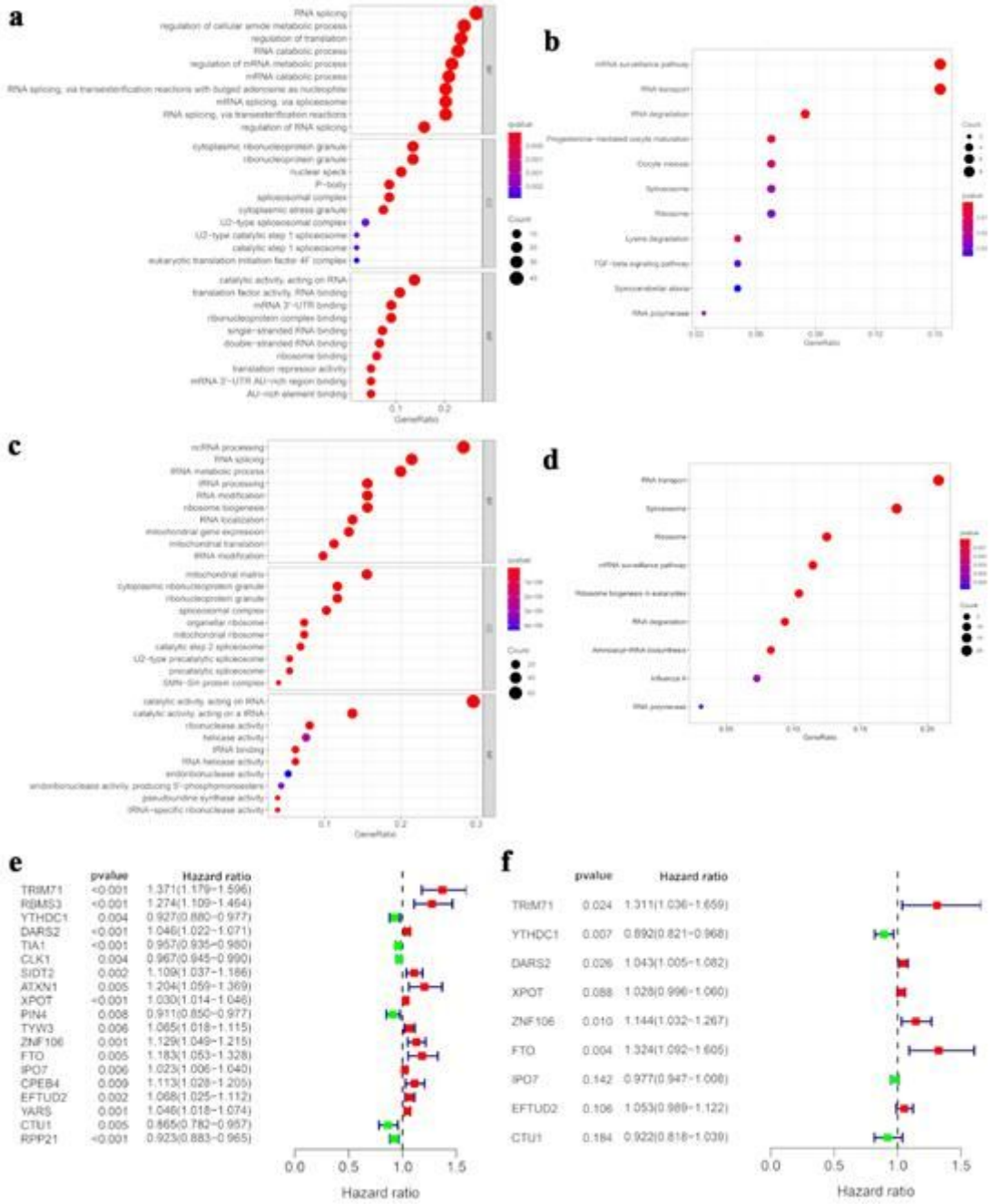

Figure 3

Enrichment analysis and Cox regression analysis of the DERBPs. a, b GO and KEGG enrichment analysis of downregulated DERBPs. c, d GO and KEGG enrichment analysis of upregulated DERBPs. e Univariate Cox regression analysis for identification of prognosis-related candidate hub RBPs. f Multivariate Cox regression analysis to identify prognosis-related RBPs

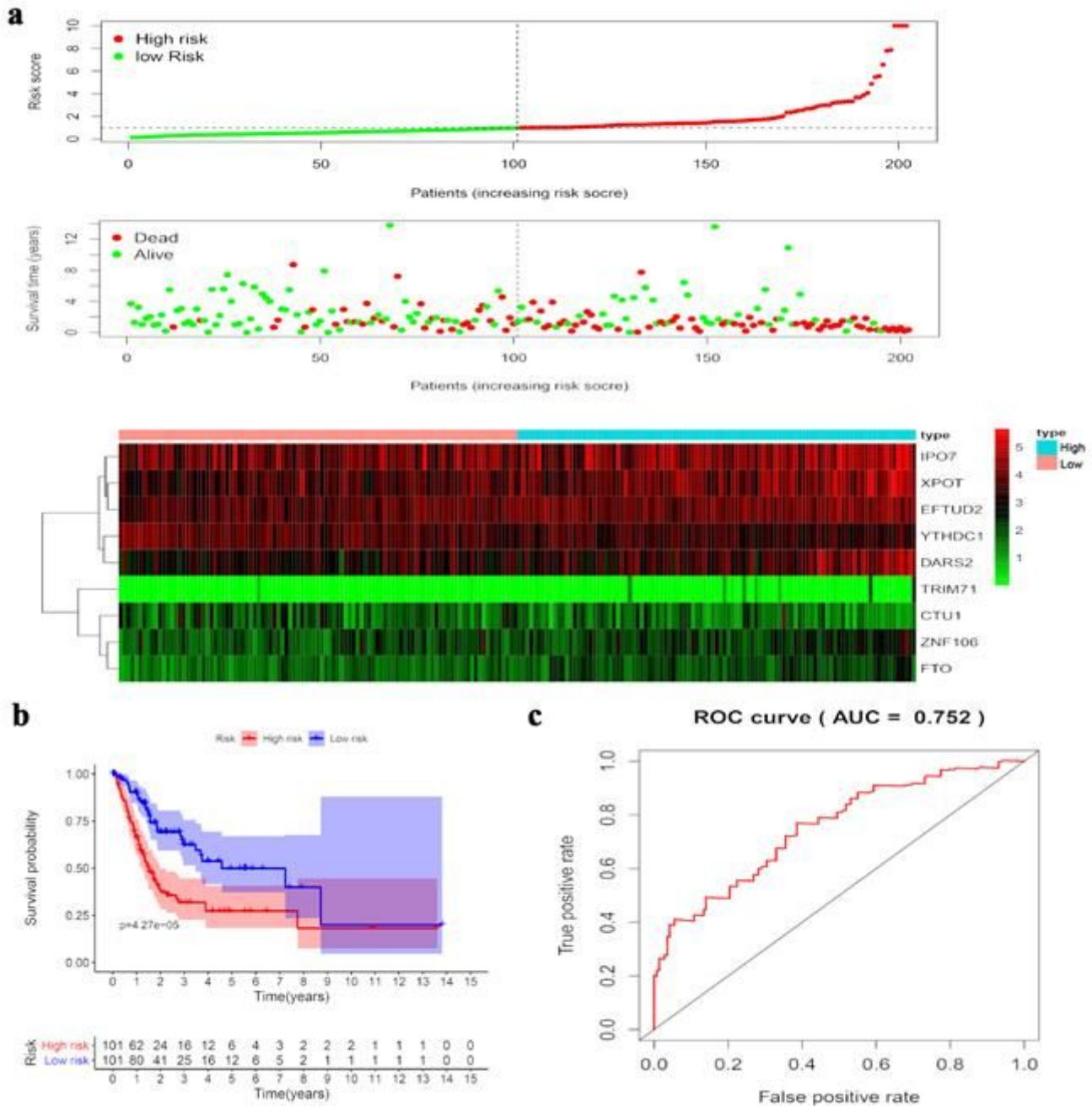

Figure 4

Construction of the prognostic signature based on the training group. a The distribution of risk scores; the distribution of survival time and survival status in the low- and high-risk cohorts; heatmap of the nine prognosis-related RBPs expression between low- and high-risk cohorts. b The patients in the high-risk cohort had significantly shorter OS than those in the low-risk cohort. c The ROC curve of model for forecasting OS based on risk score

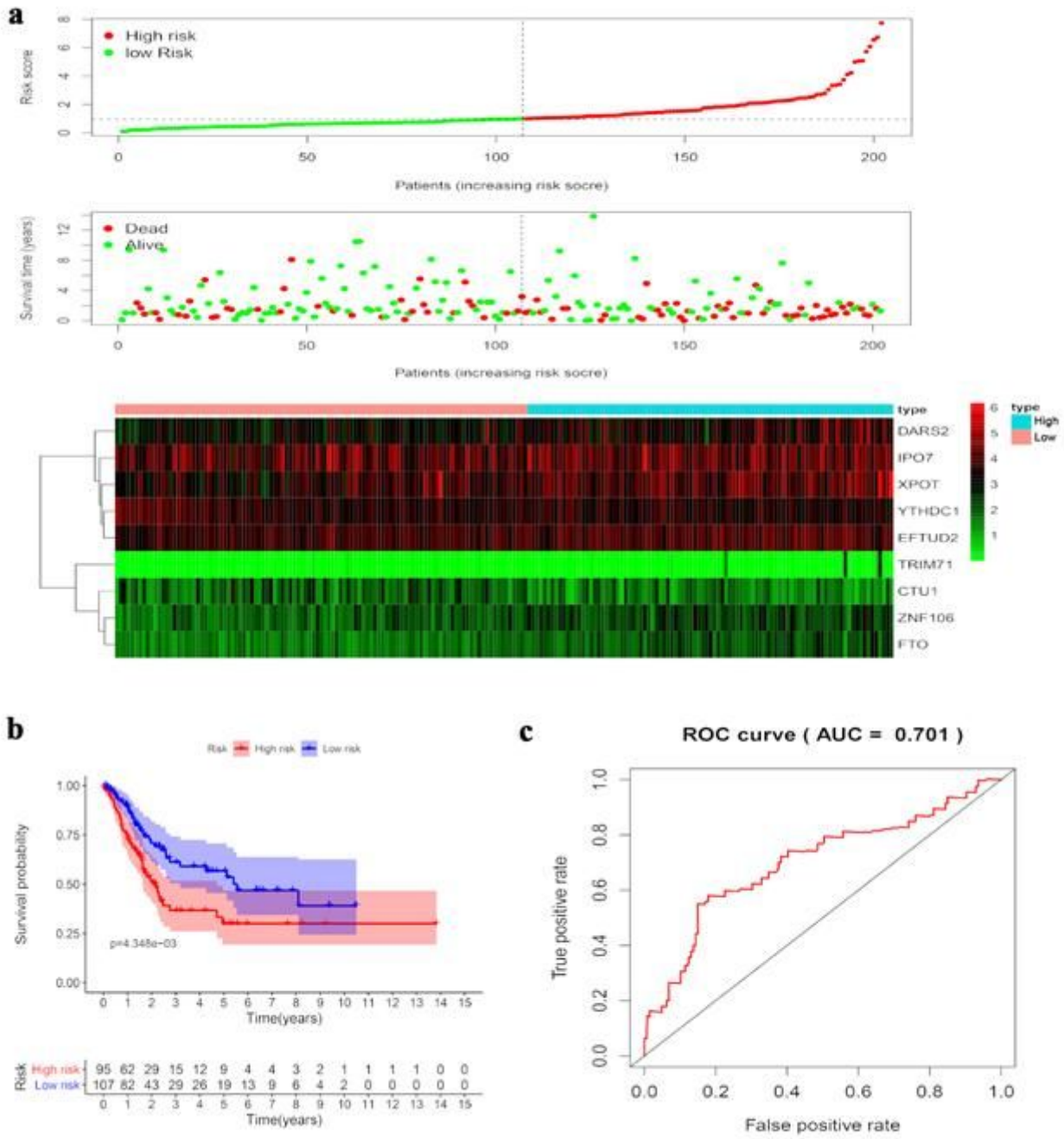

Figure 5

Validation of the prognostic signature in the testing group. a The distribution of risk scores; the distribution of survival time and survival status in the low- and high-risk cohorts; heatmap of the nine prognosis-related RBPs expression between low- and high-risk cohorts. b The patients in the high-risk cohort had significantly shorter OS than those in the low-risk cohort. c The ROC curve of model for forecasting OS based on risk score

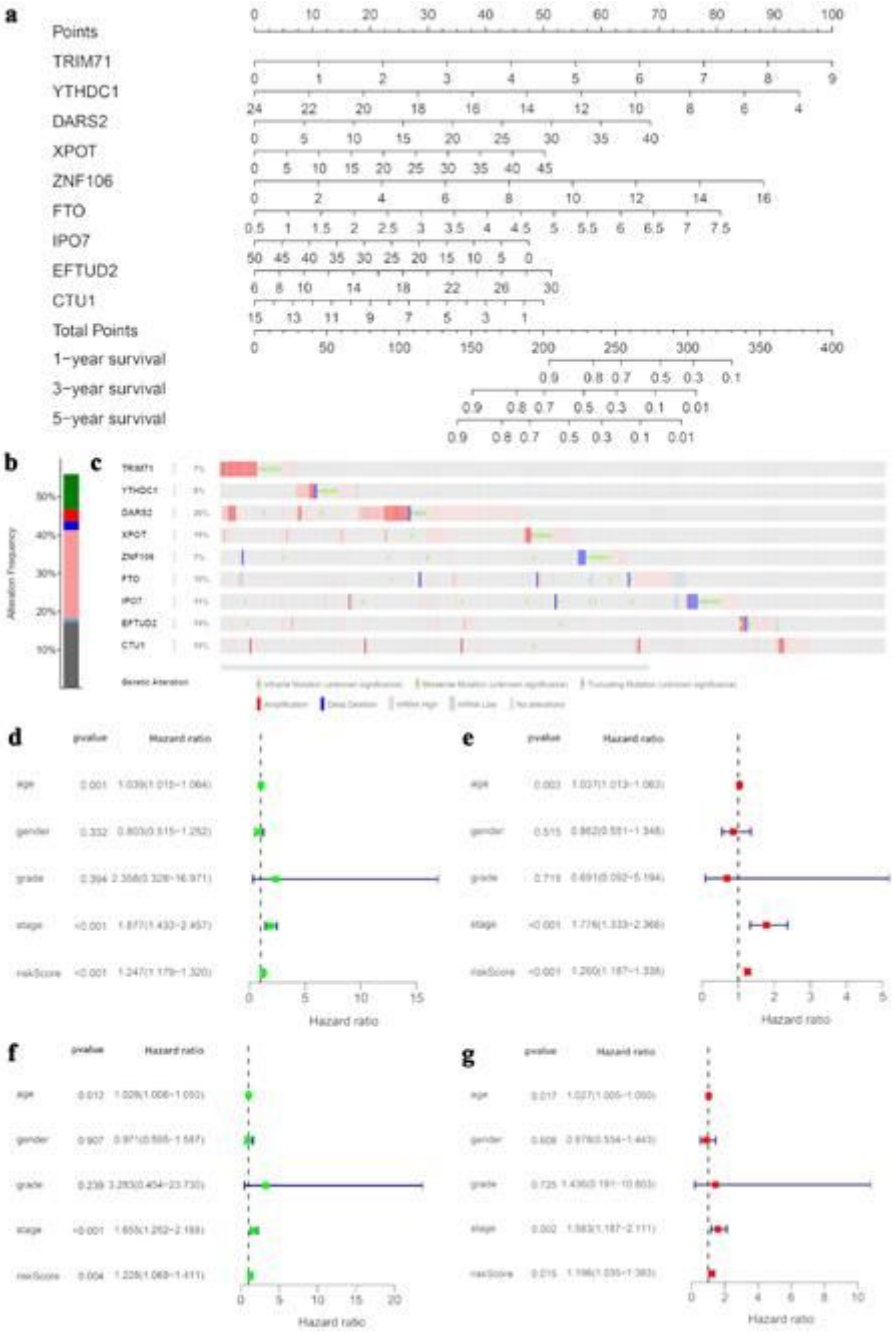

Figure 6

Nomogram and mutation analysis of nine RBPs, and the prognostic effect of different clinical parameters. a Nomogram model for predicting the probability of 1-, 3-, and 5-year OS in BLCA patients. b Mutation frequency of hub RBP genes. c Mutation frequency of each RBP gene. Age, tumor stage, and risk score were correlated with OS of BLCA patients by univariate analysis in the training (d) and testing (f) group. Age, tumor stage, and risk score were the independent prognostic indicators by multivariate analysis in the training (e) and testing (g) group

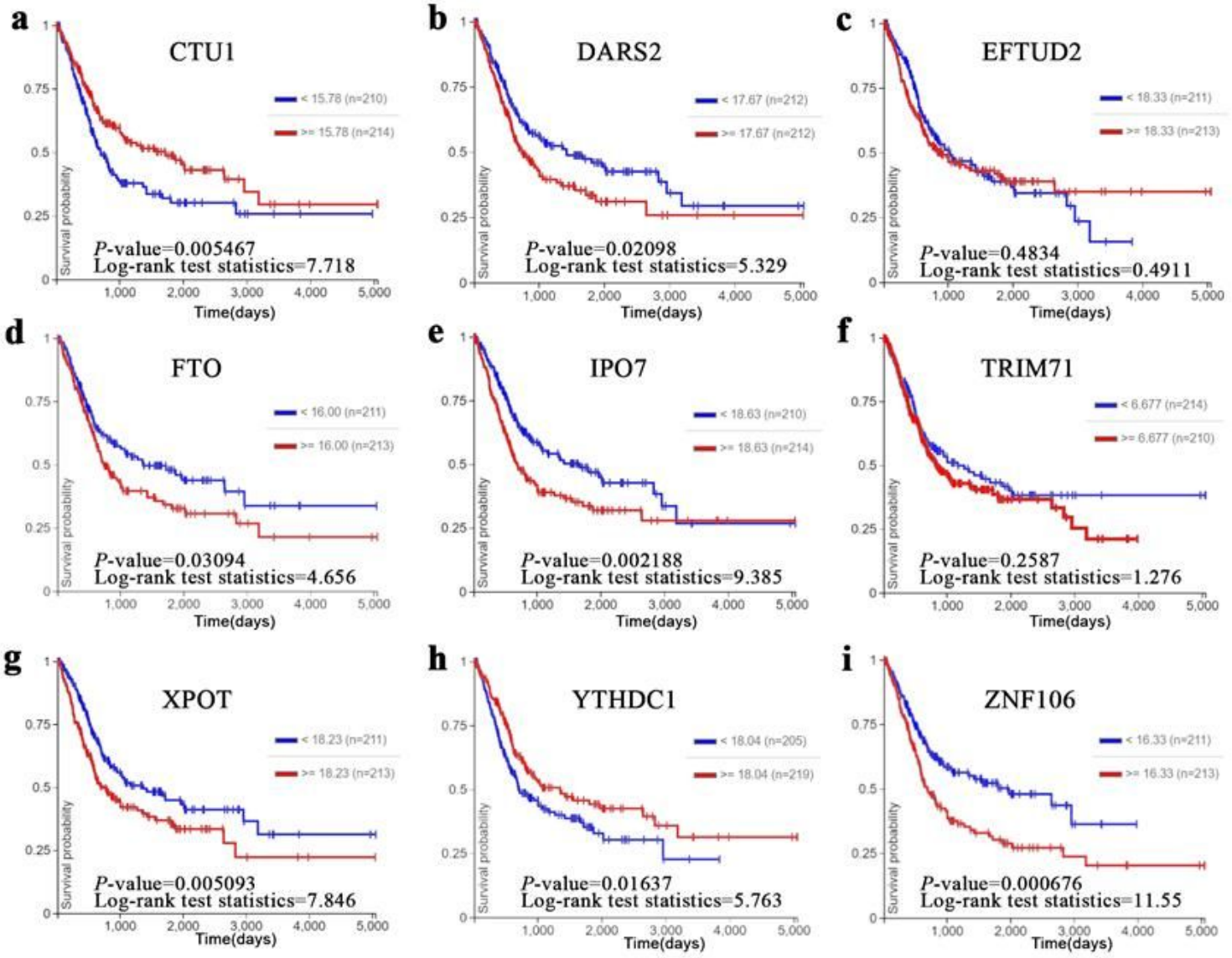

Figure 7

Survival analysis of nine RBPs in BLCA. High DARS2 (b), FTO (d), IPO7 (e), XPOT (g), and ZNF106 (i) expression had remarkably worse overall survival. Low CTU1 (a) and YTHDC1 (h) expression had remarkably worse overall survival

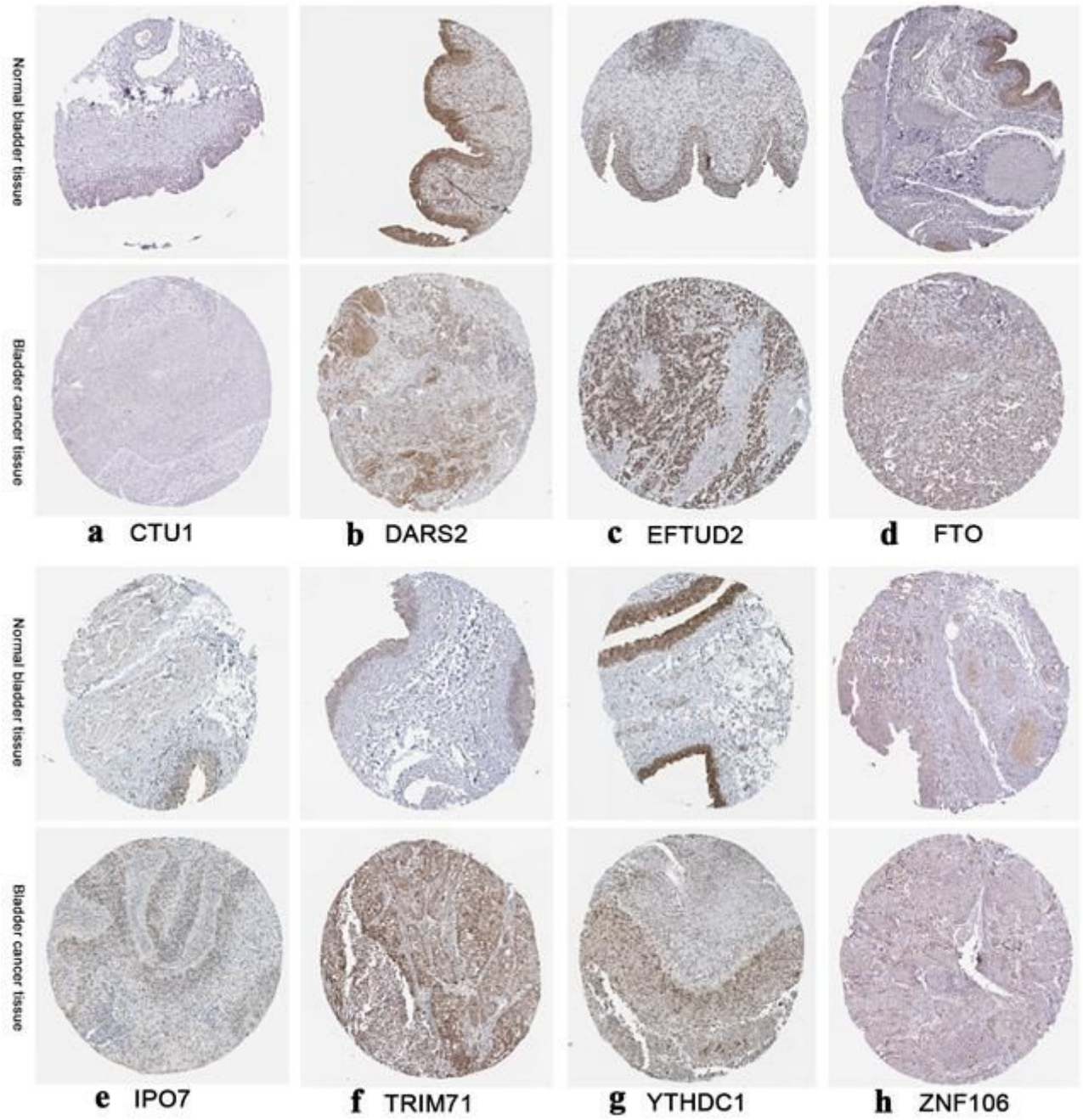

Figure 8

Verification of hub RBPs expression in BLCA and normal bladder tissue. DARS2 (b), EFTUD2 (c), FTO (d), TRIM71 (f), and ZNF106 (h) in bladder cancer tissues were remarkably higher than those in normal bladder tissues. The antibody staining levels of CTU1 (a), IPO7 (e) and YTHDC1 (g) in bladder cancer tissues were relatively reduced