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

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Abstract: 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. 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. A total of 388 DERBPs were identified, including 219 upregulated and 169 downregulated RBPs. All RBPs were screened for the 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 receiver operating characteristic (ROC) curve was 0.752 in the training group and 0.701 in the testing group, which supports the strength of its predictive ability. A nomogram was established according to nine prognosis-related RBPs, which showed strong predictive ability for BLCA. The C-indices of the nomogram were 0.7033 in the training group, and 0.6295 in the testing group. 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 for BLCA.

Keywords: RNA-binding proteins, bladder urothelial cancer, differentially expressed RBPs, overall survival, predictive ability

Introduction
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 related deaths are estimated to have occurred in 2018; 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 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 very rarely originates 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.
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These proteins, among other diverse biological functions, facilitate regulation by miRNAs and sRNAs [7, 8]. To 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 their involvement in carcinogenesis. IGF2BP1 causes an 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 which may be potential biomarkers for the diagnosis and prognosis of BLCA.

Material 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. We used the Wilcoxon test in R to select DERBPs between normal bladder and BLCA tissues, considering $|\log_2 \text{FC} (\text{fold change})| \geq 0.5$ and FDR (false discovery rate) < 0.05. Finally, we applied R and the heatmap R package to draw a volcano map and heatmap of the DERBPs.

Prognosis-related RBP identification

Univariate Cox regression analysis or the 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 the univariate Cox regression test. The Kaplan-Meier test was used to evaluate the prognostic value of DERBPs, and a $p$-value $< 0.05$ was considered to indicate candidate hub RBPs related to prognosis. Thereafter, a 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 a training 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 [16], survminer, and survivalROC packages in R. Using the model, we calculated the risk score of each patient with BLCA based on the following formula: 

$$\text{Risk score} = \beta_1 \times \text{Exp}_1 + \beta_2 \times \text{Exp}_2 + \cdots + \beta_i \times \text{Exp}_i,$$

where $\beta$ is regression coefficient and Exp is expression level.

On the basis of median risk score from the formula, the training group was divided into a low- and high-risk cohort; thereafter, the testing group was also divided into a low- 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 a 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 the survival ROC R package to evaluate the predictive ability of the model in both training and testing groups, and the heatmap R package was used to draw the risk plot and heatmap. Finally, based on the nine hub RBPs, a nomogram was constructed to predict

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the possibility of OS using the rms R package. C-indices were used to estimate the predictive performance of the nomogram.

**Mutation analysis and prognostic value of clinical parameters**

Mutation analysis of nine hub RBPs was executed using the cBioPortal platform (http://www.cbioportal.org) [17]. 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 validation**

We also analyzed the hub RBP’s expression level of TCGA-BLCA using the GEPIA online tool (http://gepia.cancer-pku.cn/index.html) [18]. The online database Human Protein Atlas (http://www.proteinatlas.org/) was utilized to explore the expression of hub RBPs at a translational level [19].

**Results**

**Differentially expressed RBP identification**

The research design is shown in Figure 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 Figure 1B, 1C.

**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 (Figure 2A). Multivariate Cox regression analysis was performed on the 19 RBPs, of which 9 hub RBPs were identified as independent predictors of BLCA (Figure 2B; Table 1). Among them, four RBPs (FTO, IPO7, YTHDC1, and ZNF106) were downregulated, and five RBPs (CTU1, DARS2, EFTUD2, TRIM71, and XPOT) were upregulated.

**Prognosis-related model construction and analysis**

A total of 404 patients with BLCA were randomly divided into a training (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 \times \text{ExpTRIM71} + (-0.1148 \times \text{ExpYTHDC1}) + (0.0417 \times \text{DARS2}) + (0.0272 \times \text{ExpXPOT}) + (0.1341 \times \text{ExpZNF106}) + (0.2806 \times \text{ExpFTO}) + (-0.023 \times \text{ExpIPO7}) + (0.0521 \times \text{ExpEFTUD2}) + (-0.0812 \times \text{ExpCTU1}).

Next, we aimed to evaluate the predictive ability. Results in the training group indicated that patients in the high-risk cohort had a worse OS than those in the low-risk cohort (Figure 3B). ROC analysis demonstrated the prognostic value of the nine hub RBPs. Area under the ROC curve (AUC) of the model was 0.752 in the training group (Figure 3C), suggesting it had better diagnostic capability. For the training group, Figure 3A shows the expression heatmap, patient survival status, and risk scores for the low- and high-risk cohorts based on nine RBPs.

**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 (Figure 5A). The C-indices of the nomogram were 0.7033 in the training group, and 0.6295 in the testing group (validation cohort) (Figure 5B). Based on multivariate Cox regression analysis, the point scale in the 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
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A

BLCA clinical information in TCGA
BLCA RNA sequencing data in TCGA

Differently expressed RNA binding proteins (RBPs) between BLCA and normal bladder tissue

Univariate cox regression analysis to select significant candidate hub RBPs

Validation of hub RBPs expression

Multivariate cox regression analysis to identify prognosis related hub RBPs

Mutation analysis

Multivariate cox proportional hazards regression model

Validation cohort TCGA BLCA (Testing group)

ROC
Nomogram
Log-rank test
KM-plotter

Diagnostic value analysis
Clinical decision-making analysis
Prognostic risk assessment
Validation the prognostic value

B

C

Volcano

Significant
Down
Unchange
Up

-log10(p-value)
-logFC

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estimated overall survival rate of 1, 3, and 5 years, which could eventually help doctors 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%) (Figure 6A, 6B). 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 the OS of patients with BLCA, in both training and testing groups (Figure 6C, 6E). 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 (Figure 6D, 6F).

**Hub RBP expression levels and validation**

We analyzed the hub RBP expression levels in TCGA-BLCA using the GEPIA online tool, and the result indicated that CTU1, DARS2, EFTUD2, TRIM71, and XPOT expression levels in BLCA tissue were significantly higher than those in...
normal bladder tissue. Whereas, FTO, IPO7, YTHDC1, and ZNF106 expression levels in BLCA tissue were significantly lower than those in normal bladder tissue (Figure 7). We used the immunohistochemical results of Human Protein Atlas database to explore the expression of hub RBPs in BLCA, and found that CTU1, DARS2, EFTUD2, TRIM71, and XPOT levels in bladder cancer tissues were significantly higher than in normal bladder tissues. However, the antibody staining levels of FTO, IPO7, YTHDC1, and ZNF106 in bladder cancer tissues were relatively reduced (Figure 8).

Discussion

Although early diagnosis and multimodal treatment of bladder cancer have recently achieved promising results, metastatic diseases are usually incurable, and the 5-year survival rate remains only 15% [20]. Metastasis and recurrence are the main causes of death in patients with bladder cancer, especially MIBC [21]. Therefore, it would be highly significant to understand the molecular mechanism of bladder cancer further, and develop effective early-screening and diagnostic approaches to
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Enhance treatment effectiveness 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 [22]. RBPs and RNAs assemble into a dynamic complex, called ribonucleoprotein (RNP), which regulates almost every stage of RNA lifecycle [23]. An important regulatory mechanism of IncRNAs is RNA-binding protein-mediated post-transcriptional regulation [24]. 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 [25], prostate cancer [26], glioblastoma [27], ovarian cancer [28], and melanoma [29] have been reported to be related to RNA-binding proteins.

Figure 4. 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 expression of nine prognosis-related RBPs 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.
However, understanding of the mechanisms of RBPs in BLCA is currently very limited.

In our study, RNA sequencing data of BLCA were integrated to identify the DERBPs between bladder cancer tissues and normal bladder tissues. 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 from the training group, the risk score model was constructed with the 9 RBPs to predict the prognosis of patients with BLCA. In the training group, the ROC curve of the nine-RBP risk score model also had a moderate ability to predict OS (AUC = 0.752), and high-risk patients with BLCA showed remarkably worse overall survival time. The C-indices of the nomogram were 0.7033 in the training group, and 0.6295 in the testing group. 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 the risk score model, high-risk score patients had worse prognosis, suggesting that the treatment plan and individualized treatment would possibly require adjustment. We further demonstrated that CTU1, DARS2, EFTUD2, TRIM71, and XPOT expression levels in BLCA tissue were significantly higher than those in normal bladder tissue. Whereas, FTO, IPO7, YTHDC1, and ZNF106 expression levels in BLCA tissue were significantly lower than those in normal bladder tissue. Moreover, CTU1, DARS2, EFTUD2, TRIM71, and XPOT expression

Figure 5. Nomogram construction and assessment. A. Nomogram model for predicting the probability of 1-, 3-, and 5-year OS in BLCA patients. B. The C-index of the nomogram in two cohorts.
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A

B

C

D

E

F

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Figure 6. Mutation analysis of nine RBPs, and the prognostic effect of different clinical parameters. (A) Mutation frequency of hub RBP genes. (B) 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 (C) and testing (E) group. Age, tumor stage, and risk score were the independent prognostic indicators by multivariate analysis in the training (D) and testing (F) group.

Figure 7. Expression level of nine RBPs in TCGA-BLCA using the GEPIA online tool. CTU1, DARS2, EFTUD2, TRIM71, and XPOT expression levels in BLCA tissue were significantly higher than those in normal bladder tissue. In contrast, expression levels of FTO, IPO7, YTHDC1, and ZNF106 were the opposite.
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Figure 8. Validation of hub RBPs expression in BLCA and normal bladder tissue. Expression of CTU1, DARS2, EFTUD2, and TRIM71 in bladder cancer tissues were higher than those in normal bladder tissues. The antibody staining levels of FTO, IPO7, YTHDC1, and ZNF106 in bladder cancer tissues were relatively lower.

was significantly higher in bladder cancer tissues than in normal bladder tissues using Human Protein Atlas database. However, the staining levels of FTO, IPO7, YTHDC1, and ZNF106 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) mcm5^s2^-tRNA modification, are upregulated and promote metastasis in human breast cancers [30]. CTU1 copy number amplifications were identified in 25% of myxopapillary ependymomas [31]. Qin et al. demonstrated DARS2 as a hepatocarcinoma gene that could promote the progression of the hepatocarcinoma cell cycle and inhibit the apoptosis of hepatocarcinoma cells [32]. EFTUD2 gene expression was upregulated in hepatocarcinoma, and had prognostic significance in patients with hepatocarcinoma [33]. 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 progno-
sis. In terms of function, FTO silencing inhibited the growth and migration of ESCC cells in CCK8 and Transwell assays, whereas FTO overexpression showed an opposite result [34]. Inhibition of IPO7 by siRNA is known to lead to reduced proliferation of prostate cancer cells [35]. 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 [36]. XPOT belongs to the Ran-GTPase exportin family that mediates export of tRNA from the nucleus to the cytoplasm, and high expression of XPOT in hepatocellular carcinoma is associated with worse prognosis [37]. Celona et al. had reported ZFP106 knockout mice to have severe degeneration of motoneurons while transgenic recovery of ZFP106 specifically inhibited the degeneration [38].

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 limitations. Firstly, our findings are only based on RNA sequencing without other omics data. Secondly, the risk score model was established based on the TCGA BLCA data, and prospective studies 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 are required to verify the claims.

Conclusion

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 for BLCA.

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Disclosure of conflict of interest

None.

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References

[1] Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA and Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394-424.
[2] Van Batavia J, Yamany T, Molotkov A, Dan H, Mansukhani M, Batourina E, Schneider K, Oyon D, Dunlop M, Wu XR, Cordon-Cardo C and Mendelsohn C. Bladder cancers arise from distinct urothelial sub-populations. Nat Cell Biol 2014; 16: 982-5.
[3] Crabb SJ and Douglas J. The latest treatment options for bladder cancer. Br Med Bull 2018; 128: 85-95.
[4] Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, Newling DW and Kurth K. Predicting recurrence and progression in individual patients with stage Ta T1 bladder cancer using EORTC risk tables: a combined analysis of 2596 patients from seven EORTC trials. Eur Urol 2006; 49: 466-477.
[5] Wołącewicz M, Hryniewicz R, Grywałska E, Suchojad T, Lekowska T, Rolifski J and Niedźwiedzka-Rystwej P. Immunotherapy in bladder cancer: current methods and future perspectives. Cancers (Basel) 2020; 12: 1181.
[6] Corley M, Burns MC and Yeo GW. How RNA-binding proteins interact with RNA: molecules and mechanisms. Mol Cell 2020; 78: 9-29.
[7] Klisch T, Sträßer K, Kunetsky V and Ankö ML. From parts lists to functional significance-RNA-protein interactions in gene regulation. Wiley Interdiscip Rev RNA 2020; 11: e1582.
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[8] Nechay M and Kleiner RE. High-throughput approaches to profile RNA-protein interactions. Curr Opin Chem Biol 2020; 54: 37-44.

[9] Gerstberger S, Hafner M and Tuschl T. A census of human RNA-binding proteins. Nat Rev Genet 2014; 15: 829-845.

[10] Elcheva IA, Wood T, Chiarolanzio K, Chim B, Wong M, Singh V, Gowda CP, Lu Q, Hafner M, Dovat S, Liu Z, Muljo SA and Spiegelman VS. RNA-binding protein IGFBP2BP1 maintains leukemia stem cell properties by regulating HOXB4, MYB, and ALDH1A1. Leukemia 2020; 34: 1354-1363.

[11] Yi C, Li G, Ivanov DN, Wang Z, Velasco MX, Hernández G, Kaundal S, Villarreal J, Gupta YK, Qiao M, Hubert CG, Hart MJ and Penalva LOF. Luteolin inhibits Musashi1 binding to RNA and disrupts cancer phenotypes in glioblastoma cells. RNA Biol 2018; 15: 1440-1452.

[12] Schuscherl K, Helwig M, Hüttelmaier S, Heckl D, Klusmann JH and Hoei JJ. RNA-binding proteins in acute leukemias. Int J Mol Sci 2020; 21: 3409.

[13] Masuda K and Kuwano Y. Diverse roles of RNA-binding proteins in cancer traits and their implications in gastrointestinal cancers. Wiley Interdiscip Rev RNA 2019; 10: e1520.

[14] García-Cárdenas JM, Guerrero S, López-Cortés A, Armendáriz-Castillo I, Guevara-Ramírez P, Pérez-Villa A, Yumiceba V, Zambrano AK, Leone PE and Paz-Y-Miño C. Post-transcriptional regulation of colorectal cancer: a focus on RNA-binding proteins. Front Mol Biosci 2019; 6: 65.

[15] Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W and Smyth GK. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res 2015; 43: e47.

[16] Friedman J, Hastie T and Tibshirani R. Regularization paths for generalized linear models via coordinate descent. J Stat Softw 2010; 33: 1-22.

[17] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larson E, Cerami E, Sander C and Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6: pl1.

[18] Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 2017; 45: W98-W102.

[19] Thul PJ, Åkesson L, Wiking M, Mahdessian D, Geladaki A, Ait Blal E, Alm T, Asplund A, Björk L, Breckels LM, Bäckström A, Danielsson F, Fagerberg L, Fall J, Gatto L, Gann C, Hober S, Hjelmare M, Johansson F, Lee S, Lindskog C, Mulder J, Mulvey CM, Nilsson P, Oksvold P, Rockberg J, Schutten R, Schwenk JM, Sivertsson Å, Sjöstedt E, Skogs M, Stadler C, Sullivan DP, Tegel H, Winsnes C, Zhang C, Zwhalen M, Mardinglu A, Pontén F, von Felitzen K, Lilley KS, Uhlem M and Lundberg E. A subcellular map of the human proteome. Science 2017; 356: eaal3321.

[20] Nadal R and Bellmunt J. Management of metastatic bladder cancer. Cancer Treat Rev 2019; 76: 10-21.

[21] Richters A, Aben KKH and Kiemeneij LALM. The global burden of urinary bladder cancer: an update. World J Urol 2020; 38: 1895-1904.

[22] Mohibi S, Chen X and Zhang J. Cancer the ‘RBP’eutics-RNA-binding proteins as therapeutic targets for cancer. Pharmacol Ther 2019; 203: 107390.

[23] Beckmann BM, Castello A and Medenbach J. The expanding universe of ribonucleoproteins: of novel RNA-binding proteins and unconventional interactions. Pflugers Arch 2016; 468: 1029-1040.

[24] Jonas K, Calin GA and Pichler M. RNA-binding proteins as important regulators of long non-coding RNAs in cancer. Int J Mol Sci 2020; 21: 2969.

[25] Chatterji P and Rustgi AK. RNA binding proteins in intestinal epithelial biology and colorectal cancer. Trends Mol Med 2018; 24: 490-506.

[26] Busà R, Paronetto MP, Farini D, Pierantozzi E, Botti F, Angelini DF, Attisani F, Vespasiani G and Sette C. The RNA-binding protein Sam68 contributes to proliferation and survival of human prostate cancer cells. Oncogene 2007; 26: 4372-4382.

[27] Janiszewska M, Suvà ML, Riggi N, Houtkooper RH, Auwerx J, Clément-Schatlo V, Radovanic I, Rheinbay E, Provero P and Stamenkovic I. Imp2 controls oxidative phosphorylation and is crucial for preserving glioblastoma cancer stem cells. Genes Dev 2012; 26: 1926-1944.

[28] Hopkins TS, Mura M, Al-Ashtal HA, Lahr RM, Abd-Latip N, Sweeney K, Lu H, Weir J, El-Bahrawy M, Steel JH, Ghaem-Maghami S, Aboagye EO, Berman AJ and Blagden SP. The RNA-binding protein LARP1 is a post-transcriptional regulator of survival and tumorigenesis in ovarian cancer. Nucleic Acids Res 2016; 44: 1227-1246.

[29] Wurth L, Papasaikas P, Olmeda D, Bley N, Calvo GT, Guerrero S, Cereo-Willis D, Martinez-Userso J, García-Fernández M, Hüttelmaier S, Soengas MS and Gebauer F. UNR/CSDE1 drives a post-transcriptional program to promote melanoma invasion and metastasis. Cancer Cell 2016; 30: 694-707.

[30] Delaunay S, Rapino F, Tharun L, Zhou Z, Heukamp L, Termathe M, Shostak K, Klevernic I,
Florin A, Desmecht H, Desmet CJ, Nguyen L, Leidel SA, Willis AE, Büttner R, Chariot A and Close P. Elp3 links tRNA modification to IRES-dependent translation of LEF1 to sustain metastasis in breast cancer. J Exp Med 2016; 213: 2503-2523.

[31] Zhang M, Iyer RR, Azad TD, Wang Q, Garzon-Muvdi T, Wang J, Liu A, Burger P, Eberhart C, Rodriguez FJ, Sciubba DM, Wolinsky JP, Gokaslan Z, Groves ML, Jallo GI and Bettegowda C. Genomic landscape of intramedullary spinal cord gliomas. Sci Rep 2019; 9: 18722.

[32] Qin X, Li C, Guo T, Chen J, Wang HT, Wang YT, Xiao YS, Li J, Liu P, Liu ZS and Liu QY. Upregulation of DARS2 by HBV promotes hepatocarcinogenesis through the miR-30e-5p/MAPK/NFAT5 pathway. J Exp Clin Cancer Res 2017; 36: 148.

[33] Chi Q, Geng X, Xu K, Wang C and Zhao H. Potential targets and molecular mechanism of miR-331-3p in hepatocellular carcinoma identified by weighted gene coexpression network analysis. Biosci Rep 2020; 40: BSR20200124.

[34] Liu S, Huang M, Chen Z, Chen J, Chao Q, Yin X and Quan M. FTO promotes cell proliferation and migration in esophageal squamous cell carcinoma through up-regulation of MMP13. Exp Cell Res 2020; 389: 111894.

[35] Szczyrba J, Nolte E, Hart M, Döll C, Wach S, Taubert H, Keck B, Kremmer E, Stöhr R, Hartmann A, Wieland W, Wullich B and Grässer FA. Identification of ZNF217, hnRNP-K, VEGF-A and IPO7 as targets for microRNAs that are downregulated in prostate carcinoma. Int J Cancer 2013; 132: 775-84.

[36] Torres-Fernández LA, Jux B, Bille M, Port Y, Schneider K, Geyer M, Mayer G and Kolanus W. The mRNA repressor TRIM71 cooperates with nonsense-mediated decay factors to destabilize the mRNA of CDKN1A/p21. Nucleic Acids Res 2019; 47: 11861-11879.

[37] Lin J, Hou Y, Huang S, Wang Z, Sun C, Wang Z, He X, Tam NL, Wu C and Wu L. Exportin-T promotes tumor proliferation and invasion in hepatocellular carcinoma. Mol Carcinog 2019; 58: 293-304.

[38] Celona B, Dollen JV, Vatsavayai SC, Kashima R, Johnson JR, Tang AA, Hata A, Miller BL, Huang EJ, Krogan NJ, Seeley WW and Black BL. Suppression of C9orf72 RNA repeat-induced neurotoxicity by the ALS-associated RNA-binding protein Zfp106. Elife 2017; 6: e19032.